Renal Cancer

Mark H Smith

Queens, New York 11418, USA
mark20082009@gmail.com

Abstract: Cancer is the cells that grow out of control. Cancer cells can also invade other tissues. Growing out of control and invading other tissues are what makes a cell a cancer cell. Involved in more than 100 diseases, the cancer can cause serious illness and death. Normally, the cells become cancer cells because of DNA damage. This material is a literature collection of the researches on the renal cancer.
[Smith MH. **Renal Cancer .** *Cancer Biology* 2013;3(1):180-247]. (ISSN: 2150-1041). http://www.cancerbio.net. 4

Keywords: cancer; biology; life; disease; research; literature; renal

1. Introduction

Cancer is the general name for a group of more than 100 diseases. Although there are many kinds of cancer, all cancers start because abnormal cells grow out of control. Untreated cancers can cause serious illness and death.

The body is made up of trillions of living cells. Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace worn-out or dying cells or to repair injuries.

Literatures

Alam, N. A., S. Olpin, et al. (2005). "Fumarate hydratase mutations and predisposition to cutaneous leiomyomas, uterine leiomyomas and renal cancer." Br J Dermatol **153**(1): 11-7.

Germline heterozygous loss-of-function mutations of fumarate hydratase (FH) predispose to the autosomal dominant syndrome of multiple cutaneous and uterine leiomyomatosis (MCUL). Forty-five distinct FH mutations have been identified in 76 of 89 (85%) reported probands with skin leiomyomas. This suggests that MCUL is a genetically homogeneous condition and that most patients presenting with skin leiomyomas will have underlying FH mutations. FH mutations identified include 26/45 (58%) missense; 12/45 (27%) frameshift, 4/45 (9%) nonsense changes and 3/45 (7%) different whole gene deletions. In MCUL kindreds, the majority of females with FH mutations have both skin and uterine leiomyomas. A proportion of individuals with FH mutations have associated renal cancer, a variant known as hereditary leiomyomatosis and renal cell cancer (HLRCC). If selection bias is removed, the prevalence of renal cancer in MCUL lies between one of 46 (2%) families who were not radiologically screened, and two of 32 (6%) families who were radiologically screened. Truncating, particularly frameshift, mutations appear to be significantly associated with renal cancer (P = 0.003), suggesting a possible basis for selective screening. There may also be a significantly increased rate of renal cancer in females (P = 0.004), suggesting a possible role for hormonal factors. Review of the literature suggests that, unlike most individuals presenting with skin leiomyomas, the majority of patients presenting with uterine leiomyomas or renal cancer will not have underlying FH mutations.

Alam, N. A., A. J. Rowan, et al. (2003). "Genetic and functional analyses of FH mutations in multiple cutaneous and uterine leiomyomatosis, hereditary leiomyomatosis and renal cancer, and fumarate hydratase deficiency." Hum Mol Genet **12**(11): 1241-52.

Germline mutations of the fumarate hydratase (FH, fumarase) gene are found in the recessive FH deficiency syndrome and in dominantly inherited susceptibility to multiple cutaneous and uterine leiomyomatosis (MCUL). We have previously reported a number of germline FH mutations from MCUL patients. In this study, we report additional FH mutations in MCUL and FH deficiency patients. Mutations can readily be found in about 75% of MCUL cases and most cases of FH deficiency. Some of the more common FH mutations are probably derived from founding individuals. Protein-truncating FH mutations are functionally null alleles. Disease-associated missense FH changes map to highly conserved residues, mostly in or around the enzyme's active site or activation site; we predict that these mutations severely compromise enzyme function. The mutation spectra in FH deficiency and MCUL are similar, although in the latter mutations tend to occur earlier in the gene and, perhaps, are more likely to result in a truncated or absent protein. We have found that not all mutation-carrier parents of FH deficiency children have a strong predisposition to leiomyomata. We have confirmed that renal carcinoma is sometimes

part of MCUL, as part of the variant hereditary leiomyomatosis and renal cancer (HLRCC) syndrome, and have shown that these cancers may have either type II papillary or collecting duct morphology. We have found no association between the type or site of FH mutation and any aspect of the MCUL phenotype. Biochemical assay for reduced FH functional activity in the germline of MCUL patients can indicate carriers of FH mutations with high sensitivity and specificity, and can detect reduced FH activity in some patients without detectable FH mutations. We conclude that MCUL is probably a genetically homogeneous tumour predisposition syndrome, primarily resulting from absent or severely reduced fumarase activity, with currently unknown functional consequences for the smooth muscle or kidney cell.

Allory, Y., Y. Matsuoka, et al. (2005). "The L1 cell adhesion molecule is induced in renal cancer cells and correlates with metastasis in clear cell carcinomas." Clin Cancer Res **11**(3): 1190-7.

PURPOSE: The L1 cell adhesion molecule is overexpressed in many human carcinomas. The objectives of the study were to provide a comprehensive description of L1 distribution in human kidney and to establish the prognostic relevance of L1 expression in renal cell carcinomas (RCC). EXPERIMENTAL DESIGN: Using two antibodies to the extracellular part and the cytoplasmic domain, respectively, we first compared L1 expression in normal kidney and renal tumors of diverse histopathologic origin, then we studied L1 expression together with tumor stage, grade, molecular prognostic biomarkers, and metastatic behavior. RESULTS: In normal kidney, L1 immunoreactive with both antibodies was expressed in all epithelial cells originating from the ureteric bud except for intercalated cells. In renal tumors, L1 was mainly detected in those originating from cells that do not express L1 in the normal kidney [i.e., 33 of 72 clear cell RCC (ccRCC) and 25 of 88 papillary RCC (papRCC)]. Both in ccRCC and papRCC, L1 reacted only with the antibody to the extracellular domain, suggesting that the protein was truncated. In these carcinomas, L1 expression was strongly correlated with Ki-67 proliferation index (ccRCC, P = 0.0059; papRCC, P = 0.0039), but only in ccRCC, the presence of L1 was associated with the risk of metastasis (P = 0.0121). This risk was higher if cyclin D1 was concurrently absent in tumor cells (P < 0.0001). The L1(+)/cyclin D1(-) profile was an independent prognostic factor of metastasis occurrence in multivariate analysis (P = 0.0023). CONCLUSION: We have found a combination of markers that can serve to identify a subgroup of high-risk patients with ccRCC that may require more aggressive therapies.

Arya, M., D. Chao, et al. (2004). "Allogeneic hematopoietic stem-cell transplantation: the next generation of therapy for metastatic renal cell cancer." Nat Clin Pract Oncol **1**(1): 32-8.

The management of metastatic renal cell carcinoma (mRCC) remains a therapeutic challenge; less than 10% of patients survive for longer than 5 years. The resistance of renal cancer to chemotherapy may be explained by high levels of the multidrug resistance gene, MDR1. Immune-based treatments for renal cancer have been explored because of their unusual susceptibility to immunological assault. However, response rates to cytokines such as interleukin-2 and interferon-alpha have ranged from only 10% to 20%, prompting other immunotherapy approaches, such as allogeneic stem-cell transplantation, to be investigated. Several clinical trials have provided evidence of partial or complete disease regression in refractory mRCC following nonmyeloablative stem-cell transplantation. This effect is because of a donor antimalignancy effect mediated by immunocompetent donor T cells, called graft-versus-tumor effect. Unfortunately, less than 30% of patients who could have this procedure will have a human-leukocyte-antigen-compatible sibling, and attention is focusing on alternative donors such as matched unrelated donors and partially mismatched related donors. Despite the improved safety of nonmyeloablative conditioning regimens, transplant-related toxic effects (particularly graft-versus-host disease) remain obstacles to the safe and effective use of this treatment. Regardless of these limitations, innovative approaches have attempted to harness the potential of the graft-versus-tumor effect in mRCC and other solid tumors.

Ashida, S., H. Okuda, et al. (2000). "Detection of circulating cancer cells with von hippel-lindau gene mutation in peripheral blood of patients with renal cell carcinoma." Clin Cancer Res **6**(10): 3817-22.

Mutations of the von Hippel-Lindau (VHL) tumor suppressor gene have been detected in up to 60% of sporadic clear cell renal carcinomas (RCCs). Even patients with RCCs believed to be curable with radical nephrectomy sometimes develop distant metastasis 5-10 years after surgery, suggesting hematogenous circulation of cancer cells. Useful tumor markers have not yet been established for RCC. To detect patients at high risk of metastasis after surgery, we developed a highly sensitive and specific nested reverse transcription-PCR method using VHL gene mutation to detect circulating cancer cells. We screened 29 sporadic clear cell RCCs from patients for

mutations of the VHL gene by direct sequencing. We next examined blood samples from patients with the VHL gene mutation using mutation-specific nested reverse transcription-PCR. Somatic mutations were detected in 20 of 29 (69.0%) sporadic clear cell RCCs. The VHL gene mutations were detected in peripheral and/or renal venous blood from 15 of 20 (75%) patients. The mutations were detected in the peripheral blood in 2 of 17 (11.8%) patients before surgery, 6 of 16 (37.5%) patients within 24 h after surgery, 3 of 16 (18.8%) patients on day 7 after surgery, and 2 of 11 (18.2%) patients on day 30 after surgery. In seven of nine (77.8%) patients, mutations were detected in renal venous blood during surgery. These findings indicate the presence of circulating cancer cells with VHL gene mutation. Although much larger studies are needed to determine the clinical significance, our study shows that this technique is feasible for detecting circulating RCC cells.

Atkins, M., M. Regan, et al. (2005). "Carbonic anhydrase IX expression predicts outcome of interleukin 2 therapy for renal cancer." Clin Cancer Res **11**(10): 3714-21.

PURPOSE: Renal cancer response to interleukin 2 (IL-2) therapy and patient survival has been correlated with tumor histology and carbonic anhydrase IX (CAIX) expression. In an effort to confirm and expand these observations, we examined CAIX expression in pathology specimens from renal cancer patients who had previously received IL-2 therapy. EXPERIMENTAL DESIGN: Paraffin-embedded tissue sections of renal cancer were immunostained with the MN-75 monoclonal antibody to CAIX and expression levels were correlated with histologic findings and clinical outcome. RESULTS: Tissue specimens were obtained from 66 patients; 27 of whom (41%) had responded to IL-2-based therapy. Fifty-eight specimens were assessed as clear cell, with 56, 33, and 4 having alveolar, granular, and papillary features, respectively. Twenty-four (36%), 31 (47%), and 11 (17%) were classified into good, intermediate, and poor prognosis groups according to the Upton pathology model. Forty-one specimens (62%) had high CAIX expression. Twenty-one of 27 (78%) responding patients had high CAIX expressing tumors compared with 20 of 39 (51%) nonresponders (odds ratio, 3.3; P = 0.04). Median survival was prolonged (P = 0.04) and survival >5 years was only seen in high CAIX expressers. In patients with intermediate pathologic prognosis, all nine responders had high CAIX expression versus 11 of 22 nonresponders. A resultant group with good pathologic prognosis alone or with intermediate pathologic prognosis and high CAIX contained 26 of 27 (96%) responders compared with 18 of 39 (46%) nonresponders (odds ratio, 30; P < 0.01) and exhibited longer median survival (P < 0.01). CONCLUSIONS: CAIX expression seems to be an important predictor of outcome in renal cell carcinoma patients receiving IL-2-based therapy and may enhance prognostic information obtained from pathology specimens.

Badeloe, S., A. J. van Geest, et al. (2008). "Absence of fumarate hydratase mutation in a family with cutaneous leiomyosarcoma and renal cancer." Int J Dermatol **47 Suppl 1**: 18-20.

A 41-year-old man was diagnosed with a cutaneous leiomyosarcoma on the left shoulder. Family history revealed that his brother had died of a metastatic kidney tumor at young age. Although apparently rare, the familial occurrence of cutaneous leiomyosarcoma with renal cancer has been described in the context of hereditary cutaneous leiomyomatosis and renal cell cancer (HLRCC). This rare genetic syndrome is caused by heterozygous mutations in the fumarate hydratase (FH) gene. Hence, the manifestation of these two rare malignancies within one family was strongly suggestive of a common underlying genetic defect. However, mutation analysis in the FH gene excluded HLRCC in this family. Although the familial occurrence of these rare tumors might be coincidental, it cannot be ruled out that, beside FH, mutations in another as yet unknown gene could give rise to both leiomyosarcoma and kidney cancer.

Banks, R. E., P. Tirukonda, et al. (2006). "Genetic and epigenetic analysis of von Hippel-Lindau (VHL) gene alterations and relationship with clinical variables in sporadic renal cancer." Cancer Res **66**(4): 2000-11.

Genetic and epigenetic changes in the von Hippel-Lindau (VHL) tumor suppressor gene are common in sporadic conventional renal cell carcinoma (cRCC). Further insight into the clinical significance of these changes may lead to increased biological understanding and identification of subgroups of patients differing prognostically or who may benefit from specific targeted treatments. We have comprehensively examined the VHL status in tissue samples from 115 patients undergoing nephrectomy, including 96 with sporadic cRCC. In patients with cRCC, loss of heterozygosity was found in 78.4%, mutation in 71%, and promoter methylation in 20.4% of samples. Multiplex ligation-dependent probe amplification identified intragenic copy number changes in several samples including two which were otherwise thought to be VHL-noninvolved. Overall, evidence of biallelic inactivation was found in 74.2% of patients with cRCC. Many of the mutations were novel and approximately two-thirds were potentially truncating. Examination of these and other published

findings confirmed mutation hotspots affecting codons 117 and 164, and revealed a common region of mutation in codons 60 to 78. Gender-specific differences in methylation and mutation were seen, although not quite achieving statistical significance (P = 0.068 and 0.11), and a possible association between methylation and polymorphism was identified. No significant differences were seen between VHL subgroups with regard to clinicopathologic features including stage, grade, tumor size, cancer-free and overall survival, with the exception of a significant association between loss of heterozygosity and grade, although a possible trend for survival differences based on mutation location was apparent.

Barbero, G., F. Carta, et al. (2006). "Protein/RNA coextraction and small two-dimensional polyacrylamide gel electrophoresis for proteomic/gene expression analysis of renal cancer biopsies." Anal Biochem **349**(1): 62-71.

A small amount of bioptic tissue (approximately 5-10mg of fresh tissue) usually does not contain enough material to extract protein and RNA separately, to obtain preparative two-dimensional polyacrylamide gel electrophoresis (2-DE), and to identify a large number of separated proteins by MS. We tested a method, on small renal cancer specimens, for the coextraction of protein and RNA coupled with 2-DE and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) or quadrupole time-of-flight (Q-TOF) analysis. We coextracted 0.28+/-0.05mg of proteins and 2.5+/-0.33microg of RNA for each 10mg of renal carcinoma tissue. Small and large 2-DE gels were compared: they showed a similar number of spots, and it was possible to match each other; using small format gels, one-fifth of the protein amount was required to identify, by Q-TOF analysis, the same number of proteins identifiable in large-format gel using MALDI-TOF analysis. Quality of RNA coextracted with the proteins was tested by real-time PCR on a set of housekeeping genes. They were quantified with high amplification efficiency and specificity. In conclusion, using 5 to 10mg of fresh tissue, it was possible to perform comprehensive parallel proteomic and genomic analysis by high-resolution, small-format 2-DE gels, allowing approximately 300 proteins identification and 1000 genes expression analysis.

Bardi, E., A. V. Olah, et al. (2004). "Late effects on renal glomerular and tubular function in childhood cancer survivors." Pediatr Blood Cancer **43**(6): 668-73.

BACKGROUND: Late nephrotoxicity among childhood cancer survivors is poorly documented. METHODS: We investigated 115 patients and 86 controls assessing serum cystatin C concentration (CysC), urinary N-acetyl-beta-D-glucosaminidase activity (NAG), and microalbuminuria. Proteinuria was quantified and electrophoresis performed. Polymorphism of the angiotensin convertase enzyme (ACE) gene was determined by genomic PCR. RESULTS: CysC was elevated in Wilms tumor (WT) patients. Gross proteinuria was observed in 30 patients including three patients with progressive proteinuria who improved on ACE-inhibitor treatment. Neither patients with proteinuria nor the entire study population differed from controls with respect to ACE polymorphism. Pathologically elevated urinary NAG was noted in 38% of leukemia/lymphoma, 54% of solid tumor, 20% of WT survivors. A similar distribution of pathological microalbuminuria was found. CONCLUSIONS: Mild-to-moderate subclinical glomerular and tubular damage can be identified in many childhood cancer survivors. However, most patients experience some spontaneous recovery from acute nephrotoxicity.

Beales, P. L., H. A. Reid, et al. (2000). "Renal cancer and malformations in relatives of patients with Bardet-Biedl syndrome." Nephrol Dial Transplant **15**(12): 1977-85.

BACKGROUND: Bardet-Biedl syndrome (BBS) is an autosomal recessive disorder with five loci identified thus far. The spectrum of disease includes diverse malformations of the kidney and lower urinary tract. The incidence of BBS is approximately 1/100,000 with a predicted heterozygote frequency of 1/160, and it has been suggested that heterozygotes are at increased risk of obesity and hypertension. METHODS: We describe renal disease in relatives of 109 UK BBS patients. Using PCR with fluorescent microsatellite markers we amplified DNA derived from renal tumours of affected parents to determine whether there was loss of heterozygosity at any of four BBS loci and two other gene loci associated with clear cell renal cell carcinoma (CC-RCC). RESULTS: CC-RCC was diagnosed in three of 180 BBS parents and there was loss of heterozygosity at BBS1 (11q13) in the tumour tissue of one of these subjects. In addition, there was a high incidence of renal agenesis in siblings of BBS patients and two BBS families were identified with apparently dominant inheritance of renal malformations. In one family we were able to demonstrate that renal malformations segregated with the BBS2 locus (16q21). CONCLUSIONS: Since all parents and two-thirds of siblings of BBS patients must be heterozygous for BBS mutations, our observations may implicate BBS genes in the pathogenesis of both renal cancer and malformations,

both disorders of precursor cell growth and differentiation. We suggest these observations may have important implications for screening potential BBS carriers for kidney disease and may lead to a greater understanding of the aetiology of renal disease in the general population.

Bilim, V., T. Kawasaki, et al. (2000). "Altered expression of beta-catenin in renal cell cancer and transitional cell cancer with the absence of beta-catenin gene mutations." Clin Cancer Res **6**(2): 460-6.

Loss of normal beta-catenin expression and the beta-catenin gene mutations have been shown to contribute to the malignant character of various cancers. Using PCR-single-strand conformation polymorphism and DNA direct sequencing, we examined the presence of genetic alterations within the third exon of beta-catenin, which are frequently observed in other tumors, in transitional cell cancer (TCC) and renal cell cancer (RCC) cell lines, and in tumor specimens. The degrees of expression and intracellular distribution of beta-catenin were detected by immunohistochemical staining in 77 primary and 12 metastatic RCCs and in 81 primary TCCs. Western blot analysis was also applied to confirm the degree of beta-catenin expression in the cell lines and some tumor samples. We failed to reveal any genetic alterations, at least in the third exon of the beta-catenin gene, in RCC and TCC. Reduced membranous immunoreactivity of beta-catenin was observed in portions of RCC (15.5%) and TCC (24.7%) and was correlated with advanced stages and nodal involvement in RCC and with advanced stages and multiple tumors in TCC. Within the power limitations of this small study, beta-catenin abnormal expression was not correlated with recurrence or survival in either RCC or TCC. Interstitial deletions and mutations in the third exon of beta-catenin do not play a significant role in RCC or TCC tumorigenesis. Down-regulation of normal beta-catenin expression might contribute to the malignant character of RCC and TCC and result in tumor progression. However, this event is not an independent prognostic factor for recurrence or tumor specific survival.

Blish, K. R., W. Wang, et al. (2008). "A human bone morphogenetic protein antagonist is down-regulated in renal cancer." Mol Biol Cell **19**(2): 457-64.

We analyzed expression of candidate genes encoding cell surface or secreted proteins in normal kidney and kidney cancer. This screen identified a bone morphogenetic protein (BMP) antagonist, SOSTDC1 (sclerostin domain-containing-1) as down-regulated in kidney tumors. To confirm screening results, we probed cDNA dot blots with SOSTDC1. The SOSTDC1 message was decreased in 20/20 kidney tumors compared with normal kidney tissue. Immunohistochemistry confirmed significant decrease of SOSTDC1 protein in clear cell renal carcinomas relative to normal proximal renal tubule cells ($p < 0.001$). Expression of SOSTDC1 was not decreased in papillary and chromophobe kidney tumors. SOSTDC1 was abundantly expressed in podocytes, distal tubules, and transitional epithelia of the normal kidney. Transfection experiments demonstrated that SOSTDC1 is secreted and binds to neighboring cells and/or the extracellular matrix. SOSTDC1 suppresses both BMP-7-induced phosphorylation of R-Smads-1, -5, and -8 and Wnt-3a signaling. Restoration of SOSTDC1 in renal clear carcinoma cells profoundly suppresses proliferation. Collectively, these results demonstrate that SOSTDC1 is expressed in the human kidney and decreased in renal clear cell carcinoma. Because SOSTDC1 suppresses proliferation of renal carcinoma cells, restoration of SOSTDC1 signaling may represent a novel target in treatment of renal clear cell carcinoma.

Board, R. E., F. C. Thistlethwaite, et al. (2007). "Anti-angiogenic therapy in the treatment of advanced renal cell cancer." Cancer Treat Rev **33**(1): 1-8.

Metastatic renal cell cancer is associated with a poor prognosis and is resistant to traditional chemotherapy agents. The majority of tumours are associated with inactivation of the von Hippel-Lindau gene and subsequent overexpression of proangiogenic factors, including vascular endothelial growth factor (VEGF). Drugs targeting these pathways have undergone clinical testing in renal cell cancer with encouraging results. This type of therapy is set to revolutionise the treatment of renal cell cancer and this review outlines recent evidence from clinical trials investigating the most promising of these agents.

Bodmer, D., M. Schepens, et al. (2003). "Disruption of a novel gene, DIRC3, and expression of DIRC3-HSPBAP1 fusion transcripts in a case of familial renal cell cancer and t(2;3)(q35;q21)." Genes Chromosomes Cancer **38**(2): 107-16.

Previously, we identified a family with renal cell cancer and a t(2;3)(q35;q21). Positional cloning of the chromosome 3 breakpoint led to the identification of a novel gene, DIRC2, that spans this breakpoint. Here we have characterized the chromosome 2 breakpoint in detail and found that another novel gene, designated DIRC3, spans this breakpoint. In addition, we found that the first two exons of DIRC3 can splice to the second exon of HSPBAP1, a JmjC-Hsp27 domain gene that maps proximal to the breakpoint on chromosome 3. This splice results in the formation of DIRC3-HSPBAP1 fusion transcripts. We propose that these fusion transcripts may affect normal HSPBAP1

function and concomitant chromatin remodeling and/or stress response signals within t(2;3)(q35;q21)-positive kidney cells. As a consequence, familial renal cell cancer may develop.

Bodmer, D., W. van den Hurk, et al. (2002). "Understanding familial and non-familial renal cell cancer." Hum Mol Genet **11**(20): 2489-98.

Molecular genetic analysis of familial and non-familial cases of conventional renal cell carcinoma (RCC) revealed a critical role(s) for multiple genes on human chromosome 3. For some of these genes, e.g. VHL, such a role has been firmly established, whereas for others, definite confirmation is still pending. Additionally, a novel role for constitutional chromosome 3 translocations as risk factors for conventional RCC development is rapidly emerging. Also, several candidate loci have been mapped to other chromosomes in both familial and non-familial RCCs of distinct histologic subtypes. The MET gene on chromosome 7, for example, was found to be involved in both forms of papillary RCC. A PRCC-TFE3 fusion gene is typically encountered in t(X;1)-positive non-familial papillary RCCs and results in abrogation of the cell cycle mitotic spindle checkpoint in a dominant-negative fashion, thus leading to RCC. Together, these data turn human RCC into a model system in which different aspects of both familial and non-familial syndromes may act as novel paradigms for cancer development.

Boni, J. P., C. Leister, et al. (2005). "Population pharmacokinetics of CCI-779: correlations to safety and pharmacogenomic responses in patients with advanced renal cancer." Clin Pharmacol Ther **77**(1): 76-89.

OBJECTIVE: Our objective was to estimate the pharmacokinetic parameters of CCI-779 and its metabolite, sirolimus, and evaluate associations of exposure parameters with safety and clinical activity. Exposure parameters were also correlated with pharmacogenomic responses in peripheral blood mononuclear cells (PBMCs). METHODS: In this randomized, double-blind, multicenter trial, once-weekly intravenous doses of 25, 75, or 250 mg CCI-779 were administered to patients with advanced renal cancer. Whole blood for CCI-779 and sirolimus concentrations was drawn. Population pharmacokinetic analyses yielded Bayesian-predicted exposure metrics that were correlated with severity and duration of adverse events and survival. PBMC samples taken before and after treatment were examined for pharmacogenomic responses. Ribonucleic acid samples were converted to labeled probes and hybridized to oligonucleotide arrays containing more than 12,600 human sequences. RESULTS: The final population pharmacokinetic models of CCI-779 and sirolimus included 235 and 305 observations, respectively, from 50 patients. For CCI-779, dose, single versus multiple dose, and body surface area were significant pharmacokinetic covariates. For sirolimus, dose and hematocrit were significant covariates. Age, sex, or race did not influence drug disposition. CCI-779 area under the curve correlated with adverse event severity for thrombocytopenia ($P = .007$), pruritus ($P = .011$), and hyperlipemia ($P = .040$). Exposure (CCI-779 cumulative area under the curve) correlated with a specific subset of gene transcripts in PBMCs following 16 weeks after therapy ($P < .001$, Spearman correlation). CONCLUSIONS: Concentrations of CCI-779 and sirolimus were adequately described with a population model incorporating factors for dose, attenuated exposure of multiple doses, body surface area, and hematocrit. Correlations with adverse event severity and duration profiles were provided to aid in the detection of treatment-emergent effects. Pharmacogenomic profiling of PBMCs identified altered ribonucleic acid transcript expression levels that correlate with exposure. These transcripts represent potential biomarkers of CCI-779 exposure in peripheral blood.

Bonne, A., L. Vreede, et al. (2007). "Mapping of constitutional translocation breakpoints in renal cell cancer patients: identification of KCNIP4 as a candidate gene." Cancer Genet Cytogenet **179**(1): 11-8.

Our group and others had previously developed a high throughput procedure to map translocation breakpoints using chromosome flow sorting in conjunction with microarray-based comparative genomic hybridization (arrayCGH). Here we applied both conventional positional cloning and integrated arrayCGH procedures to the mapping of constitutional chromosome anomalies in four patients with renal cell cancer (RCC), three with a chromosome 3 translocation, and one with an insertion involving chromosome 3. In one of these patients, who was carrying a t(3;4)(p13;p15), the KCNIP4 gene was found to be disrupted. KCNIP4 belongs to a family of potassium channel-interacting proteins and is highly expressed in normal kidney cells. In addition, KCNIP4 splice variants have specifically been encountered in RCC.

Bonne, A. C., D. Bodmer, et al. (2004). "Chromosome 3 translocations and familial renal cell cancer." Curr Mol Med **4**(8): 849-54.

Renal cell carcinomas (RCCs) occur in both sporadic and familial forms. In a subset of families the occurrence of RCCs co-segregates with the presence

of constitutional chromosome 3 translocations. Previously, such co-segregation phenomena have been widely employed to identify candidate genes in various hereditary (cancer) syndromes. Here we survey the translocation 3-positive RCC families that have been reported to date and the subsequent identification of its respective candidate genes using positional cloning strategies. Based on allele segregation, loss of heterozygosity and mutation analyses of the tumors, a multi-step model for familial RCC development has been generated. This model is relevant for (i) understanding familial tumorigenesis and (ii) rational patient management. In addition, a high throughput microarray-based strategy is presented that will enable the rapid identification of novel positional candidate genes via a single step procedure. The functional consequences of the (fusion) genes that have been identified so far, the multi-step model and its consequences for clinical diagnosis, the identification of persons at risk and genetic counseling in RCC families are discussed.

Bonsdorff, T. B., J. H. Jansen, et al. (2008). "Second hits in the FLCN gene in a hereditary renal cancer syndrome in dogs." Mamm Genome **19**(2): 121-6.

In this study, samples from multifocal renal tumors from two dogs affected with renal cystadenocarcinoma and nodular dermatofibrosis (RCND) were collected for detection of putative second hits in the FLCN gene. Genomic DNA from the samples was typed at the previously identified disease-associated missense mutation and cDNA representing the entire coding region of the FLCN gene was sequenced for mutation detection. Second hits with predicted functional implications for the wild-type FLCN allele were observed in 12 of 17 (71%) of the kidney tumor samples. The type of mutation of the second hits varied between the tumors. Different alternative splice mutations were detected, as well as loss of heterozygosity at the germline mutation and loss of transcription product of the wild-type FLCN allele. In total, the frequency and wide spectrum of second hits identified in the tumor samples suggests a tumor suppressor function of FLCN in the kidneys of RCND-affected dogs. No mutations were detected in skin nodules sampled from the two dogs. This shows that the skin tumors of RCND-affected dogs may be caused by haploinsufficiency of the FLCN gene product.

Brauch, H., G. Weirich, et al. (2004). "VHL mutations in renal cell cancer: does occupational exposure to trichloroethylene make a difference?" Toxicol Lett **151**(1): 301-10.

Occupational exposures have long been suspected to play a role in the incidence of renal cell carcinoma (RCC). Especially, the carcinogenicity of the industrial solvent trichloroethylene (TCE) has been controversially debated, both with respect to the epidemiological and the molecular studies. In order to further elucidate this issue, it appeared important to compare suitable RCC patient groups, i.e., TCE-exposed versus non-TCE-exposed patients. We evaluated RCC from a previous German study that had described differences in RCC risks between TCE-exposed (n=17) and non-exposed patients (n=21). We compared age at diagnosis and histopathologic parameters of tumors as well as somatic mutation characteristics in the kidney cancer causing VHL tumor suppressor gene. RCC did not differ with respect to histopathological characteristics in both patient groups. We noticed a younger age at diagnosis in TCE-exposed patients compared to non-exposed patients (P=0.01). Moreover, the non-TCE-exposed patients did not share the somatic VHL mutation characteristics of TCE-exposed patients such as the previously identified hot spot mutation 454 C > T P81S or multiple mutations. These data support the notion of a putative genotoxic effect of TCE leading to VHL gene damage and subsequent occurrence of RCC in highly exposed subjects.

Bregni, M., A. Dodero, et al. (2002). "Nonmyeloablative conditioning followed by hematopoietic cell allografting and donor lymphocyte infusions for patients with metastatic renal and breast cancer." Blood **99**(11): 4234-6.

The feasibility and toxicity of allogeneic stem cell transplantation after nonmyeloablative conditioning including thiotepa, fludarabine, and cyclophosphamide have been investigated in 6 patients with breast cancer and 7 patients with renal cell cancer. The program included the use of escalating doses of donor lymphocyte infusions (DLI) and/or interferon alpha (IFNalpha) for patients showing no tumor response and no graft-versus-host disease (GVHD). Patients were at high risk of transplant-related mortality (TRM) because of age, advanced stage, and previous treatments. We observed a partial remission in 4 renal cancer and in 2 breast cancer patients (one at the molecular level in the bone marrow), occurring after cyclosporine withdrawal or after DLI and/or IFNalpha. All the responses were accompanied by the occurrence of acute GVHD. We conclude that reduced-intensity allogeneic stem cell transplantation is a feasible procedure in renal and breast cancer, and that the exploitation of graft-versus-tumor effect after DLI is a promising finding.

Brenner, W., F. Benzing, et al. (2004). "Regulation of beta1 integrin expression by PKCepsilon in renal cancer cells." Int J Oncol **25**(4): 1157-63.

Polarized cell movement represents an essential prerequisite for the progression and metastasis of malignant diseases. Protein kinase C (PKC) which physically associates with integrins has been implicated in the promotion of a migratory cell phenotype. In order to identify a direct link between PKC and integrins in renal cell carcinoma (RCC) the influence of PKC isoforms on integrin expression and possible consequences on proliferation and cell migration was analyzed in RCC cells. The constitutive expression of the PKC isoforms alpha, betaI, betaII, gamma, delta, epsilon, eta, theta, xi, lambda and micro was determined in the RCC cell line CCF-RC1. In addition, the influence of PKC inhibitors RO31-8220, GF109203X and GO6976 on the beta1, beta2 and beta3 integrin expression and cell proliferation of RCC cells was investigated by flow cytometry and by BrdU incorporation, respectively. Furthermore, the motility of CCF-RC1 cells was assessed through chamber chemotaxis analysis. All PKC isoforms tested were expressed in CCF-RC1 cells with the exception of PKClambda and theta. The PKC inhibitor RO31-8220 reduced beta1 integrin expression by 92% and inhibited proliferation by 42% of untreated cells, whereas cell migration remained uninfluenced by RO31-8220. GF109203X and GO6976 reduced beta1 integrin expression to approximately 50% of untreated cells. In contrast, beta2 and beta3 integrins were only weakly affected by RO31-8220, GF109203X and GO6976 treatment. The most significant influence on beta1 integrin expression was obtained by the PKC inhibitor RO31-8220. This leads to the assumption that PKCepsilon is involved in the regulation of beta1 integrin expression. Downregulation of beta1 integrins by RO31-8220 was associated with reduced proliferation, but did not influence migration. These findings provide a conceptual basis for treatment of renal cell carcinoma by interfering with tumor cell proliferation.

Catherino, W. H., C. M. Mayers, et al. (2007). "Compensatory alterations in energy homeostasis characterized in uterine tumors from hereditary leiomyomatosis and renal cell cancer." Fertil Steril **88**(4 Suppl): 1039-48.

OBJECTIVE: To determine the molecular alterations that maintain energy homeostasis in hereditary leiomyomatosis and renal cell cancer (HLRCC) uterine tumors with disrupted fumarate hydratase, compared with nonsyndromic uterine tumors. DESIGN: Laboratory study. SETTING: Tertiary academic university hospital. PATIENT(S): Eleven nonsyndromic leiomyoma-myometrium pairs and three HLRCC leiomyoma-myometrium pairs were obtained from patients who were recruited at national and military research centers in the United States. INTERVENTION(S): Molecular analysis. MAIN OUTCOME MEASURE(S): Hereditary leiomyomatosis and renal cell cancer and nonsyndromic leiomyomas were compared with patient-matched myometrium for relative glycolysis and Krebs cycle gene expression. RESULT(S): By microarray analysis, we confirmed that fumarate hydratase messenger RNA (mRNA) was underexpressed in HLRCC fibroids, compared with matched myometrium. Consistent with the possibility that alterations in fumarate hydratase represented a change to a more anaerobic state, we found that HLRCC fibroids overexpressed genes such as phosphofructokinase, aldolase, phosphoglycerate kinase, enolase, and pyruvate kinase. Expression of these genes was not altered in nonsyndromic leiomyomas. Furthermore, there were no overt changes in expression of Krebs cycle enzyme gene expression, with the exception of fumarate hydratase. CONCLUSION(S): Our findings demonstrate that alterations in fumarate hydratase are compensated for by increases in glycolysis enzyme expression in HLRCC.

Chang, I. W., H. Y. Huang, et al. (2009). "Melanotic Xp11 translocation renal cancer: a case with PSF-TFE3 gene fusion and up-regulation of melanogenetic transcripts." Am J Surg Pathol **33**(12): 1894-901.

Melanotic Xp11 translocation renal cancer is a recently recognized aggressive epithelioid neoplasm with features overlapping between PEComa, carcinoma, and melanoma. We describe morphologic and immunohistochemical characteristics of a melanotic Xp11 translocation renal cancer occurring in an 18-year-old girl and perform molecular genetic studies to analyze its genetic alterations and related melanogenetic activities. The tumor was composed of solid nests of epithelioid cells bearing abundant clear to finely granular eosinophilic cytoplasm and separated by delicate vascular septa. Finely granular and nonrefractile brown melanin pigments, highlighted by Fontana-Masson stain, were scattered through the tumor. By immunohistochemistry, the tumor was diffusely and strongly labeled by TFE3 and focally stained by HMB45 in a patchy pattern. In contrast, all other applied immunomarkers, including cytokeratins, epithelial membrane antigen, vimentin, CD10, S-100, smooth muscle actin, desmin, c-kit, CD68, and microphthalmia-associated transcription factor, were nonreactive to the tumor. Reverse transcription-polymerase chain reaction and validating sequencing demonstrated PSF-TFE3 gene fusion, a novel exon composition juxtaposing PSF exon 9 to TFE3 exon 5. Up-regulations of melanogenesis-associated regulators, including microphthalmia-associated transcription factor, tyrosinase (TYR), and

tyrosinase-related protein 1 (TYRP1), were identified in the tumor by semiquantitative reverse transcription-polymerase chain reaction. The morphologic and immunohistochemical discrepancies between this intriguing melanotic tumor and other documented renal cell carcinomas bearing identical PSF-TFE3 gene fusion may suggest melanotic Xp11 translocation renal cancer is a distinct entity among the MiT/TFE family neoplasms.

Cho, D., S. Signoretti, et al. (2007). "The role of mammalian target of rapamycin inhibitors in the treatment of advanced renal cancer." Clin Cancer Res **13**(2 Pt 2): 758s-763s.

Inhibitors of the mammalian target of rapamycin (mTOR) have shown promising efficacy in early-stage trials in patients with advanced renal cell carcinoma (RCC). Most RCCs have been shown to possess biallelic alterations in the von Hippel-Lindau (VHL) gene, resulting in accumulation of hypoxia-inducible factors 1alpha and 2alpha, as well as their downstream targets including vascular endothelial growth factor (VEGF). The observed clinical efficacy of mTOR inhibitors in patients with RCC may be mediated in part by the dependence of efficient hypoxia-inducible factor translation on the mTOR pathway. mTOR inhibitors have entered more advanced phase clinical trials either as single agents or in combination with other targeted agents or IFN, which might ultimately result in regulatory approval of one or more agents. Given the likely nonoverlapping mechanism of action of mTOR inhibitors and VEGF pathway-targeted agents, mTOR inhibitors may prove useful if administered in combination or after resistance to VEGF inhibitors. With an increasing number of active agents for treatment of patients with RCC, efforts must continue to develop patient selection models based on predictive biomarkers to direct therapy to appropriate patients.

Choyke, P. L. (2003). "Imaging of hereditary renal cancer." Radiol Clin North Am **41**(5): 1037-51.

Over the past 5 years there have been dramatic developments in the extent of knowledge of hereditary renal cancers. In addition to VHL, which is associated with clear cell carcinoma, one can now list HPRC (associated with type I papillary renal cancer) and HLRCC (associated with type II papillary renal cancer). BHD and FRO are associated with chromophobe carcinoma and oncocytomas, although other histologic tumor types have been found in BHD. Medullary carcinoma of the kidney is associated with sickle cell trait. Although the genes associated with these tumors have been discovered, the exact mechanisms by which they cause renal cancer remain to be elucidated. It is quite likely that other genes also are involved in this process. Using VHL as an example, research is now underway on targeting mutant pVHL or excess HIF for diagnostic and therapeutic purposes. Understanding the mechanisms leading to cancer may open new targets of opportunity for drug development. This improved knowledge of the biogenetic pathways used to form tumors will impact the development of new therapeutic techniques for treating renal cancers in hereditary and nonhereditary forms of the disease.

Costa, V. L., R. Henrique, et al. (2007). "Quantitative promoter methylation analysis of multiple cancer-related genes in renal cell tumors." BMC Cancer **7**: 133.

BACKGROUND: Aberrant promoter hypermethylation of cancer-associated genes occurs frequently during carcinogenesis and may serve as a cancer biomarker. In this study we aimed at defining a quantitative gene promoter methylation panel that might identify the most prevalent types of renal cell tumors. METHODS: A panel of 18 gene promoters was assessed by quantitative methylation-specific PCR (QMSP) in 85 primarily resected renal tumors representing the four major histologic subtypes (52 clear cell (ccRCC), 13 papillary (pRCC), 10 chromophobe (chRCC), and 10 oncocytomas) and 62 paired normal tissue samples. After genomic DNA isolation and sodium bisulfite modification, methylation levels were determined and correlated with standard clinicopathological parameters. RESULTS: Significant differences in methylation levels among the four subtypes of renal tumors were found for CDH1 ($p = 0.0007$), PTGS2 ($p = 0.002$), and RASSF1A ($p = 0.0001$). CDH1 hypermethylation levels were significantly higher in ccRCC compared to chRCC and oncocytoma ($p = 0.00016$ and $p = 0.0034$, respectively), whereas PTGS2 methylation levels were significantly higher in ccRCC compared to pRCC ($p = 0.004$). RASSF1A methylation levels were significantly higher in pRCC than in normal tissue ($p = 0.035$). In pRCC, CDH1 and RASSF1A methylation levels were inversely correlated with tumor stage ($p = 0.031$) and nuclear grade ($p = 0.022$), respectively. CONCLUSION: The major subtypes of renal epithelial neoplasms display differential aberrant CDH1, PTGS2, and RASSF1A promoter methylation levels. This gene panel might contribute to a more accurate discrimination among common renal tumors, improving preoperative assessment and therapeutic decision-making in patients harboring suspicious renal masses.

Cozar, J. M., J. M. Romero, et al. (2007). "High incidence of CTLA-4 AA (CT60) polymorphism in renal cell cancer." Hum Immunol **68**(8): 698-704.

Polymorphism in genes encoding T-cell regulatory proteins and cytokines may influence inflammation and cancer development via regulation of antitumor immune response. In the current study we analyzed genotypic frequencies of cytotoxic T-lymphocyte antigen-4 (CTLA-4)/CT60, CTLA-4/A49G, interleukin (IL)-4, and IL-10 polymorphisms in 117 renal cell carcinoma patients, 96 patients with colorectal cancer, and 196 healthy controls to test for an association between polymorphism in these genes and the risk of renal and colon cancer in a Spanish group of patients. In the case-control study, DNA samples from cancer patients and controls were analyzed using a TaqMan single nucleotide polymorphism genotyping assay. The distribution of IL-4 and IL-10 polymorphisms was similar between renal cancer patients and controls. However, a higher incidence of CTLA-4/CT60-AA genotype ($p = 0.005$; odds ratio (OR)= 2.12 with 95% confidence interval (CI): 1.28-3.50) and CTLA-4/A49G-AA ($p = 0.022$; OR = 1.76 with 95% CI: 1.11-2.80) genotype was observed in renal cancer patients than in controls. In addition, we observed a positive correlation between the AA genotype in both CTLA-4 polymorphisms and RCC grade, suggesting a role for the CTLA4 gene in tumor development. Therefore, our data suggest the CTLA-4 gene may be a candidate as a renal adenocarcinoma susceptibility gene, but does not play an important role in colon cancer.

Crnkovic-Mertens, I., N. Wagener, et al. (2007). "Targeted inhibition of Livin resensitizes renal cancer cells towards apoptosis." Cell Mol Life Sci **64**(9): 1137-44.

Cancer cells are typically characterized by apoptosis deficiency. In order to investigate a possible role for the anti-apoptotic livin gene in renal cell cancer (RCC), we analyzed its expression in tumor tissue samples and in RCC-derived cell lines. In addition, we studied the contribution of livin to the apoptotic resistance of RCC cells by RNA interference (RNAi). Livin gene expression was detected in a significant portion of RCC tumor tissue specimens (13/14, 92.9%) and tumor-derived cell lines (12/15, 80.0%). Moreover, targeted inhibition of livin by RNAi markedly sensitized RCC cells towards proapoptotic stimuli, such as UV irradiation or the chemotherapeutic drugs etoposide, 5-fluorouracil, and vinblastine. These effects were specific for livin expressing tumor cells. We conclude that livin can contribute significantly to the apoptosis resistance of RCC cells. Targeted inhibition of livin could represent a novel therapeutic strategy to increase the sensitivity of renal cancers towards pro-apoptotic agents.

da Silva, N. F., D. Gentle, et al. (2003). "Analysis of the Birt-Hogg-Dube (BHD) tumour suppressor gene in sporadic renal cell carcinoma and colorectal cancer." J Med Genet **40**(11): 820-4.

Germline mutations in the BHD gene cause the dominantly inherited cancer susceptibility disorder, Birt-Hogg-Dube (BHD) syndrome. Individuals with BHD are reported to have an increased risk of renal cell carcinoma (RCC) and of colorectal polyps and cancer. The BHD gene maps to 17p11.2, and to investigate whether somatic inactivation of the BHD gene region is implicated in the pathogenesis of sporadic RCC and colorectal cancer (CRC), we performed mutation analysis in 30 RCC primary tumours and cell lines, and 35 CRCs and cell lines. A somatic missense mutation (Ala444Ser) with loss of the wild type allele (consistent with a two hit mechanism of tumorigenesis) was detected in a primary clear cell RCC, and a further missense mutation (Ala238Val) was identified in a clear cell RCC cell line for which matched normal DNA was not available. A somatic missense substitution (Arg392Gly) was identified in a primary CRC, and the same change was detected in three RCCs (all oncocytomas) for which matched normal DNA was not available. A germline Arg320Gln missense variant detected in a primary CRC was not detected in 40 control individuals or in a further 159 familial and sporadic CRC cases. However, AA homozygotes for an intronic single nucleotide polymorphism (c.1517+6 G-->A) were under-represented in familial cases compared with controls ($p = 0.03$). For some tumour suppressor genes, epigenetic silencing is a more common mechanism of inactivation than somatic mutations. However, we did not detect evidence of epigenetic silencing of BHD in 19 CRC and RCC cell lines, and BHD promoter region hypermethylation was not detected in 20 primary RCCs. These findings suggest that BHD inactivation occurs in a subset of clear cell RCC and CRC.

Datta, D., A. G. Contreras, et al. (2009). "Calcineurin inhibitors activate the proto-oncogene Ras and promote protumorigenic signals in renal cancer cells." Cancer Res **69**(23): 8902-9.

The development of cancer is a major problem in immunosuppressed patients, particularly after solid organ transplantation. We have recently shown that calcineurin inhibitors (CNI) used to treat transplant patients may play a critical role in the rapid progression of renal cancer. To examine the intracellular signaling events for CNI-mediated direct

tumorigenic pathway(s), we studied the effect of CNI on the activation of proto-oncogenic Ras in human normal renal epithelial cells (REC) and renal cancer cells (786-0 and Caki-1). We found that CNI treatment significantly increased the level of activated GTP-bound form of Ras in these cells. In addition, CNI induced the association of Ras with one of its effector molecules, Raf, but not with Rho and phosphatidylinositol 3-kinase; CNI treatment also promoted the phosphorylation of the Raf kinase inhibitory protein and the downregulation of carabin, all of which may lead to the activation of the Ras-Raf pathway. Blockade of this pathway through either pharmacologic inhibitors or gene-specific small interfering RNA significantly inhibited CNI-mediated augmented proliferation of renal cancer cells. Finally, it was observed that CNI treatment increased the growth of human renal tumors in vivo, and the Ras-Raf pathway is significantly activated in the tumor tissues of CNI-treated mice. Together, targeting the Ras-Raf pathway may prevent the development/progression of renal cancer in CNI-treated patients.

Datta, D., A. G. Contreras, et al. (2008). "Calcineurin inhibitors modulate CXCR3 splice variant expression and mediate renal cancer progression." J Am Soc Nephrol **19**(12): 2437-46.

Calcineurin inhibitors (CNI) are used to prevent inflammatory diseases and allograft rejection. However, little is known about the mechanism(s) underlying their ability to promote the development and recurrence of cancer. Recent studies suggested that the chemokine receptor CXCR3 may play important roles in tumorigenesis. CXCR3 has two splice variants with opposite functions: CXCR3-A promotes cell proliferation, and CXCR3-B inhibits cell growth. Here, we explored the effects of CNI on the expression and function of CXCR3 splice variants. Compared with normal renal tissues and renal epithelial cells, human renal cancer tissues and renal cancer cell lines demonstrated higher expression of CXCR3-A and markedly lower expression of CXCR3-B. In human renal cancer cells (786-0 and Caki-1) and renal epithelial cells, CNI markedly downregulated the expression of CXCR3-B, whereas expression of CXCR3-A was unchanged. This CNI-mediated downregulation of CXCR3-B resulted in increased proliferation and migration of renal cancer cells; CNI-mediated cell proliferation involved signaling through G(i) proteins, perhaps via CXCR3-A. Finally, it was observed that CNI treatment increased the growth of human renal tumors in vivo, and the expression of CXCR3-B was significantly decreased in these tumors. In summary, these observations suggest that CNI may mediate the progression of human renal cancer by downregulating CXCR3-B and by promoting proliferative signals, likely through CXCR3-A. Targeting CXCR3 splice variants or the signaling pathways downstream of CXCR3 receptors may provide a therapeutic strategy for the prevention of CNI-mediated renal cancer progression.

Datta, K., J. Li, et al. (2004). "Protein kinase C zeta transactivates hypoxia-inducible factor alpha by promoting its association with p300 in renal cancer." Cancer Res **64**(2): 456-62.

Hydroxylation at an asparagine residue at the COOH-terminal activation domain of hypoxia-inducible factor (HIF)-1/2 alphas is essential for its inactivation under normoxic condition. To date, the mechanism by which HIF-alpha avoids the inhibitory effect of asparagine hydroxylase in renal cell carcinoma (RCC) in normoxia is undefined. We have shown herein that protein kinase C (PKC) zeta has an important role in HIF-alpha activation in RCC. By using dominant negative mutant and small interference RNA approaches, we have demonstrated that the association between HIF-alpha and p300 is modulated by PKCzeta. Moreover, a novel signaling pathway involving phosphatidylinositol 3'-kinase and PKCzeta has been shown to be responsible for the activation of HIF-alpha by inhibiting the mRNA expression of FIH-1 (factor inhibiting HIF-1) in RCC and thereby promoting the transcription of hypoxia-inducible genes such as vascular permeability factor/vascular endothelial growth factor.

Datta, K., R. Nambudripad, et al. (2000). "Inhibition of insulin-like growth factor-I-mediated cell signaling by the von Hippel-Lindau gene product in renal cancer." J Biol Chem **275**(27): 20700-6.

Insulin-like growth factor-I (IGF-I)-mediated signaling is thought to be involved in the regulation of multiple cellular functions in different tumors including renal cell carcinoma (RCC). Blocking IGF-I signaling by any of the several strategies abolishes or delays the progression of a variety of tumors in animal models. Herein, we demonstrate that in RCC cell lines, IGF-I-mediated signaling is found to be inhibited in the presence of wild type von Hippel-Lindau (VHL) tumor suppresser gene. Moreover, molecular modeling and biochemical approaches have revealed that beta-domain of the VHL gene product by interacting directly with protein kinase Cdelta inhibits its association with IGF-IR for downstream signaling. We also demonstrated that RCC has IGF-I-mediated invasive activity where protein kinase Cdelta is an important downstream molecule, and this invasiveness can be blocked by wild type VHL. These experiments thus elucidate a novel tumor suppresser function of VHL with its unique kinase inhibitory domain.

Del Monte, G., P. Ferroni, et al. (2008). "Interleukin-2 inhalation therapy in renal cell cancer: a case report and review of the literature." In Vivo **22**(4): 481-8.

Renal cell carcinoma (RCC) is the most common malignancy of the kidney. One third of RCC presents metastatic disease at the time of diagnosis, usually leading to a fatal outcome. Small response rates were seen with most cytotoxic agents including gemcitabine and vinorelbine, whereas systemic therapy with high doses of interleukin 2 (IL-2) has been shown to provide durable complete remissions. However, in consideration of its severe toxicity, IL-2 immunotherapy is restricted to selected patients. Aerosol IL-2 has been introduced as an alternative therapy in cancer patients. However, only very few data are available on its use in patients with pulmonary metastatic RCC. This paper briefly summarizes current clinical experience with the use of inhaled IL-2 therapy, either as a single therapy or in combination with other treatments. In addition, we report on a male patient with pulmonary metastasized RCC who achieved a durable complete response to combined gemcitabine/vinorelbine and interleukin-2 inhalation therapy.

Diaconu, I., L. Denby, et al. (2009). "Serotype chimeric and fiber-mutated adenovirus Ad5/19p-HIT for targeting renal cancer and untargeting the liver." Hum Gene Ther **20**(6): 611-20.

Despite some advances, patients with advanced renal cell carcinoma (RCC) cannot usually be cured. Alteration of the natural tropism of adenoviruses may permit more specific gene transfer to target tissues. The aim of this study was to use novel targeting moieties for adenoviral gene therapy of RCC. Previous work in rats suggested that use of Ad5/19p (Ad5 capsid with Ad19p fiber) with kidney vascular targeting moieties HTTHREP (HTT), HITSLLS (HIT), and APASLYN (APA) placed into the fiber knob might be useful for targeting kidney vasculature. Therefore, we sought to investigate the utility of Ad5/19p variants for gene delivery to human RCC cell lines, clinical samples, and orthotopic murine models of metastatic RCC. Six different human RCC cell lines were infected but only Ad5/19p-HIT showed increased transduction, and only in one cell line. Thus, we analyzed human normal and cancerous kidney specimens fresh from patients, which might better mimic the three-dimensional architecture of clinical tumors and found that Ad5/19p-HIT showed transduction levels similar to Ad5. In mice, we found that intraperitoneal and intravenous Ad5/19p-HIT transduced tumors at levels comparable to Ad5, and that intratumoral Ad5/19p-HIT was superior to Ad5. Liver tropism was significantly reduced in comparison with Ad5. Improvements in tumor-to-liver transduction ratios suggested that Ad5/19p-HIT may be promising for systemic gene delivery to kidney tumors.

Donald, C. D., C. Q. Sun, et al. (2003). "Cancer-specific loss of beta-defensin 1 in renal and prostatic carcinomas." Lab Invest **83**(4): 501-5.

In a previous large-scale gene expression profiling study of renal epithelial neoplasms, human beta-defensin-1 (DEFB1) was found to be significantly down-regulated in conventional clear cell (renal) carcinoma. We have now completed an expanded expression analysis of this gene. We performed immunohistochemical analysis for the DEFB1 protein in clinical specimens of both renal cell carcinoma and prostate cancer. In a subset of prostate cancers, we performed laser capture microdissection and RT-PCR to correlate mRNA levels with protein levels. Overall, 82% of prostate cancers exhibit either complete loss of protein expression or only minimal expression, whereas the adjacent benign epithelium retained expression in all cases. Similarly, 90% of renal cell carcinomas show cancer-specific loss of DEFB1 protein. In the prostate cancer subset analysis, mRNA levels correlate with protein levels. We have thus demonstrated the cancer-specific down-regulation of DEFB1 in a large sample of prostatic and renal carcinomas and validated one of the key findings of previous cancer gene profiling studies of prostatic and renal neoplasia.

Dong, L. M., P. Brennan, et al. (2009). "An analysis of growth, differentiation and apoptosis genes with risk of renal cancer." PLoS One **4**(3): e4895.

We conducted a case-control study of renal cancer (987 cases and 1298 controls) in Central and Eastern Europe and analyzed genomic DNA for 319 tagging single-nucleotide polymorphisms (SNPs) in 21 genes involved in cellular growth, differentiation and apoptosis using an Illumina Oligo Pool All (OPA). A haplotype-based method (sliding window analysis of consecutive SNPs) was used to identify chromosome regions of interest that remained significant at a false discovery rate of 10%. Subsequently, risk estimates were generated for regions with a high level of signal and individual SNPs by unconditional logistic regression adjusting for age, gender and study center. Three regions containing genes associated with renal cancer were identified: caspase 1/5/4/12(CASP 1/5/4/12), epidermal growth factor receptor (EGFR), and insulin-like growth factor binding protein-3 (IGFBP3). We observed that individuals with CASP1/5/4/12 haplotype (spanning area upstream of CASP1 through exon 2 of CASP5) GGGCTCAGT were at higher risk

of renal cancer compared to individuals with the most common haplotype (OR:1.40, 95% CI:1.10-1.78, p-value = 0.007). Analysis of EGFR revealed three strong signals within intron 1, particularly a region centered around rs759158 with a global p = 0.006 (GGG: OR:1.26, 95% CI:1.04-1.53 and ATG: OR:1.55, 95% CI:1.14-2.11). A region in IGFBP3 was also associated with increased risk (global p = 0.04). In addition, the number of statistically significant (p-value<0.05) SNP associations observed within these three genes was higher than would be expected by chance on a gene level. To our knowledge, this is the first study to evaluate these genes in relation to renal cancer and there is need to replicate and extend our findings. The specific regions associated with risk may have particular relevance for gene function and/or carcinogenesis. In conclusion, our evaluation has identified common genetic variants in CASP1, CASP5, EGFR, and IGFBP3 that could be associated with renal cancer risk.

Eleveld, M. J., D. Bodmer, et al. (2001). "Molecular analysis of a familial case of renal cell cancer and a t(3;6)(q12;q15)." Genes Chromosomes Cancer **31**(1): 23-32.

We identified a novel familial case of clear-cell renal cancer and a t(3;6)(q12;q15). Subsequent cytogenetic and molecular analyses showed the presence of several abnormalities within tumour samples obtained from different patients. Loss of the der(3) chromosome was noted in some, but not all, of the samples. A concomitant VHL gene mutation was found in one of the samples. In addition, cytogenetic and molecular evidence for heterogeneity was obtained through analysis of several biopsy samples from one of the tumours. Based on these results and those reported in the literature, we conclude that loss of der(3) and subsequent VHL gene mutation may represent critical steps in the development of renal cell cancers in persons carrying the chromosome 3 translocation. Moreover, preliminary data suggest that other (epi)genetic changes may be related to tumour initiation.

Farker, K., M. H. Lehmann, et al. (2000). "Analysis of point mutation in exon 2 of CYP2E1 gene in renal cell/urothelial cancer patients in comparison with control population." Int J Clin Pharmacol Ther **38**(1): 30-4.

OBJECTIVE: Genetic polymorphisms of human cytochrome P450s have been implicated to be of importance for susceptibility to different cancers. Recently, a point mutation was found in the exon 2 of the CYP2E1 gene (CYP2E1*2) [Hu et al. 1997]. In order to evaluate a possible link between the point mutation in exon 2 of the CYP2E1 gene and the susceptibility to renal cell/urothelial cancer, we developed a screening method based on the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). MATERIAL: DNA of peripheral white blood cells was isolated from 158 renal cell/urothelial cancer patients as well as from 150 controls. METHOD: Primers for PCR were designed by the Primer 3 release 0.1 program. The PCR yield a product of 215 base pairs (bp), which was digested with the restriction enzyme Hha I. The DNA fragments were separated on a 3% agarose gel stained with ethidium bromide. Restriction enzyme digestion of the PCR product obtained from the wild-type DNA resulted in the appearance of a 66 bp, a 43 bp, a 40 bp, a 39 bp and a 28 bp DNA fragment. In contrast to the wild-type, the digestion of the PCR product from DNA carrying the point mutation resulted in the loss of the 39 bp and 40 bp fragments and the appearance of an additional 79 bp fragment. Therefore, the loss of one Hha I restriction site caused by a single nucleotide exchange is suitable for the identification of the point mutation in exon 2 of CYP2E1 gene. RESULTS: However, we could not detect any point mutation in any of the 158 renal cell/urothelial cancer patients or the 150 controls. The distribution of the point mutation in exon 2 of CYP2E1 gene did not show any difference in renal cell/urothelial cancer patients and controls. CONCLUSION: This might indicate a lack of association between this CYP2E polymorphism (CYP2E1*2) and renal cell/urothelial cancer.

Feijoo-Cuaresma, M., F. Mendez, et al. (2008). "Inadequate activation of the GTPase RhoA contributes to the lack of fibronectin matrix assembly in von Hippel-Lindau protein-defective renal cancer cells." J Biol Chem **283**(36): 24982-90.

The von Hippel-Lindau (VHL) tumor suppressor gene regulates extracellular matrix deposition. In VHL negative renal cancer cells, VHL(-), the lack of fibronectin matrix assembly is thought to promote and maintain tumor angiogenesis allowing vessels to infiltrate tumors. Therefore, and considering the importance of this process in tumor growth, we aimed to study why VHL(-) renal cancer cells fail to form a proper extracellular matrix. Our results showed that VHL(-) cells were not defective in fibronectin production and that the fibronectin produced by these cells was equally functional in promoting cell adhesion and matrix assembly as that produced by VHL+ cells. We have previously reported that VHL(-) cells fail to form beta1 integrin fibrillar adhesions and have a diminished organization of actin stress fibers; therefore, we aimed to study if the small GTPase family is involved in this process. We found that activation of the RhoA GTPase was defective in

VHL(-) cells, and this was possibly mediated by an increased activation of its inhibitor, p190RhoGAP. Additionally, the expression of constitutively active RhoA in VHL(-) cells resulted in formation of a fibronectin matrix. These results strongly suggest an important role for RhoA in some of the defects observed in renal cancer cells.

Fergelot, P., N. Rioux-Leclercq, et al. (2005). "[Molecular pathways of tumour angiogenesis and new targeted therapeutic approaches in renal cancer]." Prog Urol **15**(6): 1021-9.

The conventional form of renal cell carcinoma (RCC) is a highly vascular tumour with an extremely poor prognosis in the presence of metastases. Significant progress has recently been made in the understanding of the molecular mechanisms leading to the vascular phenotype of renal cancer In particular, VHL disease constitutes a useful study model, as inactivation of the VHL gene leads to accumulation of HIF factor, inducing activation of genes such as: VEGF, PDGF, EPO, CaIX and TGF-alpha. The fact that VHL inactivation has been found in about 70% of sporadic renal cancers constitutes the best rationale to target the products of these genes. Candidate drugs currently target VEGF, VEGFR, PDGFR and tyrosine kinase receptors, which are necessary for intracellular signal transduction. The preliminary results of phase II trials in metastatic renal cancer, usually as second-line therapy, are very encouraging. The results of phase III trials will soon be available, but many studies are already evaluating these drugs either as first-line or in combination. Urologists have an opportunity to become familiar with these drugs by actively participating in trials of adjuvant therapy that will be initiated in the near future.

Fleming, S. (1999). "Renal cancer genetics: von Hippel Lindau and other syndromes." Int J Dev Biol **43**(5): 469-71.

There have been significant advances in our understanding of the genetic basis of renal carcinogenesis. In particular, research in the last five years has demonstrated a central role for the inactivation of the von Hippel-Lindau gene by mutation or hypermethylation in the formation of the conventional type of renal cell carcinoma. The von Hippel-Lindau syndrome is characterised by germ-line inactivating mutation whereas sporadic renal carcinoma is associated with somatic mutations. Tumour formation is accompanied by loss of the remaining wild-type allele. The biology of the von Hippel-Lindau gene and its normal function continued to be unravelled but a role has been demonstrated for it in the regulation of gene transcription, the regulation of oxygen-dependent genes and their expression and the control of tumour angiogenesis acting via the vascular endothelial growth factor. Another form of familial renal cancer, the hereditary papillary renal cell carcinoma, has been shown to be consequent upon activating mutations of the c-met proto-oncogene. The genetic data continue to enhance our understanding of the biology of this common set of neoplasms.

Franco, O. E., T. Onishi, et al. (2003). "Phenylacetate inhibits growth and modulates cell cycle gene expression in renal cancer cell lines." Anticancer Res **23**(2B): 1637-42.

BACKGROUND: Phenylacetate (PA), an aromatic fatty acid, is now undergoing evaluation as a potential anticancer reagent. Our previous study showed that PA induces cell growth inhibition in prostate cancer cells. Here, we investigated whether PA is effective against three renal cancer cell lines in vitro. MATERIALS AND METHODS: The cell viability of PA-treated renal carcinoma cell lines (Caki-1, Os-RC-2 and RCC10) was assessed by trypan-blue exclusion and cell cycle distribution by flow cytometry. The cell cycle-regulatory protein expression was evaluated by Western blot, immunoprecipitation and kinase assay. RESULTS: Growth inhibition occurred with PA treatment at a dose of 2-5 mM and an increased percentage of cells in G1 after 24 hours of exposure. Reduced phosphorylation of the retinoblastoma protein (Rb) and CDK2 activity, increased expression of p21Cip1 and enhanced binding of p21Cip1 to CDK2 were observed following treatment with PA. CONCLUSION: Overall, these results suggest that p21Cip1 is a critical target in PA-mediated cell growth inhibition in RCC cells playing a key role in CDK2 inactivation, hypophosphorylation of pRb and subsequent G1 cell cycle arrest.

Franzini, A., S. C. Picozzi, et al. (2007). "A case of renal cancer with TFE3 gene fusion in an elderly man. Clinical, radiological and surgical findings." Urol Int **78**(2): 179-81.

Sporadic cases of a particular group of renal cancers associated with a translocation involving Xp11.2, known as the TFE3 transcription factor gene, have been reported in the last 20 years. The group was also classified in 2004 WHO kidney carcinoma classifications. A 79-year-old male patient was investigated at the outpatient department for gross intermittent hematuria. Sonography showed a spherical left kidney with increased total size, without evidence of the corticomedullary differentiation due to parenchymal dyshomogeneity with a neoplasm aspect. CT confirmed the sonographic left kidney findings and showed gross node involvement. Angiography did

not show any pathological arterial circulation, but massive thrombotic involvement of the renal vein was evident. Radical nephrectomy with thrombectomy and staging lymphectomy were performed. At pathological examination the kidney parenchyma was completely substituted by white firm tissue. Microscopically the tumor was composed of papillary structures lined by epithelial cells with a clear cytoplasm. Multiple node metastases were found. Immunohistochemical examination showed negativity for epithelial markers (cytokeratin and epithelial membrane antigen) and reactivity for CD10 and TFE3. The genetic and histological aspects of this rare tumor are reported. In addition, we describe clinical, radiological and surgical findings.

Franzke, A., J. Buer, et al. (2001). "HLA phenotype and cytokine-induced tumor control in advanced renal cell cancer." Cancer Biother Radiopharm **16**(5): 401-9.

BACKGROUND: The natural history of malignancies, the response to cytokine-based therapy and survival of patients may be partly determined by the human leukocyte antigen (HLA) phenotype. Here, we investigated in a retrospective analysis the correlation of the HLA phenotype of 73 prognostic favored patients with advanced renal cell carcinoma to (a) the expected HLA distribution in Caucasians, (b) the susceptibility or resistance to metastatic sites, (c) response to cytokine-based therapy and (d) sustained cytokine-induced effective tumor control. METHODS: We retrospectively determined the MHC class I and II antigens in patients with metastatic renal cell carcinoma selected by survival. Antigens were serologically typed by standard lymphocytotoxicity techniques. For statistical analysis, we calculated the probability of the presented HLA antigens in correlation to the expected Caucasian HLA phenotypes. An independent confirmation was performed by using the chi-square and two-tailed Fisher's exact test. RESULTS: Various HLA antigens deviated significantly from the normal distribution in the Caucasian population. HLA.B44 was the only antigen associated ($p < 0.01$) with the absence of lung and presence of bone metastases, while it did not impact on overall survival or response to therapy. A1 ($p < 0.0001$, $p < 0.002$) and B8 ($p < 0.009$, $p < 0.04$) alleles were more frequently expressed in responding patients than expected from the normal distribution in Caucasians and that observed in non-responding patients, respectively. The HLA analysis of patients achieving a durable complete remission showed a significantly higher frequency of expression of the A1 and B8 antigens and furthermore of the B14 antigen ($p < 0.05$). CONCLUSIONS: Our data underline the pivotal role of the MHC complex in controlling and regulating the cellular immune response in renal cell cancer. We could identify HLA antigens, which correlate with response to cytokine-treatment, with a long-lasting effective tumor control and prolonged overall survival.

Fritzsche, F. R., K. Wassermann, et al. (2008). "ADAM9 is highly expressed in renal cell cancer and is associated with tumour progression." BMC Cancer **8**: 179.

BACKGROUND: A Disintegrin And Metalloprotease (ADAM) 9 has been implicated in tumour progression of various solid tumours, however, little is known about its role in renal cell carcinoma. We evaluated the expression of ADAM9 on protein and transcript level in a clinico-pathologically characterized renal cell cancer cohort. METHODS: 108 renal cancer cases were immunostained for ADAM9 on a tissue-micro-array. For 30 additional cases, ADAM9 mRNA of microdissected tumour and normal tissue was analyzed via quantitative RT-PCR. SPSS 14.0 was used to apply crosstables (Fisher's exact test and chi2-test), correlations and univariate as well as multivariate survival analyses. RESULTS: ADAM9 was significantly up-regulated in renal cancer in comparison to the adjacent normal tissue on mRNA level. On protein level, ADAM9 was significantly associated with higher tumour grade, positive nodal status and distant metastasis. Furthermore, ADAM9 protein expression was significantly associated with shortened patient survival in the univariate analysis. CONCLUSION: ADAM9 is strongly expressed in a large proportion of renal cell cancers, concordant with findings in other tumour entities. Additionally, ADAM9 expression is significantly associated with markers of unfavourable prognosis. Whether the demonstrated prognostic value of ADAM9 is independent from other tumour parameters will have to be verified in larger study cohorts.

Fujimoto, E., H. Sato, et al. (2005). "A Src family inhibitor (PP1) potentiates tumor-suppressive effect of connexin 32 gene in renal cancer cells." Life Sci **76**(23): 2711-20.

Connexin (Cx) genes exert negative growth effects on tumor cells with certain cell specificity. We have recently reported that Cx32 acts as a tumor suppressor gene in renal cancer cells due to the inhibition of Src-dependent signaling. In line with the previous study, here we examined if a Src family inhibitor (PP1) could potentiate tumor-suppressive effect of Cx32 in Caki-2 cell from human renal cell carcinoma. In order to clarify the potentialization of PP1, using Cx32-transfected Caki-2 cells and mock-transfected Caki-2 cells, we estimated difference in

cytotoxic effect of PP1 on the two cell clones in vitro as well as in vivo. PP1 showed more cytotoxic effect on Caki-2 cells having Cx32 positive expression than that of Cx32 negative expression at lower doses. This potentialization was also observed in xenograft model of nude mice. The potentialization of the effect mainly depended on the induction of apoptosis but not the control of cell growth. In conjugation with this event, the reduction of anti-apoptotic molecules (Bcl-2 and Bcl-xL) was caused by the combination of Cx32 expression and PP1 treatment in Caki-2 cells. These results suggest that PP1 potentiates tumor-suppressive effect of connexin 32 gene in renal cancer cells through the reduction of anti-apoptotic molecules.

Fujimoto, E., T. Yano, et al. (2005). "Cytotoxic effect of the Her-2/Her-1 inhibitor PKI-166 on renal cancer cells expressing the connexin 32 gene." J Pharmacol Sci **97**(2): 294-8.

We have reported that connexin (Cx) 32 acts as a tumor suppressor gene in renal cancer cells partly due to Her-2 inactivation. Here, we determined if a Her-2/Her-1 inhibitor (PKI-166) can enhance the tumor-suppressive effect of Cx32 in Caki-2 cells from human renal cell carcinoma. The expression of Cx32 in Caki-2 cells was required for PKI-166-induced cytotoxic effect at lower doses. The cyctotoxicity was dependent on the occurrence of apoptosis and partly mediated by Cx32-driven gap junction intercellular communications. These results suggest that PKI-166 further supports the tumor-suppressive effect of the Cx32 gene in renal cancer cells through the induction of apoptosis.

Gattinoni, L., M. Alu, et al. (2003). "Renal cancer treatment: a review of the literature." Tumori **89**(5): 476-84.

Renal carcinoma represents about 3% of all adult tumors, with an estimate of 31,900 new cases diagnosed in 2003 in the United States. In the early phase of its natural history, renal cancer is potentially curable by surgery, but if the disease presents any signs of metastasis, the chances of survival are remote, even though anecdotal cases characterized by long survival have been reported. In fact, the treatment of metastatic renal cancer remains unsatisfactory. Systemic treatment with single agents and with polychemotherapy, with or without cytokine-based immunotherapy, has not been successful, obtaining very low response rates without a significant benefit in overall survival. This review highlights the most interesting issues regarding conventional therapeutic strategies, in localized and in advanced disease. New approaches such as monoclonal antibodies, vaccines, gene therapy, angiogenesis inhibitors and allogeneic cell transplantation and their possible clinical applications are also discussed.

Godinot, C., E. de Laplanche, et al. (2007). "Actuality of Warburg's views in our understanding of renal cancer metabolism." J Bioenerg Biomembr **39**(3): 235-41.

More than 50 years ago, Warburg proposed that the shift in glucose metabolism from oxidative phosphorylation (OXPHOS) to glycolysis occurring in spite of an adequate oxygen supply was at the root of cancer. This hypothesis often disregarded over the following years has recently stirred up much interest due to progress made in cancer genetics and proteomics. Studies related to renal cancers have been particularly informative to understand how abnormal use of glucose and decrease in OXPHOS are linked to cell proliferation in tumors. Indeed, in aggressive tumors such as clear cell renal carcinoma, the von Hippel-Lindau factor invalidation stabilizes the hypoxia-inducible factor (HIF) in the presence of oxygen. HIF stimulating glycolytic gene expression increases the glycolytic flux. Deficiencies in genes involved in oxidative phosphorylation that can explain the down-regulation of OXPHOS components also begin to be identified. These findings are important in the search for novel therapeutic approaches to cancer treatment.

Gordon, M. S. (2004). "Novel antiangiogenic therapies for renal cell cancer." Clin Cancer Res **10**(18 Pt 2): 6377S-81S.

Renal cell cancer remains a disease for which highly effective therapy for the majority of patients with metastatic disease is lacking. The biology of clear cell carcinomas and their association with mutations of the von Hippel-Lindau gene and its resultant increased expression of vascular endothelial growth factor (VEGF) make angiogenesis a potentially pathophysiologic mechanism for tumor development. As a result, the use of antiangiogenic therapy is an intriguing concept for the treatment of renal cell cancer. Various agents, aside from the inhibitors of VEGF, have been studied, including thalidomide, low-dose interferon, and novel antiangiogenic agents such as the thrombospondin-1 mimetics. Use of these agents has been associated with some degree of objective response or prolonged stabilization of disease, and their true value needs to be assessed in ongoing prospective studies. Combinations of antiangiogenic agents either with other similarly acting drugs or as a component of a "cocktail" with other noncytotoxic therapies should be explored in this patient population.

Gordon, M. S., M. Hussey, et al. (2009). "Phase II study of erlotinib in patients with locally advanced or metastatic papillary histology renal cell cancer: SWOG S0317." J Clin Oncol **27**(34): 5788-93.

PURPOSE: Patients with advanced papillary renal cell cancer (pRCC) have poor survival after systemic therapy; the reported median survival time is 7 to 17 months. In this trial, we evaluated the efficacy of erlotinib, an oral epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor in patients with advanced pRCC, a tumor type associated with wild-type von Hippel Lindau gene. PATIENTS AND METHODS: Patients with histologically confirmed, advanced, or metastatic pRCC were treated with erlotinib 150 mg orally once daily. A RECIST (Response Evaluation Criteria in Solid Tumors) response rate (RR) of > or = 20% was considered a promising outcome. Secondary end points included overall survival and 6-month probability of treatment failure. RESULTS: Of 52 patients registered, 45 were evaluable. The overall RR was 11% (five of 45 patients; 95% CI, 3% to 24%), and the disease control rate was 64% (ie five partial response and 24 stable disease). The median overall survival time was 27 months (95% CI, 13 to 36 months). Probability of freedom from treatment failure at 6 months was 29% (95% CI, 17% to 42%). There was one grade 5 adverse event (AE) of pneumonitis, one grade 4 thrombosis, and nine other grade 3 AEs. CONCLUSION: Although the RECIST RR of 11% did not exceed prespecified estimates for additional study, single-agent erlotinib yielded disease control and survival outcomes of interest with an expected toxicity profile. The design of future trials of the EGFR axis in pRCC should be based on preclinical or molecular data that define appropriate patient subgroups, new drug combinations, or potentially more active alternative schedules.

Gratama, J. W., A. H. Zea, et al. (1999). "Restoration of expression of signal-transduction molecules in lymphocytes from patients with metastatic renal cell cancer after combination immunotherapy." Cancer Immunol Immunother **48**(5): 263-9.

A decrease in lymphocyte signal-transduction molecules, described in cancer patients and patients with chronic infectious diseases, has been proposed as a possible mechanism leading to an impaired immune response in cancer patients. Here we report the effects of combination immunotherapy on the levels of T cell receptor zeta chain and p56lck tyrosine kinase in a retrospective study of cryopreserved lymphocytes from 26 metastatic renal cell carcinoma patients treated with high-dose interleukin-2 (IL-2), interferon alpha (IFNalpha) and ex vivo IL-2-activated lymphocytes. Of the 26 patients, 12 were responders (5 complete and 7 partial) and 14 were non-responders (6 stable and 8 with progressive disease). Prior to treatment, 21 of 26 patients (81%) and 13 of 21 patients (62%) respectively expressed zeta chain and p56lck at less than 50% of the levels observed in healthy controls. During therapy, this low zeta chain and p56lck expression increased to at least 50% of normal in 13 of the 21 patients (62%) and in 6 of the 13 patients (46%) respectively; in the remaining patients expression levels remained at 50% of normal or more, or declined. Although, in this limited study, pretreatment levels of and p56lck did not show significant correlation with antitumor response, 4 of 5 patients that achieved a complete response (80%) corrected both zeta chain and p56lck levels to at least 50% of normal, while restoration of both signal-transduction molecules to such levels was only observed in 3 of 7 partial responders (43%), 1 of 5 patients with stable disease (20%) and 2 of 7 patients with progressive disease (29%). Thus, these results suggest that analysis of changes in signal-transduction molecules may a be useful tool for immunological monitoring of patients throughout immunotherapy, and could provide important information for designing new clinical trials that restore impaired signal transduction while activating T cell responses.

Guillen-Ahlers, H. (2008). "Wnt signaling in renal cancer." Curr Drug Targets **9**(7): 591-600.

About one fourth of people diagnosed with kidney cancer in 2007, are expected to die of this disease within 5 years from the date of diagnosis. Recent years have produced novel drugs, some with FDA approval, and many in clinical trials, all showing very discrete results. Failure in finding effective treatments to improve survival with drugs mainly targeting VEGF and its downstream effectors, urges to shift the drug development targets to other unexploited pathways shown to be also involved in renal cancer. Several studies show alterations in the Wnt signaling pathway, many of which differ from those implicated in other human cancers. Unlike colorectal or hepatocellular carcinomas, where APC and axin mutations, respectively, are the main Wnt signaling deregulating event, renal carcinomas seem to be affected by other factors. Recent studies have presented VHL, a tumor suppressor gene strongly associated with renal cell carcinoma, as a beta-catenin target. This confirms that Wnt signaling is likely playing a central role during renal carcinoma development, which needs to be considered and addressed to treat this disease. This review outlines briefly the molecular biology of the most common renal cancers and the drug treatments currently used to treat the disease. The canonical Wnt pathway is reviewed more carefully adding specific features in a

renal carcinoma context, which present potential targets for drug development and biomarker use.

Guse, K., T. Ranki, et al. (2007). "Treatment of metastatic renal cancer with capsid-modified oncolytic adenoviruses." Mol Cancer Ther **6**(10): 2728-36.

Renal cancer is a common and deadly disease that lacks curative treatments when metastatic. Here, we have used oncolytic adenoviruses, a promising developmental approach whose safety has recently been validated in clinical trials. Although preliminary clinical efficacy data exist for selected tumor types, potency has generally been less than impressive. One important reason may be that expression of the primary receptor, coxsackie-adenovirus receptor, is often low on many or most advanced tumors, although not evaluated in detail with renal cancer. Here, we tested if fluorescence-assisted cell sorting could be used to predict efficacy of a panel of infectivity-enhanced capsid-modified marker gene expressing adenoviruses in renal cancer cell lines, clinical specimens, and subcutaneous and orthotopic murine models of peritoneally metastatic renal cell cancer. The respective selectively oncolytic adenoviruses were tested for killing of tumor cells in these models, and biodistribution after locoregional delivery was evaluated. In vivo replication was analyzed with noninvasive imaging. Ad5/3-Delta24, Ad5-Delta24RGD, and Ad5.pK7-Delta24 significantly increased survival of mice compared with mock or wild-type virus and 50% of Ad5/3-Delta24 treated mice were alive at 320 days. Because renal tumors are often highly vascularized, we investigated if results could be further improved by adding bevacizumab, a humanized antivascular endothelial growth factor antibody. The combination was well tolerated but did not improve survival, suggesting that the agents may be best used in sequence instead of together. These results set the stage for clinical testing of oncolytic adenoviruses for treatment of metastatic renal cancer currently lacking other treatment options.

Gutwein, P., A. Schramme, et al. (2009). "Tumoural CXCL16 expression is a novel prognostic marker of longer survival times in renal cell cancer patients." Eur J Cancer **45**(3): 478-89.

The aim of our study was to analyse the expression of CXCL16, ADAM10 and CXCR6 in renal cell carcinoma (RCC) tissue and to correlate the expression pattern with clinicopathologic data, including patient survival. Furthermore, we investigated CXCL16, ADAM10 and CXCR6 expressions by FACS, immunofluorescence and ELISA analysis in renal carcinoma cell lines. Our immunohistochemical analysis on tissue microarray of renal cancer samples of 104 patients revealed that ADAM10 correlated significantly with tumour stage, pathological nodal status, M status and lymphangiosis carcinomatosa. CXCL16, CXCR6 and ADAM10 were significantly increased in papillary carcinomas. Importantly, high levels of CXCL16 expression in renal cancer tissue correlated with better survival of patients, and CXCL16 correlated inversely to the tumour stage. In addition, inhibition of CXCL16 induced the migration of renal cancer cells assuming an anti-migratory function of transmembrane CXCL16. Taken together, our data demonstrate that downregulation of CXCL16 plays an important role in renal cancer development and progression, and that CXCL16 in RCC is an independent prognostic marker for better patient survival.

Hansel, D. E. and B. I. Rini (2008). "Molecular genetics of hereditary renal cancer: new genes and diagnostic and therapeutic opportunities." Expert Rev Anticancer Ther **8**(6): 895-905.

Renal cell carcinoma may be sporadic or occur in the setting of an inherited cancer syndrome, such as von Hippel-Lindau or Birt-Hogg-Dube syndrome. Although the clinical spectrum of heritable renal cancer syndromes varies significantly, commonalities include the often young age of presentation, multifocal and bilateral nature of renal lesions, and autosomal dominant pattern of inheritance. Molecular studies have recently begun to elucidate the genetic abnormalities and subsequent alterations in downstream intracellular signaling cascades that underlie the development of these syndromes. This review will highlight the clinicopathologic and molecular features associated with the diverse array of heritable renal cancer syndromes and emphasize the potential cellular pathways that may be utilized to develop novel treatment strategies for patients with these syndromes.

Hao, D., S. D. Huan, et al. (2000). "A pilot study of low dose hydroxyurea as a novel resistance modulator in metastatic renal cell cancer." J Chemother **12**(4): 360-6.

Mechanisms of chemoresistance in renal cell carcinoma include P-glycoprotein, overexpression of multidrug resistance-1 (mdr1) gene, and unstable chromosomal aberrations. In vitro exposure of resistant tumor cells to low dose hydroxyurea causes loss of chromosomal aberrations, decrease in the mdr1 gene copies, and increased sensitivity to vinblastine. Patients received continuous hydroxyurea 500 mg every Monday, Wednesday and Friday. Vinblastine 5 mg/m2 was given intravenously on days 1 and 8 every 21 days. Seventeen patients with a median age of 63 (range 40-80) received a median of 3 courses of vinblastine (range 1-14). Toxicities included: > or =

grade 3 non-hematologic toxicity (1) and febrile neutropenia (2). No treatment related mortality occurred. Three patients (17.6%) had partial responses. The median survival was 38.0 weeks (95% CI = 26.9-49.1 weeks). The addition of hydroxyurea given at the dose of 500 mg orally three times weekly had no major impact on the expected antitumor effect of vinblastine.

Haviv, Y. S., J. L. Blackwell, et al. (2002). "Adenoviral gene therapy for renal cancer requires retargeting to alternative cellular receptors." Cancer Res **62**(15): 4273-81.

Metastatic renal cell carcinoma (RCC) is one of the most treatment-resistant malignancies in humans. Therefore, the identification of new agents with better antitumor activity merits a high priority in the treatment of advanced RCC. In this regard, gene therapy with adenoviral (Ad) vectors is a promising new modality for cancer. However, a primary limiting factor for the use of Ad vectors for cancer gene therapy is their critical dependence on cellular expression of the primary Ad receptor, the coxsackie and adenovirus receptor (CAR), known to be down-regulated in many cancer types. Following the identification of CAR deficiency in RCC lines, we have found abundant membrane expression of alpha(v)beta 3 and alpha(v)beta 5 integrins and of the putative receptor to Ad serotype 3 (Ad3). As an alternative gene therapy approach for RCC that would circumvent CAR deficiency, we employed retargeting of replication-incompetent Ad vectors and replication-competent Ad viruses to alpha(v)beta 3 and alpha(v)beta 5 integrins and to the putative Ad3 receptor. These strategies to genetically alter Ad tropism were based on either the insertion of a cysteine-aspartate-cysteine-arginine-glycine-aspartate-cysteine-phenylalanine-cys teine (RGD) motif into the HI loop of the Ad fiber knob domain or on generation of a chimeric Ad fiber composed of adenovirus serotype 5 shaft/Ad3 knob. Both strategies proved highly efficient to circumvent CAR deficiency and enhance gene delivery into RCC cells. Furthermore, in the context of replication-competent Ad, tropism alteration resulted in distinct capacity of the retargeted viruses to infect, replicate, and lyse RCC models in vitro and in vivo. The retargeting strategies were particularly beneficial in the context of replication-competent Ad. These findings underscore the importance of CAR-independent cellular entry mechanisms in RCC and are highly consequential for the development of viral antitumor agents for RCC and other CAR-negative tumors.

Haviv, Y. S. and D. T. Curiel (2008). "Gene therapy for renal cancer." Contrib Nephrol **159**: 135-50.

Recent advances in understanding the molecular events associated with renal cell carcinoma (RCC) are revolutionizing the therapeutic options offered for patients with advanced-stage RCC. These targeted approaches for RCC are based primarily on antiangiogenesis and/or specific kinase inhibitors targeting the vascular-endothelial growth factor and platelet-derived growth factor receptors, Raf and mammalian target of rapamycin inhibitor. In this context, characterization of the molecular events unique to RCC is also of critical significance for gene therapy endeavors. The attributes of gene therapy for RCC may include true targeting to cancer cells, transfer of immunomodulatory or antiangiogenic genes and novel nonapoptotic cancer cell killing mechanisms. Gene therapy may thus become a promising new adjuvant modality for RCC and expand the therapeutic armamentarium against RCC. Beyond the current stage of preclinical proof of principle and toxicological analysis in animal models, the utility of RCC gene therapy will depend on safety and efficacy trials in human subjects. These trials will determine whether targeted therapy for RCC employing genome-based strategies will broaden the current therapeutic spectrum for RCC comprising kinome-based, immunomodulatory and antiangiogenesis strategies.

Haviv, Y. S., W. J. van Houdt, et al. (2004). "Transcriptional targeting in renal cancer cell lines via the human CXCR4 promoter." Mol Cancer Ther **3**(6): 687-91.

Metastatic renal cell carcinoma (RCC) is often resistant to standard treatment, thereby requiring new therapeutic strategies. In this regard, tumor cell migration and metastasis have recently been shown to be regulated by chemokines and their respective receptors (e.g., SDF-1alpha/CXCR4). In the context of RCC, up-regulation of CXCR4 expression is closely related to the development of invasive cancer. Thus, we hypothesized that the CXCR4 pathway could be exploited for RCC targeting with gene therapy vectors. In this regard, targeting adenoviral vectors to tumor cells is critically dependent on tumor-specific gene expression. Toward the end of RCC tumor targeting, we evaluated the utility of the CXCR4 promoter in an adenoviral context. First, overexpression of CXCR4 was confirmed in several RCC cell lines. Next, an adenoviral vector was constructed, whereby the human CXCR4 promoter drives the expression of a reporter gene. We tested the activity of the CXCR4 promoter in vitro and in vivo in relevant models. Our data indicate that the human CXCR4 promoter is highly active in RCC cells but not in normal human cells. Finally, biodistribution studies in mice demonstrated dramatic repression of the

CXCR4 promoter in the liver but not in the kidney. In conclusion, the unique activity of the CXCR4 promoter in RCC lines and its repression in normal human cells and in the murine liver underscore its potential utility as a novel candidate for transcriptional targeting of RCC.

Hervouet, E., H. Simonnet, et al. (2007). "Mitochondria and reactive oxygen species in renal cancer." Biochimie **89**(9): 1080-8.

In most cancer cells, the ATP necessary for survival and proliferation is derived from glycolysis rather than from oxidative phosphorylations (OXPHOS) even when oxygen supply would be adequate to sustain them. This phenomenon, named "aerobic glycolysis" by Warburg many years ago, can now be explained by a mechanism up-regulating the expression of genes involved in glucose transport, glucose metabolism, lactate formation and exit from the cell. In clear cell renal carcinoma, this mechanism is due to the stabilization of the hypoxia-inducible transcription factor HIF occurring when the tumor suppressor gene vhl is invalidated. HIF increases the transcription of genes involved in glycolysis and lactate metabolism. Although respiratory chain complex activities and subunit amounts are severely diminished, the transcription of genes involved in the structure and biogenesis of these complexes does not seem to be significantly decreased in these cancers but reactive oxygen species (ROS) production is increased. In this review, we discuss the roles that ROS may play in the decrease of OXPHOS in cancer and in the regulation of the mitochondria-induced initiation of apoptosis.

Hirata, H., Y. Hinoda, et al. (2009). "Wnt antagonist gene DKK2 is epigenetically silenced and inhibits renal cancer progression through apoptotic and cell cycle pathways." Clin Cancer Res **15**(18): 5678-87.

PURPOSE: Wnt/beta-catenin signaling is involved in renal cancer. DKK2, a Wnt antagonist, is silenced in some cancers, although its function has not been investigated. We hypothesized that DKK2 may be epigenetically silenced and inhibits progression of renal cell carcinoma (RCC). EXPERIMENTAL DESIGN: RCC cell lines and a normal kidney cell line were used for methylation and chromatin immunoprecipitation assays. To assess various functions of DKK2, we established stable DKK2-transfected cells and examined them with regard to cell viability, colony formation, apoptosis, cell cycle, and invasive capability. A total of 52 patients with confirmed conventional RCC were enrolled in this study. RESULTS: RCC cell lines had decreased levels of DKK2, which were significantly increased after treatment with 5-Aza-2'-deoxycytidine alone or 5-Aza-2'-deoxycytidine and trichostatin A. In chromatin immunoprecipitation assay, the levels of acetyl H3, acetyl H4, and dimethylated H3K4 were decreased, whereas the level of dimethylated H3K9 was increased in RCC cell lines compared with HK2 cells. Increased methylation in RCC tissues was associated with higher grades, pathologic stages, and pathologic tumor in RCC. Functional analysis showed that the numbers of viable A498 cells were significantly decreased in DKK2-transfected cells compared with mock cells. The number of apoptotic cells and S/G(2)-M phase cells was significantly increased and decreased after DKK2 transfection, respectively. Corresponding to these results, Bcl2 and cyclin D1 expression were also decreased in DKK2-overexpressing cells. CONCLUSION: DKK2 is epigenetically silenced by methylation in higher grades and stages of RCC. These results suggest that DKK2 inhibits renal cancer progression through apoptotic and cell cycle pathways.

Hirata, H., Y. Hinoda, et al. (2009). "The bcl2 -938CC genotype has poor prognosis and lower survival in renal cancer." J Urol **182**(2): 721-7.

PURPOSE: A single nucleotide polymorphism (-938C/A, rs2279115) was found in the bcl2 gene, whose -938A allele is significantly associated with increased Bcl2 expression compared with that of the C allele. Bcl2 up-regulation was reported to be associated with longer survival in patients with renal cancer. However, to our knowledge there is currently no information on the role of the bcl2-938C/A single nucleotide polymorphism in renal cell carcinoma cases. Therefore, we investigated the polymorphism at the bcl2 -938C/A site and its effects on clinical characteristics in patients with renal cell carcinoma. MATERIALS AND METHODS: We genotyped the bcl2-938C/A single nucleotide polymorphism in 216 patients with renal cancer, and in 209 healthy age and gender matched controls. We also investigated the relationship between the bcl2 -938C/A polymorphism, Bcl2 expression, proliferation and apoptosis status in renal cell carcinoma tissues using immunohistochemistry and TUNEL assay. The association of the bcl2 -938C/A single nucleotide polymorphism with survival in patients with renal cell carcinoma was also analyzed by Kaplan-Meier curves. RESULTS: Survival in Bcl2 positive cases was significantly longer than in negative cases. On univariate and multivariate analyses the bcl2 -938CC genotype was independently associated with poor prognosis. Kaplan-Meier analysis showed that survival in patients with CC genotypes was significantly worse than in those with CA+AA genotypes. CC genotype carriers had significantly lower Bcl2 expression and higher proliferative activity

in renal cancer tissues than CA+AA genotype carriers. CONCLUSIONS: To our knowledge this is the first report to show that the bcl2 -938C/C genotype has worse prognosis and lower survival in patients with renal cell carcinoma. In addition, the bcl2 -938C/A single nucleotide polymorphism was shown to be an independent adverse prognostic factor for renal cell carcinoma.

Hirata, H., Y. Hinoda, et al. (2009). "Wnt antagonist gene polymorphisms and renal cancer." Cancer **115**(19): 4488-503.

BACKGROUND: Epigenetic silencing of several wingless-type mouse mammary tumor virus integration site (Wnt) pathway-related genes has been reported in renal cancer. Except for the T-cell factor 4 gene TCF4, there are no reports regarding Wnt pathway gene polymorphisms in renal cancer. Therefore, the authors of this report hypothesized that the polymorphisms in Wnt signaling genes may be risk factors for renal cancer. METHODS: In total, 210 patients (145 men and 65 women) with pathologically confirmed renal cell carcinoma (RCC) and 200 age-matched and sex-matched control individuals were enrolled in this study. We genotyped 14 single nucleotide polymorphisms (SNPs) in 6 genes. including Dickkopf 2 (DKK2) (reference SNP identification number 17037102 [rs17037102], rs419558, and rs447372), DKK3 (rs3206824, rs11022095, rs1472189, rs7396187, and rs2291599), DKK4 (rs2073664), secreted frizzled-related protein 4 (sFRP4) (rs1802073 and rs1802074), mothers against decapentaplegic homolog (SMAD) family member 7 or SMAD7 (rs12953717), and disheveled associated activator of morphogenesis 2 or DAAM2 (rs6937133 and rs2504106) using polymerase chain reaction-restriction fragment length polymorphism analysis and direct sequencing in the patients with RCC and in the healthy, age-matched control group. The relations also were tested between these polymorphisms and clinicopathologic data, including sex, tumor grade, tumor stage, lymph node involvement, distant metastasis, and overall survival. RESULTS: A significant decrease in the frequency of the guanine/adenine (G/A) + A/A genotypes in the DKK3 codon 335 rs3206824 was observed in the patients with RCC compared with the control group. The frequency of the rs3206824 (G/A) A-rs7396187 (guanine/cytosine [G/C]) C haplotype was significantly lower in patients with RCC compared with other haplotypes. In addition, DKK3 rs1472189 cytosine/thymine (C/T) was associated with distant metastasis, and, DKK2 rs17037102 G-homozygous patients had a decreased risk for death in multivariate Cox regression analysis. CONCLUSIONS: To the authors' knowledge, this is the first report documenting that DKK3 polymorphisms are associated with RCC and that the DKK2 rs17037102 polymorphism may be a predictor for survival in patients with RCC after radical nephrectomy.

Honke, K., M. Tsuda, et al. (1998). "Cancer-associated expression of glycolipid sulfotransferase gene in human renal cell carcinoma cells." Cancer Res **58**(17): 3800-5.

Human renal cell carcinoma (RCC) tissue and a cell line derived therefrom, SMKT-R3, showed markedly increased glycolipid sulfotransferase [cerebroside sulfotransferase (CST); EC 2.8.2.11] activity and accumulated sulfoglycolipids. Recently, we cloned a human CST cDNA from a SMKT-R3 cDNA library (K. Honke et al., J. Biol. Chem., 272: 4864-4868, 1997). In this study, we investigated the expression of the CST gene in seven human RCC lines (SMKT-R1, SMKT-R2, SMKT-R3, SMKT-R4, TOS-1, TOS-2, and ACHN) and their normal counterpart, human renal proximal tubular cells. On Northern blot analysis, a marked increase of CST mRNA was observed in every RCC line, except for ACHN, as compared with normal cells. ACHN cells showed a slightly increased level of CST mRNA. CST activity was correlated with the amount of mRNA. Sulfoglycolipid analysis revealed that expression of lactosylceramide sulfate was correlated with the CST level. Furthermore, we examined the effects of epidermal growth factor (EGF), tetradecanoylphorbol-13-acetate, and genistein, which are known to regulate CST activity in SMKT-R3 cells, on CST-gene expression in various RCC cells. On treatment with EGF, CST mRNA time-dependently increased in accord with its activity in SMKT-R3 cells. Yet, augmentation by EGF was only observed in SMKT-R3. In contrast, a reduction of CST mRNA and activity by tetradecanoylphorbol-13-acetate and genistein was observed in all of the lines examined. Taken together, these findings indicate that in human RCC cells, the CST gene is generally overexpressed via a signaling pathway involving protein kinase-C and tyrosine kinases.

Hoque, M. O., S. Begum, et al. (2004). "Quantitative detection of promoter hypermethylation of multiple genes in the tumor, urine, and serum DNA of patients with renal cancer." Cancer Res **64**(15): 5511-7.

Aberrant promoter hypermethylation of several known or putative tumor suppressor genes occurs frequently during the pathogenesis of human cancers and is a promising marker for cancer detection. We investigated the feasibility of detecting aberrant DNA methylation in the urine and serum samples of renal cancer patients. We examined the tumor and the matched urine and serum DNA for

aberrant methylation of nine gene promoters (CDH1, APC, MGMT, RASSF1A, GSTP1, p16, RAR-beta2, and ARF) from 17 patients with primary kidney cancer by quantitative fluorogenic real-time PCR. An additional 9 urine samples (total, 26) and 1 serum sample (total, 18) also were tested from renal cancer patients. Urine from 91 patients without genitourinary cancer and serum from 30 age-matched noncancer individuals were used as controls. Promoter hypermethylation of at least two of the genes studied was detected in 16 (94%) of 17 primary tumors. Aberrant methylation in urine and serum DNA generally was accompanied by methylation in the matched tumor samples. Urine samples from 91 control subjects without evidence of genitourinary cancer revealed no methylation of the MGMT, GSTP1, p16, and ARF genes, whereas methylation of RAR-beta2, RASSF1A, CDH1, APC, and TIMP3 was detected at low levels in a few control subjects. Overall, 23 (88%) of 26 urine samples and 12 (67%) of 18 serum samples from cancer patients were methylation positive for at least one of the genes tested. By combination of urine or serum analysis of renal cancer patients, hypermethylation was detected in 16 of 17 patients (94% sensitivity) with high specificity. Our findings suggest that promoter hypermethylation in urine or serum can be detected in the majority of renal cancer patients. This noninvasive high-throughput approach needs to be evaluated in large studies to assess its value in the early detection and surveillance of renal cancer.

Hueber, P. A., D. Iglesias, et al. (2008). "In vivo validation of PAX2 as a target for renal cancer therapy." Cancer Lett **265**(1): 148-55.

PAX genes are frequently overexpressed in human cancer tissue and appear to contribute to the tumor phenotype, suggesting that they may be potential targets for cancer therapy. In particular, aberrant PAX2 expression has been reported in a high proportion of primary tumors, including the majority of renal cell carcinomas (RCC). We recently demonstrated that PAX2 suppresses cisplatin-induced apoptosis in cultured RCC cells. We hypothesized that silencing of PAX2 expression might partially overcome the notorious resistance of renal cell carcinomas to chemotherapy in vivo. In this report, we show that a PAX2 shRNA successfully knocks down PAX2 mRNA and protein levels in an RCC cell line (ACHN). ACHN cells stably transfected with shRNAs targeted against the PAX2 homeodomain are 3-6-fold more susceptible to cisplatin-induced caspase-3 activation than control ACHN cells line. Furthermore, growth of subcutaneous ACHN/shPAX2 xenografts in nude mice is significantly more responsive to cisplatin therapy than control ACHN cell tumors. Our observations validate PAX2 as a potential therapeutic gene target in renal cancer and suggest that adjunctive PAX2 knockdown may enhance the efficacy of other chemotherapeutic agents.

Ibanez de Caceres, I., E. Dulaimi, et al. (2006). "Identification of novel target genes by an epigenetic reactivation screen of renal cancer." Cancer Res **66**(10): 5021-8.

Aberrant promoter hypermethylation is a common mechanism for inactivation of tumor suppressor genes in cancer cells. To generate a global profile of genes silenced by hypermethylation in renal cell cancer (RCC), we did an expression microarray-based analysis of genes reactivated in the 786-0, ACHN, HRC51, and HRC59 RCC lines after treatment with the demethylating drug 5-aza-2 deoxycytidine and histone deacetylation inhibiting drug trichostatin A. Between 111 to 170 genes were found to have at least 3-fold up-regulation of expression after treatment in each cell line. To establish the specificity of the screen for identification of genes, epigenetically silenced in cancer cells, we validated a subset of 12 up-regulated genes. Three genes (IGFBP1, IGFBP3, and COL1A1) showed promoter methylation in tumor DNA but were unmethylated in normal cell DNA. One gene (GDF15) was methylated in normal cells but more densely methylated in tumor cells. One gene (PLAU) showed cancer cell-specific methylation that did not correlate well with expression status. The remaining seven genes had unmethylated promoters, although at least one of these genes (TGM2) may be regulated by RASSF1A, which was methylated in the RCC lines. Thus, we were able to show that up-regulation of at least 6 of the 12 genes examined was due to epigenetic reactivation. The IGFBP1, IGFBP3, and COL1A1 gene promoter regions were found to be frequently methylated in primary renal cell tumors, and further study will provide insight into the biology of the disease and facilitate translational studies in renal cancer.

Iliopoulos, O. (2006). "Molecular biology of renal cell cancer and the identification of therapeutic targets." J Clin Oncol **24**(35): 5593-600.

Renal cell cancer (RCC) is a heterogeneous disease consisting of different histologic types. Major advances have been accomplished during the last 15 years in our understanding of the genetic events that initiate RCC. These advances were greatly facilitated by meticulous clinical description and registration of patients with familial predisposition to RCC. The cloning of the susceptibility genes that underline familial predisposition to RCC has offered entry points into the signaling pathways that are also

deregulated in sporadic RCC. Biochemical studies of these signaling pathways and target validation experiments have already culminated in the discovery and clinical application of small molecules with promising activity in RCC. In this article, we highlight the molecular genetic features of RCC that are more directly related to identification and validation of promising targets for molecular therapy.

Isaacs, J. S., Y. J. Jung, et al. (2005). "HIF overexpression correlates with biallelic loss of fumarate hydratase in renal cancer: novel role of fumarate in regulation of HIF stability." Cancer Cell **8**(2): 143-53.

Individuals with hemizygous germline fumarate hydratase (FH) mutations are predisposed to renal cancer. These tumors predominantly exhibit functional inactivation of the remaining wild-type allele, implicating FH inactivation as a tumor-promoting event. Hypoxia-inducible factors are expressed in many cancers and are increased in clear cell renal carcinomas. Under normoxia, the HIFs are labile due to VHL-dependent proteasomal degradation, but stabilization occurs under hypoxia due to inactivation of HIF prolyl hydroxylase (HPH), which prevents HIF hydroxylation and VHL recognition. We demonstrate that FH inhibition, together with elevated intracellular fumarate, coincides with HIF upregulation. Further, we show that fumarate acts as a competitive inhibitor of HPH. These data delineate a novel fumarate-dependent pathway for regulating HPH activity and HIF protein levels.

Ishizawa, J., S. Yoshida, et al. (2004). "Inhibition of the ubiquitin-proteasome pathway activates stress kinases and induces apoptosis in renal cancer cells." Int J Oncol **25**(3): 697-702.

The ubiquitin-proteasome pathway plays a critical role in the degradation of cellular proteins related to signal transduction. Cytokine and growth factor-dependent aberrant proliferation has been implicated in renal cell carcinoma (RCC). We hypothesized that inhibiting the proteasome function might activate a proapoptotic signal transduction by modulating the cytokine and growth factor related signal transduction pathway. We therefore investigated the effectiveness of a proteasome inhibitor in the treatment of RCC regarding the involvement of Mitogen-activated protein kinases (MAP kinases), because MAP kinases are major signal transduction molecules that are known to play a pivotal role in cancer cell proliferation or apoptosis triggered by extra-cellular cytokines and growth factors. A proteasome inhibitor, MG132 inhibited the proliferation of RCC cell lines, 786-O and KU20-01 in a time and dose-dependent manner. 786-O cells have truncated von-Hippel Lindau (VHL) tumor suppressor gene protein due to a one base pair deletion at exon 1, whereas KU20-01 cells have a wild-type VHL protein. MG132 induced apoptosis in both cell lines. The inhibition of the ubiquitin-proteasome pathways was confirmed by the accumulation of ubiquitin-tagged proteins. MG132 induced the phosphorylation of ERK at 4 h and thereafter persisted for 8 to 16 h. In contrast, JNK and p38 activation persisted for longer periods and remained enhanced until 24 h. The concomitant activation of effector caspases, caspase-3 and caspase-7 was observed in 786-O cells. The inhibition of the proteasome function can induce apoptosis in RCC irrespective of the VHL protein status. The persistence of JNK and p38 activation may therefore be a unique mechanism underlying MG132 induced apoptosis.

Joensuu, T. K., S. Nilsson, et al. (2004). "Phase I trial on sms-D70 somatostatin analogue in advanced prostate and renal cell cancer." Ann N Y Acad Sci **1028**: 361-74.

Plasma concentrations and tolerability of a novel somatostatin analogue sms-D70 were studied in patients with metastatic hormone-resistant prostate cancer (HRPC) or metastatic renal cell cancer. To overcome the limitations of the octapeptides having affinity only to somatostatin receptor subtypes 2 and 5, HRPC expressing mainly somatostatin receptors 1 and 4, a somatostatin derivative based on the natural somatostatin having affinity to all five somatostatin receptor subtypes, was developed. The in vivo stability of this dextran-conjugated derivative, somatostatin-D70, was confirmed previously in animal studies, and the nanomolar "panaffinity" has been shown in in vitro receptor binding studies on cell lines transfected with the somatostatin receptor genes. Sms-D70 was given with subcutaneous injection once a week at dose levels of 5, 10, 20, 35, and 50 mg. For pharmacokinetic studies, sms-D70 was labeled with 131I. Fourteen patients were treated, of whom 10 had prostate and 4 renal cell cancer. The kinetic data revealed high stability with a long half-life in the blood. The drug was well tolerated, and no grade 4 (WHO) toxicity was observed. The maximal tolerated dose could not be established due to the lack of dose-limiting toxicities. Objective PSA responses were not recorded in these heavily treated patients, but subjective stabilization of pain was observed and urinary symptoms were alleviated in four patients. Three patients with metastatic HRPC received 5-10-mg intravenous injections of sms-D70 once weekly for 4-14 months on a compassionate use basis. In all cases, serum PSA values decreased more than 50% from the pretreatment level, but these results are

difficult to interpret due to concomitant treatments given to these patients. In conclusion, sms-D70 was well tolerated in the treatment of metastatic prostate and renal cell cancer, but no responses were found in these heavily treated patients.

Johannesma, P. C., J. W. Lammers, et al. (2009). "[Spontaneous pneumothorax as the first manifestation of a hereditary condition with an increased renal cancer risk]." Ned Tijdschr Geneeskd **153**: A581.

Spontaneous pneumothorax can be due to Birt-Hogg-Dube syndrome (BHD syndrome), an autosomal dominant predisposition for fibrofolliculomas, multiple lung cysts, pneumothorax and renal cancer. The syndrome is the result of germline mutations in the FLCN (folliculin) gene. Its clinical presentation is highly variable. Consequently, this syndrome is probably under-diagnosed. An illustrative kindred is presented in which the index patient, a man aged 26, had recurrent episodes of pneumothorax without apparent skin lesions or renal abnormalities. He had bilateral mostly basally-located lung cysts. There was a family history of fibrofolliculomas, lung cysts, pneumothorax and clear cell renal cancer. Recognition of BHD is important since carriers of the mutation can be offered surveillance for early detection and treatment of renal cancer.

Jonasdottir, T. J., C. S. Mellersh, et al. (2000). "Genetic mapping of a naturally occurring hereditary renal cancer syndrome in dogs." Proc Natl Acad Sci U S A **97**(8): 4132-7.

Canine hereditary multifocal renal cystadenocarcinoma and nodular dermatofibrosis (RCND) is a rare, naturally occurring inherited cancer syndrome observed in dogs. Genetic linkage analysis of an RCND-informative pedigree has identified a linkage group flanking RCND (CHP14-C05.377-C05.414-FH2383-C05. 771-[RCND-CPH18]-C02608-GLUT4-TP53-ZuBe Ca6-AHT141-FH2140-FH2594) thus localizing the disease to a small region of canine chromosome 5. The closest marker, C02608, is linked to RCND with a recombination fraction (theta) of 0.016, supported by a logarithm of odds score of 16.7. C02608 and the adjacent linked markers map to a region of the canine genome corresponding to portions of human chromosomes 1p and 17p. A combination of linkage analysis and direct sequencing eliminate several likely candidate genes, including tuberous sclerosis 1 and 2 genes (TSC1 and TSC2) and the tumor suppressor gene TP53. These data suggest that RCND may be caused by a previously unidentified tumor suppressor gene and highlight the potential for canine genetics in the study of human disease predisposition.

Jones, J. and T. A. Libermann (2007). "Genomics of renal cell cancer: the biology behind and the therapy ahead." Clin Cancer Res **13**(2 Pt 2): 685s-692s.

Renal cell cancer (RCC) is the most lethal of the urological cancers and accounts for 3% of all adult malignancies. Despite numerous recent advances in diagnostic imaging, surgical therapy, and basic molecular understanding, many patients still experience metastatic disease. For metastatic disease patients, response rates to conventional therapies rarely exceed 15% to 25% and are associated with serious adverse effects. The recent development of novel targeted therapies based on the precise biological pathways deregulated in a particular patient has paved the way for individualized, targeted patient management. Nevertheless, to achieve this goal, it is important to delineate the molecular mechanisms underlying cancer development and progression. Genomic approaches have revolutionized the field of cancer research and have led to the rapid discovery of multiple, parallel disease hypotheses, which ultimately have to be validated in large cohorts of patients and in downstream biological experiments for translation into clinical applications. The variable course of RCC and, until recently, a paucity of therapeutic options in the event of metastasis have led to the search for diagnostic and prognostic markers. We and others have used transcriptional profiling to classify different subtypes of RCC and to identify subtype- and metastasis-specific gene signatures predictive for outcome. We discuss herein recent genomic approaches to RCC and the emerging biological pathways underlying RCC development and progression. We also speculate how genomics may affect drug development and the management of patients with RCC.

Jones, J., H. Otu, et al. (2005). "Gene signatures of progression and metastasis in renal cell cancer." Clin Cancer Res **11**(16): 5730-9.

PURPOSE: To address the progression, metastasis, and clinical heterogeneity of renal cell cancer (RCC). EXPERIMENTAL DESIGN: Transcriptional profiling with oligonucleotide microarrays (22,283 genes) was done on 49 RCC tumors, 20 non-RCC renal tumors, and 23 normal kidney samples. Samples were clustered based on gene expression profiles and specific gene sets for each renal tumor type were identified. Gene expression was correlated to disease progression and a metastasis gene signature was derived. RESULTS: Gene signatures were identified for each tumor type with 100% accuracy. Differentially expressed genes

during early tumor formation and tumor progression to metastatic RCC were found. Subsets of these genes code for secreted proteins and membrane receptors and are both potential therapeutic or diagnostic targets. A gene pattern ("metastatic signature") derived from primary tumor was very accurate in classifying tumors with and without metastases at the time of surgery. A previously described "global" metastatic signature derived by another group from various non-RCC tumors was validated in RCC. CONCLUSION: Unlike previous studies, we describe highly accurate and externally validated gene signatures for RCC subtypes and other renal tumors. Interestingly, the gene expression of primary tumors provides us information about the metastatic status in the respective patients and has the potential, if prospectively validated, to enrich the armamentarium of diagnostic tests in RCC. We validated in RCC, for the first time, a previously described metastatic signature and further showed the feasibility of applying a gene signature across different microarray platforms. Transcriptional profiling allows a better appreciation of the molecular and clinical heterogeneity in RCC.

Karami, S., P. Brennan, et al. (2009). "Analysis of SNPs and haplotypes in vitamin D pathway genes and renal cancer risk." PLoS One **4**(9): e7013.

In the kidney vitamin D is converted to its active form. Since vitamin D exerts its activity through binding to the nuclear vitamin D receptor (VDR), most genetic studies have primarily focused on variation within this gene. Therefore, analysis of genetic variation in VDR and other vitamin D pathway genes may provide insight into the role of vitamin D in renal cell carcinoma (RCC) etiology. RCC cases (N = 777) and controls (N = 1,035) were genotyped to investigate the relationship between RCC risk and variation in eight target genes. Minimum-p-value permutation (Min-P) tests were used to identify genes associated with risk. A three single nucleotide polymorphism (SNP) sliding window was used to identify chromosomal regions with a False Discovery Rate of <10%, where subsequently, haplotype relative risks were computed in Haplostats. Min-P values showed that VDR (p-value = 0.02) and retinoid-X-receptor-alpha (RXRA) (p-value = 0.10) were associated with RCC risk. Within VDR, three haplotypes across two chromosomal regions of interest were identified. The first region, located within intron 2, contained two haplotypes that increased RCC risk by approximately 25%. The second region included a haplotype (rs2239179, rs12717991) across intron 4 that increased risk among participants with the TC (OR = 1.31, 95% CI = 1.09-1.57) haplotype compared to participants with the common haplotype, TT. Across RXRA, one haplotype located 3' of the coding sequence (rs748964, rs3118523), increased RCC risk 35% among individuals with the variant haplotype compared to those with the most common haplotype. This study comprehensively evaluated genetic variation across eight vitamin D pathway genes in relation to RCC risk. We found increased risk associated with VDR and RXRA. Replication studies are warranted to confirm these findings.

Karumanchi, S. A., J. Merchan, et al. (2002). "Renal cancer: molecular mechanisms and newer therapeutic options." Curr Opin Nephrol Hypertens **11**(1): 37-42.

Renal cell carcinomas account for 80-85% of all primary renal neoplasms. Recent identification of VHL, c-met and TSC as candidate genes mutated in various types of renal carcinomas has greatly enhanced our understanding of the pathogenesis of renal carcinomas and has provided novel therapeutic options for patients with renal cancer. Furthermore, developments in angiogenesis and in tumor immunology have given us additional treatment modalities for cancer patients, especially those with renal cancer. This review highlights the genetic abnormalities seen in renal cell carcinomas and reviews current and future therapeutic options.

Kasahara, T., V. Bilim, et al. (2006). "Homozygous deletions of the INK4a/ARF locus in renal cell cancer." Anticancer Res **26**(6B): 4299-305.

BACKGROUND: Genetic alterations of p14ARF contribute to dysfunction of p53 pathways by disruption of MDM2-mediated inhibition of p53. P14(ARF) was investigated by focusing on the homozygous deletion (HD) in the INK4a/ARF locus and hypermethylation of the p14(ARF) promoter in renal cell cancer (RCC). MATERIALS AND METHODS: Using 6 RCC cell lines, RT-PCR and Western blotting was performed for p14(ARF). DNA from 34 RCCs was analyzed for HD in the INK4a/ARF locus, promoter hypermethylation and p53 gene mutation. RESULTS: HD was confirmed in 4 out of 6 cell lines and in 8 out of 34 (23.5%) RCC specimens, which correlated with the presence of metastasis, high tumor grade and had a tendency to more advanced stage (I vs. II-IV). No hypermethylation of the p14(ARF) promoter or p53 mutation was detected among the RCC specimens. CONCLUSION: These results indicate that the deletion in the INK4a/ARF locus might contribute to tumor progression in RCC at least partly by functional inactivation of wild-type p53.

Kawakami, T., K. Okamoto, et al. (2003). "Multipoint methylation and expression analysis of tumor

suppressor genes in human renal cancer cells." Urology **61**(1): 226-30.

OBJECTIVES: To analyze the methylation status and expression profiles of multiple tumor suppressor genes in renal cell carcinoma-derived cell lines. Aberrant promoter methylation is commonly found in human cancers. Nonetheless, it is challenging to demonstrate that methylation of a specific gene results in gene inactivation. METHODS: We simultaneously analyzed methylation and expression profiles of five putative tumor suppressor genes (p15, p16, Rb, BRCA1, and E-cadherin) in 14 different cell lines using bisulfite genomic sequencing and reverse transcriptase-polymerase chain reaction. We also used multiplex polymerase chain reaction to identify homozygous deletions at the p15 and p16 loci. RESULTS: Expression of p16, BRCA1, and E-cadherin was maintained in 4 (29%) of 14 cell lines, regardless of the presence of methylation. Aberrant methylation of p16 was observed in 2 (14%), of BRCA1 in 1 (7%), and of E-cadherin in 9 (64%) of 14 cell lines. Concurrent methylation was observed among p16 and BRCA1 (1 [7%] of 14 cell lines) and among p16 and E-cadherin (1 [7%] of 14 cell lines). We detected homozygous deletion of p16 and p15 in 11 (78%) and 6 (43%) cell lines, respectively. CONCLUSIONS: The present data shows the presence of methylation does not always contribute to the loss of expression of tumor suppressor genes. Therefore, we must be cautious in interpreting the results of methylation assays--in particular, detection of methylation by nonquantitative methods. The data also demonstrated that multiple tumor suppressor genes are simultaneously inactivated in renal cell carcinoma-derived cell lines by distinctive mechanisms.

Kempkensteffen, C., F. R. Fritzsche, et al. (2009). "Down-regulation of the pro-apoptotic XIAP associated factor-1 (XAF1) during progression of clear-cell renal cancer." BMC Cancer **9**: 276.

BACKGROUND: Decreased expression of the interferon-stimulated, putative tumour suppressor gene XAF1 has been shown to play a role during the onset, progression and treatment failure in various malignancies. However, little is yet known about its potential implication in the tumour biology of clear-cell renal cell cancer (ccRCC). METHODS: This study assessed the expression of XAF1 protein in tumour tissue obtained from 291 ccRCC patients and 68 normal renal tissue samples, utilizing immunohistochemistry on a tissue-micro-array. XAF1 expression was correlated to clinico-pathological tumour features and prognosis. RESULTS: Nuclear XAF1 expression was commonly detected in normal renal- (94.1%) and ccRCC (91.8%) samples, without significant differences of expression levels. Low XAF1 expression in ccRCC tissue, however, was associated with progression of tumour stage ($p = 0.040$) and grade ($p < 0.001$). Low XAF1 tumour levels were also prognostic of significantly shortened overall survival times in univariate analysis ($p = 0.018$), but did not provide independent prognostic information. CONCLUSION: These data suggest down-regulation of XAF1 expression to be implicated in ccRCC progression and implies that its re-induction may provide a therapeutic approach. Although the prognostic value of XAF1 in ccRCC appears to be limited, its predictive value remains to be determined, especially in patients with metastatic disease undergoing novel combination therapies of targeted agents with Interferon-alpha.

Kiuru, M. and V. Launonen (2004). "Hereditary leiomyomatosis and renal cell cancer (HLRCC)." Curr Mol Med **4**(8): 869-75.

Hereditary leiomyomatosis and renal cell cancer (HLRCC) (MIM 605839) is a recently identified autosomal dominant tumor susceptibility syndrome characterized by predisposition to benign leiomyomas of the skin and the uterus (fibroids, myomas). Susceptibility to early-onset renal cell carcinoma and uterine leiomyosarcoma is present in a subset of families. Renal cell carcinomas are typically solitary and aggressive tumors displaying papillary type 2 or collecting duct histology. The disease predisposing gene was identified as fumarate hydratase (fumarase, FH) (MIM 136850). FH encodes an enzyme that operates in the mitochondrial Krebs cycle being thus involved in cellular energy metabolism. The recent discovery of HLRCC and the predisposing gene FH has increased the present knowledge of hereditary renal cancer and enabled identification of the predisposed individuals. This review provides the present knowledge of the clinical, histopathological, and molecular features of HLRCC. Future prospects related to studies on the phenotype and molecular biology of HLRCC will also be discussed.

Kiuru, M., V. Launonen, et al. (2001). "Familial cutaneous leiomyomatosis is a two-hit condition associated with renal cell cancer of characteristic histopathology." Am J Pathol **159**(3): 825-9.

Little has been known about the molecular background of familial multiple cutaneous leiomyomatosis (MCL). We report here a clinical, histopathological, and molecular study of a multiple cutaneous leiomyomatosis kindred with seven affected members. This detailed study revealed strong features of a recently described cancer predisposition syndrome, hereditary leiomyomatosis and renal cell

cancer (HLRCC). The family was compatible with linkage to the HLRCC locus in 1q. Also, all seven cutaneous leiomyomas derived from the proband and analyzed for loss of heterozygosity displayed loss of the wild-type allele, confirming the association with a susceptibility gene in chromosome 1q. One individual had had renal cell cancer at the age of 35 years. This tumor displayed a rare papillary histopathology, which appears to be characteristic for HLRCC. The derived linkage, loss of heterozygosity, and clinical data suggest that MCL and HLRCC are a single disease with a variable phenotype. The possibility that members of leiomyomatosis families are predisposed to renal cell cancer should be taken into account.

Kiuru, M., R. Lehtonen, et al. (2002). "Few FH mutations in sporadic counterparts of tumor types observed in hereditary leiomyomatosis and renal cell cancer families." Cancer Res **62**(16): 4554-7.

Loss of function mutations in the fumarate hydratase (fumarase, FH) gene were recently identified as the cause for dominantly inherited uterine and cutaneous leiomyomas and renal cell cancer. To further evaluate the role of FH in tumorigenesis, we screened FH mutations from tumor types seen in hereditary leiomyomatosis and renal cell cancer mutation carriers-41 uterine and 10 cutaneous leiomyomas, 52 renal cell carcinomas, 53 sarcomas, 29 prostate carcinomas, and 15 lobular breast carcinomas. Few mutations were detected. Biallelic inactivation of FH was found in one uterine leiomyosarcoma, one cutaneous leiomyoma, and one soft tissue sarcoma. Whereas the two former lesions were shown to originate from a germ-line mutation, the soft tissue sarcoma is to our knowledge the first example of purely somatic inactivation of FH in tumors.

Kluijt, I., D. de Jong, et al. (2009). "Early onset of renal cancer in a family with Birt-Hogg-Dube syndrome." Clin Genet **75**(6): 537-43.

Birt-Hogg-Dube syndrome is a hereditary syndrome characterized by benign disease of skin and lungs and a risk of malignant renal tumors. We describe a clinical and genetic study of a large Dutch family with a novel mutation in the FLCN gene. Renal cancer at very young age occurred in one branch of this family, while in other branches, cutaneous and pulmonary symptoms predominated. A variety of congenital anomalies and connective tissue abnormalities were observed, possibly associated with the gene mutation.

Kobayashi, M., T. Okada, et al. (2007). "Tissue-targeted in vivo gene transfer coupled with histone deacetylase inhibitor depsipeptide (FK228) enhances adenoviral infection in rat renal cancer allograft model systems." Urology **70**(6): 1230-6.

OBJECTIVES: Although the adenoviral vector represents an efficient delivery system, hepatotropic accumulation often has detrimental effects on adenoviral vector-mediated cancer therapy. To overcome this disadvantage, we performed in vivo local gene transfer, in combination with the histone deacetylase inhibitor, depsipeptide (FK228), in a rat renal cancer model. METHODS: Renal cancer cells induced by ferric nitrilotriacetate in ACI rats were used in this study. Adenoviral vectors containing luciferase cDNA were introduced into the tumor-burdened kidney by way of a catheter placed in the renal artery. Subcutaneous tumors were treated by herpes simplex virus thymidine kinase cDNA followed by intraperitoneal ganciclovir. The levels of Coxsackie-adenovirus receptor in various tissue were determined by quantitative reverse transcriptase-polymerase chain reaction. Depsipeptide (1 mg/kg) was intravenously administered 24 hours before adenoviral vector transduction. RESULTS: The catheter-based adenoviral vector delivery enabled strong gene transduction of the tumor-burdened kidney. Moreover, depsipeptide treatment before adenoviral vector injection significantly improved transgene expression at tumor sites. Quantitative reverse transcriptase-polymerase chain reaction analysis showed that depsipeptide increased the expression levels of the Coxsackie-adenovirus receptor in the renal tumor (13-fold), but not in other normal tissues. Furthermore, the use of herpes simplex virus thymidine kinase cDNA-expressing adenoviral vector followed by ganciclovir markedly inhibited the established tumor growth in combination with depsipeptide compared with herpes simplex virus thymidine kinase cDNA alone. CONCLUSIONS: The tissue-targeted in vivo gene transfer coupled with depsipeptide significantly enhanced adenoviral infection at tumor sites. Sensitization of tumor cells with depsipeptide can improve the efficacy of adenoviral vector-mediated suicide gene therapy. Thus, application of depsipeptide could be one of the beneficial adjunct for adenoviral vector-mediated cancer gene therapy.

Kondo, Y., J. Hamada, et al. (2005). "Over expression of hypoxia-inducible factor-1alpha in renal and bladder cancer cells increases tumorigenic potency." J Urol **173**(5): 1762-6.

PURPOSE: Hypoxia-inducible factor-1alpha (HIF-1alpha) is a transcriptional factor that regulates genes involved in the response to hypoxia. We evaluated the effects of HIF-1alpha over expression on the tumorigenic potency of renal cell carcinoma VMRC cells and bladder cancer EJ cells in vitro and

in vivo. MATERIALS AND METHODS: We introduced HIF-1alpha expression vectors into VMRC and EJ cells, and generated the HIF-1alpha over expressing cell lines VMRC-HIF1alpha and EJ-HIF1alpha, and the vector only transfected cell lines VMRC-neo and EJ-neo. We then evaluated in vitro cell proliferation and in vivo tumor growth of these cell lines after subcutaneous injection into athymic nude mice. RESULTS: In vitro studies showed that HIF-1alpha over expression in VMRC and EJ cells accelerated cell proliferation during the confluent growth phase and rendered these cells resistant to hypoxic stress. Furthermore, in vivo studies revealed that all 4 types of cancer cells (VMRC-neo, VMRC-HIF1alpha, EJ-neo and EJ-HIF1alpha) formed tumors in nude mice and the size of VMRC-HIF1alpha cell derived xenografts was much larger than that of VMRC-neo cell derived xenografts. Although HIF-1alpha over expression did not affect the size of EJ cell derived xenografts, histological examination showed that there was only a small area of necrosis in EJ-HIF1alpha cell derived xenografts, whereas a large area of central necrosis was observed in EJ-neo cell derived xenografts. It was also found that HIF-1alpha over expression increased intratumor microvessel density in the xenografts. CONCLUSIONS: These results suggest that HIF-1alpha may have important roles in bladder and renal cancer angiogenesis and proliferation.

Kopper, L. and J. Timar (2006). "Genomics of renal cell cancer-- does it provide breakthrough?" Pathol Oncol Res **12**(1): 5-11.

It is a strong hope that the more we characterize the pathways in an individual tumor, the better we will be able to evaluate the response to a specific therapy. Different array technologies could be powerful tools to achieve this goal, i.e. selecting patients on the basis of the genomic and/or proteomic profiles who would really benefit from the target-designed therapy. Genomic analysis of RCC accumulated ample of data which now can be exploited in clinical management of a previously almost uncontrollable disease. Beside the previously identified genetic abnormalities (VHL, MET, EGFR), CAIX seems to be a novel molecular marker of RCC. Array studies also outlined a small set of tumor markers, vimentin, galectin-3, CD74 and parvalbumin, which can define the individual histologic subtypes of RCC. We are at the beginning to take advantage of the genomic results. Some new approaches will interfere with the progression of RCC (anti-VEGF, anti-VEGFR or anti-EGFR therapies). Further novel molecular targets are available, such as HIF, HSP90 or the IFN-regulated genes, which can be used to the fine-tuning of RCC therapy.

Koski, T. A., H. J. Lehtonen, et al. (2009). "Array comparative genomic hybridization identifies a distinct DNA copy number profile in renal cell cancer associated with hereditary leiomyomatosis and renal cell cancer." Genes Chromosomes Cancer **48**(7): 544-51.

Hereditary leiomyomatosis and renal cell cancer (HLRCC) is a tumor predisposition syndrome with cutaneous and uterine leiomyomatosis as well as renal cell cancer (RCC) as its clinical manifestations. HLRCC is caused by heterozygous germline mutations in the fumarate hydratase (fumarase) gene. In this study, we used array comparative genomic hybridization to identify the specific copy number changes characterizing the HLRCC-associated RCCs. The study material comprised formalin-fixed paraffin-embedded renal tumors obtained from Finnish patients with HLRCC. All 11 investigated tumors displayed the papillary type 2 histopathology typical for HLRCC renal tumors. The most frequent copy number changes detected in at least 3/11 (27%) of the tumors were gains in chromosomes 2, 7, and 17, and losses in 13q12.3-q21.1, 14, 18, and X. These findings provide genetic evidence for a distinct copy number profile in HLRCC renal tumors compared with sporadic RCC tumors of the same histopathological subtype, and delineate chromosomal regions that associate with this very aggressive form of RCC.

Kuefer, R., M. Autenrieth, et al. (2006). "[Translational research in renal cell cancer. Illustrated by the example of the vascular endothelial growth factor pathway]." Urologe A **45**(3): 328, 330-5.

For patients with metastatic renal cell cancer (RCC), therapeutic options after cytokine failure are rather limited. There is a considerable need to identify new substances for systemic therapy. Due to upregulation after the loss of a functional von Hippel Lindau gene product, the vascular endothelial growth factor (VEGF) pathway is a promising target for a molecular based therapy. Over the last few years, therapeutic agents have been developed which inhibit this pathway at various levels. Here, we provide an overview of the molecular background and currently used drugs which have entered clinical trials in the setting of metastatic RCC disease. Until now, the results from early clinical trials are very promising, however, the best schedule, dosage, potential combination regimens, as well as long time efficacy, are still to be determined.

Kuiper, R. P., L. Vreede, et al. (2009). "The tumor suppressor gene FBXW7 is disrupted by a

constitutional t(3;4)(q21;q31) in a patient with renal cell cancer." Cancer Genet Cytogenet **195**(2): 105-11.

FBXW7 (alias CDC4) is a p53-dependent tumor suppressor gene that exhibits mutations or deletions in a variety of human tumors. Mutation or deletion of the FBXW7 gene has been associated with an increase in chromosomal instability and cell cycle progression. In addition, the FBXW7 protein has been found to act as a component of the ubiquitin proteasome system and to degrade several oncogenic proteins that function in cellular growth regulatory pathways. By using a rapid breakpoint cloning procedure in a case of renal cell cancer (RCC), we found that the FBXW7 gene was disrupted by a constitutional t(3;4)(q21;q31). Subsequent analysis of the tumor tissue revealed the presence of several anomalies, including loss of the derivative chromosome 3. Upon screening of a cohort of 29 independent primary RCCs, we identified one novel pathogenic mutation, suggesting that the FBXW7 gene may also play a role in the development of sporadic RCCs. In addition, we screened a cohort of 48 unrelated familial RCC cases with unknown etiology. Except for several known or benign sequence variants such as single nucleotide polymorphisms (SNPs), no additional pathogenic variants were found. Previous mouse models have suggested that the FBXW7 gene may play a role in the predisposition to tumor development. Here we report that disruption of this gene may predispose to the development of human RCC.

Laing, M. E., E. Kay, et al. (2007). "Genetic factors associated with skin cancer in renal transplant patients." Photodermatol Photoimmunol Photomed **23**(2-3): 62-7.

BACKGROUND: Non-melanoma skin cancer represents a significant cause of morbidity and mortality among renal transplant recipients. Established risk factors that increase susceptibility to skin cancer after transplantation include skin type, sun exposure and level of immunosuppression. METHODS: A comprehensive literature review was carried out to discuss relevant genetic polymorphism for the development of skin cancer in organ transplant recipients. These include genetic polymorphisms in glutathione S-transferase, interleukin-10, retinoblastoma and p53 genes. We also discuss genetic polymorphisms in the folate pathway, melanocortin 1 receptor and vitamin D receptor recently discovered in our group. RESULTS: No single factor is causative in cutaneous carcinogenesis in transplant recipients. Interactions of some of the above mechanisms with known environmental factors lead to increased risk. CONCLUSION: Polymorphisms in methylenetetrahydrofolate reductase are potentially correctable with folic acid supplementation; however, further evaluation is required in adequately powered prospective clinical trials. Avoidance of known oncogenic environmental factors and genetic risk evaluation may improve outcomes in transplant patients.

Lamers, C. H., J. W. Gratama, et al. (2005). "Parallel detection of transduced T lymphocytes after immunogene therapy of renal cell cancer by flow cytometry and real-time polymerase chain reaction: implications for loss of transgene expression." Hum Gene Ther **16**(12): 1452-62.

We have started a phase I/II immunogene therapy study of metastatic renal cell cancer (RCC), using autologous T lymphocytes transduced ex vivo with a gene encoding a single-chain receptor based on the monoclonal antibody (mAb) G250 [scFv(G250)]. G250 recognizes carbonic anhydrase IX, which is overexpressed by RCC cells. We have developed and validated flow cytometric and real-time polymerase chain reaction (PCR) assays to quantitatively detect transduced T cells in patient blood. The flow assay was based on staining with the anti-G250 idiotype mAb NuH82 and showed a sensitivity of 0.06% scFv(G250)(1) cells within CD3(1) T cells. The real-time PCR method showed a sensitivity of 14 copies of scFv(G250) DNA per 100 ng of total DNA, which enabled detection of 0.008% scFv(G250)(1) T cells within leukocytes. Both assays were further validated for their specificity and reproducibility. When applied to blood samples from three RCC patients treated with intravenous infusions of scFv(G250)(1) T cells, the kinetics of scFv(G250)(1) T cell counts as detected by flow cytometry were similar to those detected by real-time PCR, although PCR allowed detection of transduced T cells over a longer period of time (i.e., for patient 3, 7 versus 32 days, respectively). Interestingly, follow-up studies of patient 3 demonstrated that the number of circulating scFv(G250)(1) T cells remained fairly constant during the first 7 days posttreatment, whereas the number of gene copies increased during the same period of time. These results suggest loss of scFv(G250) membrane expression on adoptive transfer, which would have important implications for the antitumor efficacy of this form of immunogene therapy.

Lamers, C. H., S. C. Langeveld, et al. (2007). "Gene-modified T cells for adoptive immunotherapy of renal cell cancer maintain transgene-specific immune functions in vivo." Cancer Immunol Immunother **56**(12): 1875-83.

BACKGROUND: We have treated three patients with carboxy-anhydrase-IX (CAIX) positive metastatic renal cell cancer (RCC) by adoptive

transfer of autologous T-cells that had been gene-transduced to express a single-chain antibody-G250 chimeric receptor [scFv(G250)], and encountered liver toxicity necessitating adaptation of the treatment protocol. Here, we investigate whether or not the in vivo activity of the infused scFv(G250)(+) T cells is reflected by changes of selected immune parameters measured in peripheral blood. METHODS: ScFv(G250)-chimeric receptor-mediated functions of peripheral blood mononuclear cells (PBMC) obtained from three patients during and after treatment were compared to the same functions of scFv(G250)(+) T lymphocytes prior to infusion, and were correlated with plasma cytokine levels. RESULTS: Prior to infusion, scFv(G250)(+) T lymphocytes showed in vitro high levels of scFv(G250)-chimeric receptor-mediated functions such as killing of CAIX(+) RCC cell lines and cytokine production upon exposure to these cells. High levels of IFN-gamma were produced, whilst production of TNF-alpha, interleukin-4 (IL-4), IL-5 and IL-10 was variable and to lower levels, and that of IL-2 virtually absent. PBMC taken from patients during therapy showed lower levels of in vitro scFv(G250)-receptor-mediated functions as compared to pre-infusion, whilst IFN-gamma was the only detectable cytokine upon in vitro PBMC exposure to CAIX. During treatment, plasma levels of IFN-gamma increased only in the patient with the most prominent liver toxicity. IL-5 plasma levels increased transiently during treatment in all patients, which may have been triggered by the co-administration of IL-2. CONCLUSION: ScFv(G250)-receptor-mediated functions of the scFv(G250)(+) T lymphocytes are, by and large, preserved in vivo upon administration, and may be reflected by fluctuations in plasma IFN-gamma levels.

Langbein, S., W. M. Frederiks, et al. (2008). "Metastasis is promoted by a bioenergetic switch: new targets for progressive renal cell cancer." Int J Cancer **122**(11): 2422-8.

Targeted therapies have demonstrated clinical benefit with limited impact on long-term disease specific survival in the treatment of renal cell cancer (RCC). New opportunities for the treatment of tumors that are resistant or have relapsed, are needed. Increased anaerobic glucose fermentation to lactate (aerobic glycolysis), leading to oxygen- and mitochondria-independent ATP generation is a hallmark of aggressive cancer growth. This metabolic shift results in increased lactate production via cycling through the pentose phosphate pathway (PPP), and plays an important role in tumor immune escape, progression and resistance to immune-, radiation- and chemo-therapy. This study explored the activity and impact of the oxidative and nonoxidative branches of the PPP on RCC to evaluate new therapeutic options. Activity was determined in the oxidative branch by glucose-6-phosphate-dehydrogenase (G6PD) activity, and in the nonoxidative branch by the total transketolase activity and the specific expression of the transketolase-like-1 (TKTL1) protein. Transketolase and G6PD activity were intensely elevated in tumor tissues. Transketolase, but not G6PD activity, was more elevated in metastasizing tumors and TKTL1 protein was significantly overexpressed in progressing tumors ($p = 0.03$). Lethal tumors, where surrogate parameters such as grading and staging had failed to predict progression, showed intensive TKTL1 protein expression. RCC was found to have activated oxidative and nonoxidative glucose metabolism through the PPP, displaying a bioenergetic shift toward nonoxidative glucose fermentation in progressing tumors. The coexistence of cancer cells with differentially regulated energy supplies provides new insights in carcinogenesis and novel anticancer targets.

Lau, K. W., Y. M. Tian, et al. (2007). "Target gene selectivity of hypoxia-inducible factor-alpha in renal cancer cells is conveyed by post-DNA-binding mechanisms." Br J Cancer **96**(8): 1284-92.

Inactivation of the von Hippel-Lindau tumour suppressor in renal cell carcinoma (RCC) leads to failure of proteolytic regulation of the alpha subunits of hypoxia-inducible factor (HIF), constitutive upregulation of the HIF complex, and overexpression of HIF target genes. However, recent studies have indicated that in this setting, upregulation of the closely related HIF-alpha isoforms, HIF-1alpha and HIF-2alpha, have contrasting effects on tumour growth, and activate distinct sets of target genes. To pursue these findings, we sought to elucidate the mechanisms underlying target gene selectivity for HIF-1alpha and HIF-2alpha. Using chromatin immunoprecipitation to probe binding to hypoxia response elements in vivo, and expression of chimaeric molecules bearing reciprocal domain exchanges between HIF-1alpha and HIF-2alpha molecules, we show that selective activation of HIF-alpha target gene expression is not dependent on selective DNA-binding at the target locus, but depends on non-equivalent C-terminal portions of these molecules. Our data indicate that post-DNA binding mechanisms that are dissimilar for HIF-1alpha and HIF-2alpha determine target gene selectivity in RCC cells.

Launonen, V., O. Vierimaa, et al. (2001). "Inherited susceptibility to uterine leiomyomas and renal cell cancer." Proc Natl Acad Sci U S A **98**(6): 3387-92.

Herein we report the clinical, histopathological, and molecular features of a cancer syndrome with predisposition to uterine leiomyomas and papillary renal cell carcinoma. The studied kindred included 11 family members with uterine leiomyomas and two with uterine leiomyosarcoma. Seven individuals had a history of cutaneous nodules, two of which were confirmed to be cutaneous leiomyomatosis. The four kidney cancer cases occurred in young (33- to 48-year-old) females and displayed a unique natural history. All these kidney cancers displayed a distinct papillary histology and presented as unilateral solitary lesions that had metastasized at the time of diagnosis. Genetic-marker analysis mapped the predisposition gene to chromosome 1q. Losses of the normal chromosome 1q were observed in tumors that had occurred in the kindred, including a uterine leiomyoma. Moreover, the observed histological features were used as a tool to diagnose a second kindred displaying the phenotype. We have shown that predisposition to uterine leiomyomas and papillary renal cell cancer can be inherited dominantly through the hereditary leiomyomatosis and renal cell cancer (HLRCC) gene. The HLRCC gene maps to chromosome 1q and is likely to be a tumor suppressor. Clinical, histopathological, and molecular tools are now available for accurate detection and diagnosis of this cancer syndrome.

Leach, F. S. (2004). "Linking human genetics with molecular medicine: will hereditary renal cancer play a major role?" Cancer Biol Ther **3**(5): 441-6.

An inherited or familial predisposition to form kidney tumors represents less than 4% of all renal malignancies. However, hereditary renal cancer (HRC) syndromes offer important opportunities for gene discovery and function. Basic and clinical HRC investigation often provides unique insight into regulation of cell growth, cell proliferation, tumor invasion and metastasis. The genetics, biochemistry and physiology of renal tumorigenesis has been directly impacted and significantly expanded by HRC research over the last ten years. Mutations have been identified in several genes tightly linked to increased risk for development of renal cancer. Inheritance of these mutated genes causes specific hereditary syndromes often associated with clinically significant nonrenal manifestations. Molecular and biochemical alterations of most HRC gene products are also detected in sporadic renal cancer emphasizing the importance of HRC gene function in nonhereditary carcinogenesis. Despite these important molecular findings, the clinical contribution of HRC research has generally been limited to genetic screening and prognostic assessment. HRC patients and their physicians continue to face difficult decisions regarding cancer control and quality of life despite advances in minimally invasive surgical and radiological techniques. The ultimate challenge for clinicians and scientists will be translation of molecular and genetic research into clinical tools that impact diagnosis, treatment and prevention. This bench to bedside report describes the diagnosis, genetics, pathophysiology and current cancer treatment options available for HRC syndromes.

Lee, J. K., N. Seki, et al. (2005). "Constitutive expression of functional CD40 on mouse renal cancer cells: induction of Fas and Fas-mediated killing by CD40L." Cell Immunol **235**(2): 145-52.

CD40, a member of the TNF receptor superfamily, is expressed on B cells, dendritic cells, and some tumor cells, including melanoma and bladder carcinoma. In this study, we report that both mouse and human renal carcinoma cells (RCC) also constitutively express functional CD40. Treatment of mouse RCC with CD40L induced strong expression of genes and proteins for ICAM-1 and Fas, and this expression was further enhanced by combining CD40L with IFN-gamma. Similar effects were demonstrated using an agonist anti-CD40 antibody. The increased levels of Fas expression on RCC after treatment with CD40L plus IFN-gamma resulted in potent killing by either FasL-positive effector cells or agonistic anti-Fas antibody. The combination of CD40L plus IFN-gamma also significantly enhanced killing of RCC by tumor-specific CTL lines. Our results demonstrate that constitutively expressed CD40 is functionally active and may provide a molecular target for the development of new approaches to the treatment of RCC.

Lee, T. J., J. T. Lee, et al. (2008). "Overexpression of Par-4 enhances thapsigargin-induced apoptosis via down-regulation of XIAP and inactivation of Akt in human renal cancer cells." J Cell Biochem **103**(2): 358-68.

The prostate-apoptosis-response-gene-4 (Par-4) protein has been shown to function as an effector of cell death in response to various apoptotic stimuli that trigger mitochondria and membrane receptor-mediated cell death pathways. We found that overexpressing Par-4 by stable transfection sensitizes Caki cells to induction of apoptosis by TRAIL and drugs that induce endoplasmic reticulum (ER) stress [thapsigargin (TG), tunicamycin (TU) and etoposide]. Ectopic expression of Par-4 is associated with decreased levels of XIAP protein in TG-treated cells, caused in part by XIAP protein instability and caspase activation. Levels of phospho-Akt are decreased in Caki/Par-4 cells to a significantly greater extent than

in Caki/Vector cells by treatment with TG, and this is in turn associated with decreased levels of phospho-PDK1, the kinase upstream of Akt. In conclusion, we provide evidence that ectopic expression of Par-4 sensitizes Caki cells to TG and that XIAP protein instability and inactivation of Akt are important in cellular pathways affected by Par-4.

Lehtonen, H. J., I. Blanco, et al. (2007). "Conventional renal cancer in a patient with fumarate hydratase mutation." Hum Pathol **38**(5): 793-6.

Hereditary leiomyomatosis and renal cell cancer (HLRCC) is a tumor predisposition syndrome caused by mutations in the fumarate hydratase (FH) gene. HLRCC is characterized by uterine and cutaneous leiomyomas, renal cell cancer, and uterine leiomyosarcoma. Typically, renal cell cancers in HLRCC are unilateral and display a papillary type 2 or ductal histology. We describe here a 23-year-old patient carrying a novel FH mutation (N330S) with a bilateral renal cell center. Carcinoma of the right kidney showed papillary structure, but the left tumor was diagnosed as a conventional (clear cell) renal carcinoma, a type not previously described in HLRCC. The clear cell renal carcinoma also displayed loss of the normal FH allele and the FH immunostaining. Our finding extends the number of cases in which HLRCC can be suspected, and the FH immunohistochemistry may serve as a useful tool to screen for HLRCC in young individuals with clear cell renal carcinoma.

Li, G., K. Passebosc-Faure, et al. (2001). "The expression of G250/mn/CA9 antigen by flow cytometry: its possible implication for detection of micrometastatic renal cancer cells." Clin Cancer Res **7**(1): 89-92.

Monoclonal antibody (mAb) G250 is a well characterized and specific mAb to renal cell carcinoma (RCC). The gene G250 was recently cloned and was proved to be homologous to MN/CA9. The G250/MN/CA9 antigen was recently explored as a potential marker for RCC. Flow cytometry (FCM) allows quantitative analysis of cells. The present study describes a flow cytometric method to detect this antigen in human cell lines and in malignant and normal renal tissues. Twelve human carcinoma cell lines (HeLa, Colo205, HT29, BxPC3, OVCAR3, SKOV3, ACHN, A704, CAKI-2, SKRC-59, SKRC-10, and SKRC-52), 10 specimens of normal peripheral blood mononuclear cells, and 38 malignant and 36 adjacent normal renal tissues were studied. The malignant and normal renal tissues were disaggregated mechanically into a single-cell suspension, stained by mAb G250, and analyzed by FCM. All 22 of the clear cell carcinomas, 6 of 8 mixed cell carcinomas, and 3 of 6 granular cell carcinomas were positive for G250/MN/CA9 antigen. SKRC-52 and SKRC-10 were strongly positive for G250/ MN/CA9. The G250/MN/CA9 antigen could also be detected in HeLa, SKOV3, HT29, and A704 cells. One chromophobic, one chromophilic cell carcinoma, the normal renal tissues, and normal peripheral blood mononuclear cells were considered as negative. Our results further confirmed that the G250/MN/CA9 antigen was an ideal marker for RCC, especially for clear cell carcinomas, and that this antigen was present in several types of malignant cells. FCM may serve as a fast tool of immunocytochemical detection of renal cancer cells. Flow cytometric detection of renal cancer cells by using mAb G250 should be further explored.

Lionello, I., P. Mangia, et al. (2007). "CD8(+) T lymphocytes isolated from renal cancer patients recognize tumour cells through an HLA- and TCR/CD3-independent pathway." Cancer Immunol Immunother **56**(7): 1065-76.

PURPOSE: The aim of this study was to characterize the immune response of patients affected by renal cell carcinoma (RCC). METHODS: Long-term RCC lines were established by retroviral-mediated transfer of the large T-antigen of SV40 into fresh carcinoma cells. Reactive T cell effectors were generated by iterative stimulations of patients' PBMC with autologous tumour cells. RESULTS: This protocol led to the induction of CD8(+) T cell clones reactive against the autologous tumour, but not against NK-sensitive cell lines. However, some of these effectors recognize normal renal cells, allogeneic renal carcinoma cell lines and colon and non-small cell lung carcinomas but not melanomas and lymphoblastoid lines, without evidence of shared classical HLA class I (HLA-I) molecules. Further characterization performed on the CD8(+) TCR alpha/beta(+) clone, CTL30, demonstrated that neither expression of CD1, HLA-Ia nor HLA-Ib, correlated with the T cells' recognition. Moreover, beta2m expression by target cells was not required to achieve interaction of tumour-effector cells. In agreement with this observation, the lytic activity of CTL30 was not inhibited by anti-HLA-I Ab, and antigen expression was not affected by inhibitors of antigen processing. Lytic activity of CTL30, while partially inhibited by anti-NKG2D, could not be abolished by anti-CD3 Abs. Moreover, growth and expansion of CTL30 was sustained only by T cell interaction with antigen-expressing tumour cells; unspecific mitogenic stimuli, such as anti-CD3 and PHA, did not allow T cell expansion. These results demonstrated the existence of an alpha/beta T cell population, recognizing

epithelial tumour cells through an HLA-unrestricted, CD3-independent mechanism.

Lipworth, L., R. E. Tarone, et al. (2009). "Epidemiologic characteristics and risk factors for renal cell cancer." Clin Epidemiol **1**: 33-43.

Incidence rates of renal cell cancer, which accounts for 85% of kidney cancers, have been rising in the United States and in most European countries for several decades. Family history is associated with a two- to four-fold increase in risk, but the major forms of inherited predisposition together account for less than 4% of renal cell cancers. Cigarette smoking, obesity, and hypertension are the most consistently established risk factors. Analgesics have not been convincingly linked with renal cell cancer risk. A reduced risk of renal cell cancer among statin users has been hypothesized but has not been adequately studied. A possible protective effect of fruit and vegetable consumption is the only moderately consistently reported dietary finding, and, with the exception of a positive association with parity, evidence for a role of hormonal or reproductive factors in the etiology of renal cell cancer in humans is limited. A recent hypothesis that moderate levels of alcohol consumption may be protective for renal cell cancer is not strongly supported by epidemiologic results, which are inconsistent with respect to the categories of alcohol consumption and the amount of alcohol intake reportedly associated with decreased risk. For occupational factors, the weight of the evidence does not provide consistent support for the hypotheses that renal cell cancer may be caused by asbestos, gasoline, or trichloroethylene exposure. The established determinants of renal cell cancer, cigarette smoking, obesity, and hypertension, account for less than half of these cancers. Novel epidemiologic approaches, including evaluation of gene-environment interactions and epigenetic mechanisms of inherited and acquired increased risk, are needed to explain the increasing incidence of renal cell cancer.

Liu, Y. H., C. Y. Lin, et al. (2008). "Up-regulation of vascular endothelial growth factor-D expression in clear cell renal cell carcinoma by CD74: a critical role in cancer cell tumorigenesis." J Immunol **181**(9): 6584-94.

Elevation of CD74 is associated with a number of human cancers, including clear cell renal cell carcinoma (ccRCC). To understand the role of CD74 in the oncogenic process of ccRCC, we ectopically expressed CD74 in human embryonic kidney 293 cells (HEK/CD74) and evaluated its oncogenic potential. Through overexpression of CD74 in HEK293 and Caki-2 cells and down-regulation of CD74 in Caki-1 cells, we show that vascular endothelial growth factor-D (VEGF-D) expression is modified accordingly. A significant, positive correlation between CD74 and VEGF-D is found in human ccRCC tissues (Pearson's correlation, $r = 0.65$, $p < 0.001$). In HEK/CD74 xenograft mice, CD74 significantly induced the formation of tumor masses, increased tumor-induced angiogenesis, and promoted cancer cell metastasis. Blockage of VEGF-D expression by small interference RNA resulted in a decrease in cell proliferation, invasion, and cancer cell-induced HUVEC migration enhanced by CD74. Furthermore, we provide evidence that the intracellular signaling cascade responsible for VEGF-D up-regulation by CD74 is both PI3K/AKT- and MEK/ERK-dependent, both of which are associated with NF-kappaB nuclear translocation and DNA-binding activity. These results suggest that VEGF-D is crucial for CD74-induced human renal carcinoma cancer cell tumorigenesis.

Los, M., O. A. Kerckhaert, et al. (2000). "Mutational analysis of endothelial cells derived from von Hippel-Lindau-related renal cancer." J Natl Cancer Inst **92**(20): 1688-9.

Luan, F. L., R. Ding, et al. (2003). "Rapamycin is an effective inhibitor of human renal cancer metastasis." Kidney Int **63**(3): 917-26.

Rapamycin is an effective inhibitor of human renal cancer metastasis. BACKGROUND: Human renal cell cancer (RCC) is common and is 10 to 100 times more frequent in patients with end-stage renal disease (ESRD) and candidates for renal transplantation. Treatment of metastatic RCC is largely ineffective and is further undermined by immunosuppressive therapy in transplant recipients. A treatment regimen that prevents transplant rejection while constraining RCC progression would be of high value. METHODS: We developed a human RCC pulmonary metastasis model using human RCC 786-O as the tumor challenge and the severe combined immunodeficient (SCID) beige mouse as the host. We explored the effect of rapamycin, cyclosporine, or rapamycin plus cyclosporine on the development of pulmonary metastases and survival. The effects of the drugs on tumor cell growth, apoptosis, and expression of vascular endothelial growth factor (VEGF-A) and transforming growth factor beta1 (TGF-beta1) were also investigated. RESULTS: Rapamycin reduced, whereas cyclosporine increased, the number of pulmonary metastases. Rapamycin was effective in cyclosporine-treated mice, and rapamycin or rapamycin plus cyclosporine prolonged survival. Rapamycin growth arrested RCC 786-O at the G1 phase and reduced VEGF-A expression. Immunostaining of lung tissues for von Willebrand

factor was minimal and circulating levels of VEGF-A and TGF-beta1 were lower in the rapamycin-treated mice compared to untreated or cyclosporine-treated mice. CONCLUSION: Our findings support the idea that rapamycin may be of value for patients with RCC and that its antitumor efficacy is realized by cell cycle arrest and targeted reduction of VEGF-A and TGF-beta1. A regimen of rapamycin and cyclosporine, demonstrated to be effective in reducing acute rejection of renal allografts, may prevent RCC progression as well, and has the potential to prevent mortality due to RCC in patients with ESRD who have received renal allografts.

Macher-Goeppinger, S., S. Aulmann, et al. (2009). "Prognostic value of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and TRAIL receptors in renal cell cancer." Clin Cancer Res **15**(2): 650-9.

PURPOSE: The death ligand tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and its receptors (TRAIL-R) are involved in immune surveillance and tumor development. Here, we studied a possible association between the expression of TRAIL/TRAIL-Rs and the prognosis in patients with renal cell carcinomas (RCC). EXPERIMENTAL DESIGN: A tissue microarray containing RCC tumor tissue samples and corresponding normal tissue samples from 838 patients was generated. Expression of TRAIL and TRAIL-Rs was examined by immunohistochemistry and the effect of TRAIL and TRAIL-R expression on disease-specific survival was assessed. RESULTS: High TRAIL-R2 expression levels were associated with high-grade RCCs ($P < 0.001$) and correlated negatively with disease-specific survival ($P = 0.01$). Similarly, high TRAIL expression was associated with a shorter disease-specific survival ($P = 0.01$). In contrast, low TRAIL-R4 expression was associated with high-stage RCCs ($P < 0.001$) as well as with the incidence of distant metastasis ($P = 0.03$) and correlated negatively with disease-specific survival ($P = 0.02$). In patients without distant metastasis, multivariate Cox regression analyses revealed that TRAIL-R2 and TRAIL are independent prognostic factors for cancer-specific survival (in addition to tumor extent, regional lymph node metastasis, grade of malignancy, and type of surgery). CONCLUSION: High TRAIL-R2, high TRAIL, and low TRAIL-R4 expression levels are associated with a worse disease-specific survival in patients with RCCs. Therefore, the assessment of TRAIL/TRAIL-R expression offers valuable prognostic information that could be used to select patients for adjuvant therapy studies. Moreover, our findings are of relevance for a potential experimental therapeutic administration of TRAIL-R agonists in patients with RCCs.

Madej, A., M. Puzianowska-Kuznicka, et al. (2003). "Vitamin D receptor binding to DNA is altered without the change in its expression in human renal clear cell cancer." Nephron Exp Nephrol **93**(4): e150-7.

Vitamin D co-regulates cell proliferation, differentiation and apoptosis, the processes that are disturbed in cancer tissues. It acts through the vitamin D nuclear receptor (VDR) that binds to DNA in the regulatory sequences of the target genes. As the kidney is one of the key organs for vitamin D metabolism and action, we analyzed VDR expression and its DNA binding activity in human renal clear cell cancer. 24 tumors, 24 controls that were excised from the opposite pole of the same kidney and 7 controls originating from kidneys without cancer were examined. Independently of tumor grading neither Northern blots nor immunoblotting demonstrated statistically significant differences of the mean VDR mRNA and protein amounts, respectively, in the cancer as compared to both control types. In contrast, the amount of VDR-DNA complexes was lower in 52.2% of the tumors in comparison to their corresponding controls. After normalization against VDR receptor protein amount in 34.8% of the tumors VDR-DNA binding was at least 3-4 times weaker than in the controls. However, the expression of vitamin D-dependent P21 gene on the mRNA level was not decreased in these cancers. It remains to be elucidated if altered VDR function due to its impaired binding to DNA contributes to the process of tumorigenesis, and what potential vitamin D-dependent mechanisms are involved in this process.

Maestro, M. L., V. del Barco, et al. (2000). "Loss of heterozygosity on the short arm of chromosome 3 in renal cancer." Oncology **59**(2): 126-30.

3% of human cancers are renal cell carcinomas (RCC). The most common chromosome abnormality found in this tumor is loss of heterozygosity (LOH) on the short arm of chromosome 3, which suggests that there must be one or more tumor suppressor genes between 3p14 and 3p21 near the VHL gene which play a relevant role in renal cancer development. DNA from normal and tumor tissue from 40 patients at various stages of RCC was analyzed for LOH at three microsatellites mapped to 3p (3p14.1-14.3; 3p21.2-21.3 and 3p25) by polymerase chain reaction). 42.5% of the tumors studied showed LOH on at least one locus. 30% showed LOH on only one locus; 5% on two loci and 7.5% on the three loci tested. LOH occurred only on nonpapillary tumors ($p = 0.03$). Interestingly, all the tumors with LOH on 3p21 were >/=25 mm ($p = 0.04$; relative risk 1.76, confidence interval: 1.3-2.3).

Mahajan, S., V. Dammai, et al. (2008). "Hypoxia-inducible factor-2alpha regulates the expression of TRAIL receptor DR5 in renal cancer cells." Carcinogenesis **29**(9): 1734-41.

To understand the role of hypoxia-inducible factor (HIF)-2alpha in regulating sensitivity of renal cancer cells to tumor necrosis factor-related apoptosis inducing ligand (TRAIL)-induced apoptosis, we transfected wild-type and mutant von Hippel Lindau (VHL) proteins into TRAIL-sensitive, VHL-negative A498 cells. We find that wild-type VHL, but not the VHL mutants S65W and C162F that do not degrade HIF proteins, cause TRAIL resistance. Knock down of the HIF-2alpha protein by RNA interference (short hairpin RNA) blocked TRAIL-induced apoptosis, decreased the level of TRAIL receptor (DR5) protein and inhibited the transcription of DR5 messenger RNA. By using luciferase constructs containing the upstream region of the DR5 promoter, we demonstrate that HIF-2alpha stimulates the transcription of the DR5 gene by activating the upstream region between -448 and -1188. Because HIF-2alpha is thought to exert its effect on gene transcription by interacting with the Max protein partner of Myc in the Myc/Max dimer, small interfering RNAs to Myc were used to lower the levels of this protein. In multiple renal cancer cell lines decreasing the levels of Myc blocked the ability of HIF-2alpha to stimulate DR5 transcription. PS-341 (VELCADE, bortezomib), a proteasome inhibitor used to treat human cancer, increases the levels of both HIF-2alpha and c-Myc and elevates the level of DR5 in renal cancer, sensitizing renal cancer cells to TRAIL therapy. Similarly, increasing HIF-2alpha in prostate and lung cancer cell lines increased the levels of DR5. Thus, in renal cancer cell lines expressing HIF-2alpha, this protein plays a role in regulating the levels of the TRAIL receptor DR5.

Majid, S., A. A. Dar, et al. (2009). "BTG3 tumor suppressor gene promoter demethylation, histone modification and cell cycle arrest by genistein in renal cancer." Carcinogenesis **30**(4): 662-70.

BTG3/ANA/APRO4 has been reported to be a tumor suppressor gene in some malignancies. It constitutes important negative regulatory mechanism for Src-mediated signaling, a negative regulator of the cell cycle and inhibits transcription factor E2F1. We report that BTG3 is downregulated in renal cancer and that the mechanism of inactivation is through promoter hypermethylation. Quantitative real-time polymerase chain reaction (PCR) showed that BTG3 was downregulated in cancer tissues and cells. Genistein and 5-aza-2'-deoxycytidine (5Aza-C) induced BTG3 messenger RNA (mRNA) expression in A498, ACHN and HEK-293 renal cell carcinoma (RCC) cell lines. Bisulfite-modified PCR and DNA sequencing results showed complete methylation of BTG3 promoter in tumor samples and cancer cell lines. Genistein and 5Aza-C treatment significantly decreased promoter methylation, reactivating BTG3 expression. Chromatin immunoprecipitation assay revealed that genistein and 5Aza-C increased levels of acetylated histones 3, 4, 2H3K4, 3H3K4 and RNA polymerase II at the BTG3 promoter indicative of active histone modifications. Enzymatic assays showed genistein and 5Aza-C decreased DNA Methyltransferase, methyl-CpG-binding domain 2 activity and increased HAT activity. Cell cycle and 3-(4,5-dimethylthiazole-2-yl)-2,5-biphenyl tetrazolium bromide cell proliferation assays showed that genistein has antiproliferative effect on cancer cell growth through induction of cell cycle arrest. This is the first report to show that BTG3 is epigenetically silenced in RCC and can be reactivated by genistein-induced promoter demethylation and active histone modification. Genistein had similar effects to that of 5Aza-C, which is a potent demethylating agent with high toxicity and instability. Genistein being a natural, non-toxic, dietary isoflavone is effective in retarding the growth of RCC cells, making it a promising candidate for epigenetic therapy in renal carcinoma.

Mancini, V., M. Battaglia, et al. (2008). "Current insights in renal cell cancer pathology." Urol Oncol **26**(3): 225-38.

In recent years molecular biologists and pathologists have described new entities of renal cell cancer (RCC) with a totally different morphology and biology among the histotypes of renal carcinoma, but always referring to the same renal cancer disease. The evidence of a distinct biological behavior and long-term prognosis among these makes the correct pathological diagnosis of renal cancer critically important for the clinician. Advances in understanding of the pathogenesis, behavior, and importance of prognostic factors for RCC have paved the way for a revision of its classification and staging. We reviewed the role of histological classification, microscopic tumor necrosis, microscopic venous invasion, lymph node involvement and, particularly, pathological stage. In our series of patients who underwent renal surgery for neoplasm, a retrospective study established the predictive role of tumor size on recurrence rate, compared with other known prognostic factors, and we conclude that histological grade, pathological stage and tumor size remain relevant prognosticators in early stage RCC patients. In order to optimize the management of patients with RCC it is necessary to develop an interdisciplinary approach (surgeon, radiologist, pathologist,

oncologist) and find new prognostic parameters at molecular and cellular levels. Many efforts are ongoing to integrate molecular data (from tissue microarrays) and clinical data (traditional prognosticators) into a molecular integrated staging system. In the postgenomic era, new tumor-associated antigens and molecules can be identified at the protein level using proteomics, providing a major opportunity for screening and finding novel targets that are the basis of new emerging therapies for RCC.

Margolin, K., T. W. Synold, et al. (2007). "Oblimersen and alpha-interferon in metastatic renal cancer: a phase II study of the California Cancer Consortium." J Cancer Res Clin Oncol **133**(10): 705-11.

PURPOSE: Oblimersen is an 18-base oligodeoxynucleotide encoding antisense to the gene for bcl-2, an anti-apoptotic protein that is upregulated in renal and other cancers. This study was designed to evaluate the combination of oblimersen with alpha-Interferon in advanced renal cancer. Trial endpoints were antitumor efficacy and toxicity, pharmacokinetics, and evidence of apoptosis in peripheral blood mononuclear cells. METHODS: Patients with measurable advanced renal cancer and 0-1 prior therapy were eligible. Treatment consisted of oblimersen, 7 mg/kg/day, as a continuous intravenous infusion 7 days of every 14 day cycle, plus alpha-IFN, 5 million units/m(2) subcutaneously, days 4 and 6 of the first oblimersen infusion, then thrice weekly. Blood for laboratory correlates was collected before treatment, during oblimersen, and during therapy with both agents. RESULTS: Twenty-three patients were enrolled, five of whom had prior systemic therapy (three with prior high-dose interleukin-2). The median number of treatment cycles was 4 (range 1-12). One patient had a partial response lasting 2.5 months. The Grade 3-4 toxicities were fatigue, fever, myelosuppression, hepatic enzyme and metabolic abnormalities. Laboratory analyses of CD3+ lymphocyte apoptotic markers demonstrated no change between pre-treatment and on-treatment levels of bcl-2 or Annexin/PI positivity by flow cytometry. Mean oblimersen steady-state plasma concentration and clearance was 2.3 +/- 0.9 microg/ml and 0.15 +/- 0.07 l/h/kg, respectively. CONCLUSIONS: Oblimersen given in this dose and schedule with alpha-IFN does not appear sufficiently active to warrant further study in advanced renal cancer. Combinations with newer targeted agents may show greater promise.

Marshall, F. F. (2005). "Quantitative detection of promoter hypermethylation of multiple genes in the tumor, urine, and serum DNA of patients with renal cancer." J Urol **173**(6): 1918.

Marshall, S. E., C. Bordea, et al. (2000). "Glutathione S-transferase polymorphisms and skin cancer after renal transplantation." Kidney Int **58**(5): 2186-93.

BACKGROUND: Susceptibility to skin cancer after transplantation is multifactorial, and risk factors include skin type, sun exposure, and level of immunosuppression. A major mechanism of carcinogenesis is ultraviolet radiation-induced free radical damage, and genetically determined ability to metabolize free radicals may also predispose to skin cancer. The glutathione S-transferase enzymes play a major role in limiting the toxic effects of reactive oxygen species, and this study was designed to determine whether polymorphisms in these enzymes are associated with skin cancers in renal transplant recipients. METHODS: Two hundred twenty-two long-term survivors of renal transplantation were examined for polymorphisms in the GSTM1, GSTT1, and GSTP1 genes, using a unified polymerase chain reaction with sequence specific primers (PCR-SSP) genotyping method. RESULTS: The GSTP1*C allele was associated with the development of squamous cell carcinomas (SCCs; P = 0.01). No associations of the GSTM1 null genotype or the GSTT1 null genotype were identified, and the development of basal cell carcinomas was not associated with any GST polymorphism studied. CONCLUSIONS: These results indicate that genetic variation in enzymes involved in free radical metabolism in the skin are associated with the development of skin cancer. While all renal transplant recipients should be advised to protect themselves from the sun, the identification of transplant patients with a genetic predisposition to skin tumors may permit the targeting of preventative and early intervention strategies to high-risk individuals.

Marshall, S. E., C. Bordea, et al. (2000). "p53 codon 72 polymorphism and susceptibility to skin cancer after renal transplantation." Transplantation **69**(5): 994-6.

BACKGROUND: Infection with human papillomavirus (HPV) is an important risk factor for the development of skin cancer after renal transplantation. It has recently been suggested that degradation of the tumor suppressor gene p53 is an important mechanism for human papillomavirus-induced carcinogenesis. A common genomic polymorphism occurs at codon 72 of the p53 gene, and in vitro the codon 72Arg variant appears to be particularly susceptible to degradation. METHODS: To test the hypothesis that this polymorphism predisposes to the development of human

papillomavirus-associated tumors, we studied p53 codon 72 genotype in 222 long-term survivors of renal transplantation, of whom 55 had developed at least one skin tumor. RESULTS: No differences in allele or genotype frequency were detected between individuals who had or had not developed skin tumors after transplantation, or any subgroup thereof. CONCLUSIONS: The p53 codon 72Arginine allele does not confer susceptibility to the development of skin tumors after renal transplantation.

Masuda, K., M. Ono, et al. (2003). "Downregulation of Cap43 gene by von Hippel-Lindau tumor suppressor protein in human renal cancer cells." Int J Cancer **105**(6): 803-10.

We previously identified 9 genes (i.e., thymosin beta4, secreted protein acidic and rich in cysteine, Cap43, ceruloplasmin, serum amyloid A, heat shock protein 90, LOT1, osteopontin and casein kinase Igamma) that are more highly expressed in cancerous regions than in noncancerous regions in human renal cancers. In our study, we considered the possibility that the von Hippel-Lindau (VHL) tumor suppressor gene might be able to affect the expression of these 9 genes in renal cancer cells. We first established 2 VHL-positive cell lines, 786/VHL-1 and 786/VHL-2, after the introduction of wild-type VHL into VHL-negative renal cancer 786-O cells. Of these 9 genes, expression of the Cap43 gene was specifically downregulated by VHL. Expression of Cap43 was also much lower in 4 other VHL-positive renal cancer cell lines than in VHL-negative 786-O cells. Cap43 promoter assays with several deletion or mutation constructs demonstrated that the Sp1 site in the element from -286 base pairs (bp) to -62 bp was partly responsible for VHL-induced suppression of the Cap43 gene. Immunostaining analysis with human specimens of renal cancers demonstrated that the Cap43 protein was expressed in most cancer cells and macrophages. We also observed a marked and specific increase of Cap43 mRNA levels in response to hypoxia or nickel in all VHL-positive cell lines. Cellular expression of Cap43 mRNA in response to hypoxia or nickel thus is closely associated with VHL gene expression in renal cancer cells. Although the function of the Cap43 protein remains unclear, the expression of Cap43 protein could be a molecular marker closely associated with VHL in renal cancer.

Mertz, K. D., F. Demichelis, et al. (2008). "Association of cytokeratin 7 and 19 expression with genomic stability and favorable prognosis in clear cell renal cell cancer." Int J Cancer **123**(3): 569-76.

The purpose of our study was to demonstrate that distinct cytogenetic alterations in the most common subtype of renal cell cancer, clear cell renal cell carcinoma (ccRCC), are reflected in protein expression profiles. We performed conventional cytogenetics and immunohistochemical analysis for cytokeratins (CKs) on 126 ccRCCs. Protein expression was evaluated in situ using a semiautomated quantitative system. The results were validated using an independent cohort of 209 ccRCCs with long-term follow-up. Cytogenetic alterations were identified in 96 of 126 ccRCCs, most of them involving chromosome 3 through loss, deletion or translocation. Expression of CKs and E-cadherin in ccRCC was associated with lack of cytogenetic alterations and low nuclear grade. In the validation set, CK7 and CK19 protein expression was associated with better clinical outcome. At the multivariate level, the best model included metastatic status and CK19 expression. Expression microarray analysis on 21 primary ccRCCs and 14 ccRCC metastases identified genes significantly associated with CK7 and CK19 expressing ccRCCs. Two novel ccRCC biomarkers associated with the CK7 positive ccRCC phenotype, PMS2 and MT1-MMP (MMP14), were further validated. We conclude that the variability observed for CK expression in ccRCC can be explained by genetic heterogeneity. Distinct molecular subtypes of ccRCC with prognostic relevance were identified, and the CK7/CK19 expressing subtype is associated with better outcome.

Michelsen, J., H. Thiesson, et al. (2006). "Tissue expression and plasma levels of adrenomedullin in renal cancer patients." Clin Sci (Lond) **111**(1): 61-70.

The peptide AM (adrenomedullin) is stimulated by hypoxia through HIF-1 (hypoxia-inducible factor-1). The majority of human CC-RCCs (clear cell renal cell carcinomas) display mutations in the tumour suppressor protein von Hippel-Lindau, which leads to constitutively elevated HIF-1. We hypothesized that AM is increased in CC-RCC tumours and that AM is a plasma biomarker for CC-RCC. Tumours and non-malignant kidney tissue were obtained from patients that underwent unilateral nephrectomy. Blood samples were drawn at the day of surgery, 3-6 days after surgery and 4-5 weeks after surgery. AM mRNA and peptide expression in tissue and AM plasma concentration were determined. HIF-1alpha was localized in tissue by immunohistochemistry. AM mRNA was elevated in CC-RCC compared with adjacent renal cortex (6-fold, n=18; $P<0.02$). There was no difference in AM mRNA between cortex and non-CC-RCC tissue (n=7). AM peptide concentration was elevated in CC-RCC tissue compared with adjacent cortex (4-fold, n=6; $P<0.02$), whereas there was no difference between cortex and non-CC-RCC tissue (n=5). HIF-1alpha immunoreactivity was detected in the majority of cell

nuclei in 76% of CC-RCC, consistent with constitutive stabilization. In non-CC-RCC, HIF-1alpha staining was focal. Before surgery there was no difference in plasma AM concentration between tumour types. Nephrectomy increased plasma AM significantly after 3-6 days and a similar pre-surgery level was observed after 4-5 weeks in both groups of tumour patients. We conclude that elevated tissue AM is a distinguishing feature of CC-RCC compared with other kidney tumours. Plasma AM is not suited as a tumour marker for this disease.

Mikhailenko, D. S., R. B. Kurynin, et al. (2008). "[Inactivation of the VHL gene in sporadic clear cell renal cancer]." Mol Biol (Mosk) **42**(1): 71-7.

Renal cell carcinoma is the most common variant of the kidney cancer, which accounts approximately 75% patients with this disease. The majority of those tumors are characterized by inactivation of the VHL gene suppressor as a result of mutations, allelic deletions and/or methylation. We have conducted the complex molecular-genetic analysis of 64 samples obtained from patients with the clear cell renal cancer. VHL mutations were detected by single strand conformation polymorphism and subsequent sequencing, loss of heterozygosity was analyzed using two STR-markers, methylation was tested by methylsensitive polymerase chain reaction. All revealed variations were statistically analyzed in respect to the parameters of primary tumors in various groups of patients. Seventeen VHL somatic mutations were detected, 12 from which were described for the first time. Allelic deletions of VHL were found in 31.6%, and methylation--in 7.8% samples of the renal cancer. As a whole, VHL inactivating events were presented in 46.9% cases of disease, in 51.7% -among renal cancer patients with first stage. We have not observed any association of mutations, loss of heterozygosity and methylation with clinical-pathological parameters of disease. Results of this investigation specify for expediency of further studies of molecular genetics aberrations in the VHL gene. Perhaps, it would promote renal cancer molecular markers evaluation, for example, a determination of suppressor genes methylated in renal cancer.

Moch, H. (2008). "[Molecular basis of targeted therapy in metastatic renal cancer]." Pathologe **29 Suppl 2**: 184-6.

The introduction of targeted therapy in metastatic renal cancer patients provides a whole array of individual therapeutic options. The basis for this treatment is the inactivation of the von Hippel-Lindau tumor suppressor gene, resulting in high expression of pro-angiogenic growth factors, e.g. vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF). This provides the rationale for targeting these pathways in clear cell renal cell carcinomas by small molecule inhibitors. This article gives a review on clinical trials with sunitinib, sorafenib, and temsirolimus in patients with advanced renal cell carcinoma and shows how promising treatments can emerge from an understanding of the molecular genetics and signaling pathways of tumors. However, a predictive marker, e.g. specific mutations associated with drug-resistant or responsive tumors, has not yet been identified and is paramount for the future.

Moch, H. (2008). "[Von-Hippel-Lindau (VHL) protein function by initiation and progression of renal cancer]." Pathologe **29 Suppl 2**: 149-52.

Germ line inactivation of the von-Hippel-Lindau (VHL) tumor suppressor gene causes von Hippel-Lindau hereditary cancer syndrome, and somatic mutations of this gene have been linked to the development of sporadic hemangioblastomas and clear cell renal carcinomas. The protein encoded by VHL, pVHL, has no known enzymatic activities but interacts with various partner proteins. In this review, various pVHL functions are highlighted. pVHL acts as a multi-purpose adaptor protein that controls different gene expression programs. Through its oxygen-dependent regulation of hypoxia-inducible factor alpha (HIFalpha), pVHL plays a central role in the oxygen-sensing pathway. In addition, many HIFalpha-independent functions of pVHL have recently been identified. These include microtubule-based processes, extracellular matrix assembly and suppression of kidney cyst formation. These complex pVHL functions can explain the diverse consequences of pVHL dysregulation in tumor formation and progression.

Mongiat-Artus, P., C. Miquel, et al. (2006). "Spectrum of molecular alterations in colorectal, upper urinary tract, endocervical, and renal carcinomas arising in a patient with hereditary non-polyposis colorectal cancer." Virchows Arch **449**(2): 238-43.

Hereditary nonpolyposis colon cancer (HNPCC) syndrome is the most frequent hereditary cancer syndrome predisposing to cancers of various locations, especially colon, endometrium, stomach, and upper urinary tract. Carcinomas of the kidney parenchyma are not considered as an HNPCC-related tumor. HNPCC tumors are characterized by microsatellite instability (MSI) due to a defect in mismatch repair (MMR) and carry somatic frameshift mutations in mononucleotide repeats within the coding regions of key genes. We report the first case of a papillary carcinoma of the kidney in an HNPCC patient who developed carcinomas of the upper

urinary tract, endocervix, and colon. Whereas the HNPCC-related tumors demonstrated MSI phenotype, loss of MSH2 protein expression, and frameshift mutations in several of the 13 target genes analyzed, the kidney cancer displayed MSS phenotype, normal MMR protein expression, and no frameshift mutation in target genes. Our observations do not support the possibility that papillary carcinomas are part of HNPCC syndrome.

Moore, L. E., R. Hung, et al. (2008). "Folate metabolism genes, vegetable intake and renal cancer risk in central Europe." Int J Cancer **122**(8): 1710-5.

In a multicenter case-control study of renal cell carcinoma (RCC) conducted in central and eastern Europe, we reported a strong inverse association with high vegetable intake and RCC risk. The odds ratio (OR) for high compared to the lowest tertile of vegetable intake was OR = 0.67; (95% confidence interval (CI): 0.53-0.83; p-trend < 0.001). We hypothesized that variation in key folate metabolism genes may modify this association. Common variation in 5 folate metabolism genes (CBS: Ex9+33C > T (rs234706), Ex13 +41C > T (rs1801181), Ex18 -391 G > A (rs12613); MTHFR: A222V Ex5+79C > T (rs1801133), Ex8-62A > C (rs1801131); MTR: Ex26 20A > G (rs1805087), MTRR: Ex5+136 T > C (rs161870), and TYMS:IVS2-405 C > T (rs502396), Ex8+157 C > T (rs699517), Ex8+227 A > G (rs2790)) were analyzed among 1,097 RCC cases and 1,555 controls genotyped in this study. Having at least 1 variant T allele of MTHFR A222V was associated with higher RCC risk compared to those with 2 common (CC) alleles (OR = 1.44; 95% CI: 1.17-1.77; p = 0.001). After stratification by tertile of vegetable intake, the higher risk associated with the variant genotype was only observed in the low and medium tertiles (p-trend = 0.001), but not among those in the highest tertile (p-interaction = 0.22). The association remained robust after calculation of the false discovery rate (FDR = 0.05). Of the 3 TYMS SNPs examined, only the TYMS IVS2 -405 C (rs502396) variant was associated with a significantly lower risk compared to the common genotype (OR = 0.73; 95% CI: 0.57-0.93). Vegetable intake modified the association between all 3 TYMS SNPs and RCC risk (p-interaction < 0.04 for all). In summary, these findings suggest that common variation in MTHFR and TYMS genes may be associated with RCC risk, particularly when vegetable intake is low.

Moore, L. E., R. T. Wilson, et al. (2005). "Lifestyle factors, exposures, genetic susceptibility, and renal cell cancer risk: a review." Cancer Invest **23**(3): 240-55.

Malignant kidney tumors account for approximately 2% of all new primary cancer cases diagnosed in the United States, with an estimated 30,000 cases occurring annually. Although a variety of agents, chemical and biological, have been implicated as causal agents in the development of renal cell carcinoma (RCC), the etiology remains enigmatic. The strongest association has been developed between cigarette smoking and renal cancer however consistent, positive associations between RCC and obesity, diabetes, and hypertension have also been reported. In addition, more recent investigations of familial kidney cancer syndromes indicate that a strong genetic component contributes to RCC development. Several genes have been identified through investigation of familial kidney cancer syndromes. This review article describes recent trends in RCC incidence and the currently identifiable etiological causes that account for approximately half of the RCC cases diagnoses. The remainder of this review then focuses on additional risk factors that have thus far not been well examined but may be helpful in explaining the increasing incidence trends and the geographic or racial variation observed nationally and worldwide.

Moschella, F., R. P. Catanzaro, et al. (2003). "Shifting gene expression profiles during ex vivo culture of renal tumor cells: implications for cancer immunotherapy." Oncol Res **14**(3): 133-45.

The use of cultured tumor cells rather than original tumor tissue for the preparation of therapeutic cancer vaccines represents an obvious solution to the problem of availability of adequate quantities of autologous tumor. In this study we investigated possible changes in gene expression accompanying the transition of renal cell carcinoma cells from the original tissue to cell populations in culture. In our study we employed cDNA microarray technology to compare the gene expression pattern of ex vivo cultured renal carcinoma cells to that of the original solid tumor tissue from which the cells were derived. Using this approach we detected changes in the expression of many genes mostly related to the cell lines' physiological properties. Some of the products of those genes showing differential expression between tumor-derived cell line and original tumor are known human autoantigens or tumor-associated antigens. Furthermore, analysis of overexpressed genes revealed the presence of several transcripts with restricted normal tissue distribution, representing self-antigens with potential to elicit autoimmunity. Our results suggest that adapting tumor tissue to culture can result in changes in the level of transcripts specific for known antigens and that more information regarding the composition of tumor cells and their

byproducts used in vaccine trials is needed before the efficacy and safety of such procedures can truly be determined.

Nanus, D. M., Y. Geng, et al. (2000). "Interaction of retinoic acid and interferon in renal cancer cell lines." J Interferon Cytokine Res **20**(9): 787-94.

Retinoic acid (RA) can potentiate the antitumor effect of interferons (IFN) in a variety of tumor types, including renal cell carcinoma (RCC). The mechanisms by which RA and IFN increase the antitumor effects in RCC are unknown. We used growth assays and mobility shift assays to examine the effects of combining 13-cis-retinoic acid (CRA) and IFN-alpha (plus IFN-gamma) on proliferation and on the expression of the IFN-specific transcription factor IFN-stimulated gene factor 3 (ISGF3) in RCC cell lines. Combining CRA and IFN-alpha resulted in a significant increase in growth inhibition in four cell lines compared with IFN-alpha or CRA alone. Binding of nuclear extracts from RCC cells to an IFN-stimulated response element (ISRE) oligonucleotide probe following incubation with IFN-alpha was not increased by CRA but was significantly increased by pretreatment by IFN-gamma in a time-dependent fashion. Proliferation assays showed that sequential addition of IFN-gamma and IFN-alpha significantly increased growth inhibition. IFN-alpha but not IFN-gamma or CRA increased the cellular levels Stat2 and p48 but not Statl. IFN-gamma pretreatment enhanced the upregulation of p48 levels by IFN-alpha. Combining RA and IFN results in additive growth inhibition on RCC cell lines. This increase in growth inhibition is not mediated by increased ISGF3 expression.

Noguchi, S., T. Shuin, et al. (1998). "High alkaline phosphatase activity in 3p-induced human renal cancer cells." Cancer Lett **131**(2): 223-7.

Recent studies have suggested that a tumor suppressor gene is located on the short arm of chromosome 3 (3p) for tumorigenesis of sporadic renal cell carcinoma. In this study, we introduced 3p from normal human fibroblasts into the renal cancer cell line YCR and this introduction clearly resulted in inhibited growth in vitro and in vivo. Furthermore, we investigated several enzyme activities of original YCR cells and 3p-induced clones (YCR-151 and YCR-152) and found that only alkaline phosphatase activity in 3p-induced clones was significantly increased compared to that of the original YCR cells.

Nyhan, M. J., G. C. O'Sullivan, et al. (2008). "Role of the VHL (von Hippel-Lindau) gene in renal cancer: a multifunctional tumour suppressor." Biochem Soc Trans **36**(Pt 3): 472-8.

The VHL (von Hippel-Lindau) tumour-suppressor gene is inactivated in VHL disease and in sporadic cases of CCRCC [clear-cell RCC (renal cell carcinoma)]. pVHL (VHL protein) functions as part of an E3 ubiquitin ligase complex that targets proteins for proteasomal degradation. The best-characterized substrate is HIF-alpha (hypoxia-inducible factor-alpha). Loss of pVHL and subsequent up-regulation of HIF target genes has been attributed to the highly vascular nature of these neoplasms. However, pVHL does not just function as the executioner of HIF-alpha. Additional functions of pVHL that may be important in preventing CCRCC tumorigenesis have been identified, including primary cilium maintenance, assembly of the extracellular matrix and roles in the stabilization of p53 and Jade-1 (gene for apoptosis and differentiation in epithelia). Current evidence indicates that pVHL probably requires additional co-operating signalling pathways for CCRCC initiation and tumorigenesis.

O'Connor, D. P., E. W. Kay, et al. (2001). "Altered p53 expression in benign and malignant skin lesions from renal transplant recipients and immunocompetent patients with skin cancer: correlation with human papillomaviruses?" Diagn Mol Pathol **10**(3): 190-9.

Renal transplant recipients are prone to numerous benign and malignant skin lesions. Previous work in the authors' laboratory has determined that the human papillomavirus may be the viral aetiology of these skin lesions. The p53 tumor-suppressor gene is the most frequently mutated gene in a wide range of human cancers. Here the authors describe an immunohistochemical study to evaluate the expression of p53 in benign and malignant skin lesions from renal transplant recipients and immunocompetent patients with skin cancer. The effect of p53 mutations on the expression patterns observed were examined by polymerase chain reaction-single strand conformation polymorphism analysis and direct cycle sequencing. The expression of the p53-regulated cyclin-dependant kinase inhibitor p21Waf1/Cip1 and Mdm2 was also examined in p53-positive cells. The expression of p53 in benign and malignant lesions was found to be markedly different. p53 was expressed in only 40% (6/15) of viral warts analyzed. The expression was confined to the basal layer both in the lesion and in adjacent normal skin, and the level of expression was low and only in a small number of cells (<10%). Of the cutaneous squamous cell carcinomas analyzed, 60% (9/15) showed p53 expression. Two different patterns of expression were observed. Basal layer expression in both the invasive tumor and adjacent normal skin was observed in 50% of the p53-positive squamous cell carcinomas; in the remaining 50%, p53

was expressed diffusely throughout the invasive tumor and in the basal layer of adjacent normal skin. The level of expression was high and in a large number of cells. Polymerase chain reaction-single strand conformation polymorphism analysis revealed that only one of the squamous cell carcinomas expressing p53 harbored a p53 mutation and that the accumulated p53 in the remaining tumors was wild type. No Mdm2 or p21Waf1/Cip1 expression was detected in the p53-positive squamous cell carcinomas, indicating that although the accumulated p53 is stable, it does not function effectively as a transcriptional activator. This represents a novel p53 phenotype in cutaneous squamous cell carcinoma. In addition, no correlation was seen between the presence and absence of human papillomavirus and p53 expression.

Ohba, K., Y. Miyata, et al. (2005). "Expression of nm23-H1 gene product in sarcomatous cancer cells of renal cell carcinoma: correlation with tumor stage and expression of matrix metalloproteinase-2, matrix metalloproteinase-9, sialyl Lewis X, and c-erbB-2." Urology **65**(5): 1029-34.

OBJECTIVES: To investigate the clinical significance of nm23-H1 gene product in sarcomatous cancer cells, because this is known as a tumor-metastasis suppressor. Renal cell carcinoma with sarcomatoid cancer cells is characterized by high malignant potential and a poor prognosis. METHODS: We investigated the expression of nm23-H1 gene product in the carcinomatous and sarcomatous component (CC and SC) of renal cell carcinoma using immunohistochemical techniques and the relationships between the expression and clinicopathologic features. We also examined the expression of matrix metalloproteinase (MMP)-2, MMP-9, sialyl Lewis X, and c-erbB-2 in the SC because these proteins are regulated by the nm23-H1 gene or its products. RESULTS: We examined 20 renal cell carcinoma specimens that contained an SC and CC. The CC of 12 of the 20 tumors stained positively for nm23-H1 gene product. In contrast, the SC of only 3 of the 20 stained positive. The reduced expression of nm23-H1 gene product in the SC correlated significantly with tumor invasion ($P < 0.01$), but not with tumor size or metastasis. In contrast, the expression of the nm23-H1 gene product in the CC was not associated with these pathologic features. Expression of the nm23-H1 gene product correlated negatively with MMP-2 expression ($r = -0.48$, $P = 0.03$). Other factors did not show such significant correlations with nm23-H1 gene product expression. CONCLUSIONS: Our results suggest that low expression of nm23-H1 gene products may play important roles in tumor invasion, and that this process is mediated in part by overexpression of MMP-2.

Okamura, K., H. Koike, et al. (2009). "Survivin and its spliced isoform gene expression is associated with proliferation of renal cancer cells and clinical stage of renal cancer." Cancer Epidemiol **33**(2): 137-41.

BACKGROUND: Survivin has been implicated in inhibition of apoptosis. To date, alternatively spliced isoforms, Survivin-2alpha, -2B, -DeltaEx3, -3B, have been described. We assessed the effect of survivin gene expression on the proliferation of renal cancer (RCC) cells, and studied the association of survivin and its spliced isoform gene expression levels with the clinical stage of RCC. METHODS: Gene expression of survivin and its spliced isoform in RCC cells, Caki-1, were performed by RT-PCR. We knocked down the gene expression of Survivin using small interfering RNA (siRNA), and assessed the cell proliferation by MTS assay. Next, we quantified the gene expression levels of survivin and its isoform in nephrectomy samples using quantitative real-time PCR. RESULTS: In Caki-1 cells, survivin and survivin-2alpha, -2B were expressed higher than survivin-DeltaEx3. Decrease of Survivin gene expression by transfection of siRNA was accompanied by inhibition of the proliferation of Caki-1 cell with 36% decrease in comparison with negative control transfected cells ($p<0.01$). In clinical RCC tissues, survivin expression levels in metastatic stage were significantly higher compared with those in distant metastasis stage (M0:M1=1:4.81, $p=0.014$); survivin 2B gene expression levels in pT3 tumors were associated significantly higher than those in pT1 (pT1:pT3=1:4.50, $p=0.043$). No significant differences were found in survivin-2alpha expression levels and the ratio of survivin-2B/survivin gene expression levels among any clinical stages. CONCLUSION: We first demonstrated the gene expression of survivin-2alpha in renal cancer cells, and also showed that survivin and its spliced isoforms had associations with renal cancer cell proliferation and distant metastases.

O'Kane, H. F., C. J. Watson, et al. (2006). "Targeting death receptors in bladder, prostate and renal cancer." J Urol **175**(2): 432-8.

PURPOSE: We describe key components of normal and aberrant death receptor pathways, the association of these abnormalities with tumorigenesis in bladder, prostate and renal cancer, and their potential application in novel therapeutic strategies targeted toward patients with cancer. MATERIALS AND METHODS: A MEDLINE literature search of the key words death receptors, TRAIL (tumor necrosis factor related apoptosis inducing ligand), FAS, bladder, prostate, renal and cancer was done to obtain

information for review. A brief overview of the TRAIL and FAS death receptor pathways, and their relationship to apoptosis is described. Mechanisms that lead to nonfunction of these pathways and how they may contribute to tumorigenesis are linked. Current efforts to target death receptor pathways as a therapeutic strategy are highlighted. RESULTS: Activation of tumor cell expressing death receptors by cytotoxic immune cells is the main mechanism by which the immune system eliminates malignant cells. Death receptor triggering induces a caspase cascade, leading to tumor cell apoptosis. Receptor gene mutation or hypermethylation, decoy receptor or splice variant over expression, and downstream inhibitor interference are examples of the ways that normal pathway functioning is lost in cancers of the bladder and prostate. Targeting death receptors directly through synthetic ligand administration and blocking downstream inhibitor molecules with siRNA or antisense oligonucleotides represent novel therapeutic strategies under development. CONCLUSIONS: Research into the death receptor pathways has demonstrated the key role that pathway aberrations have in the initiation and progression of malignancies of the bladder, prostate and kidney. This new understanding has resulted in exciting approaches to restore the functionality of these pathways as a novel therapeutic strategy.

Okegawa, T., J. R. Sayne, et al. (2007). "A histone deacetylase inhibitor enhances adenoviral infection of renal cancer cells." J Urol **177**(3): 1148-56.

PURPOSE: Coxsackie and adenovirus receptor is a high affinity receptor for adenovirus type 5. To our knowledge the expression profile of coxsackie and adenovirus receptor in renal cancer has not been described. We evaluated the expression of coxsackie and adenovirus receptor in human renal cancer specimens and determined whether the histone deacetylase inhibitor FK-228 (Astelas Pharmaceutical, Osaka, Japan) increases the efficiency of adenoviral infections in renal carcinoma cells in vivo and in vitro. MATERIALS AND METHODS: We used randomly selected renal cancer specimens. Specimens were analyzed for coxsackie and adenovirus receptor expression using reverse transcriptase-polymerase chain reaction and immunohistochemistry. In vitro experiments on cytotoxicity were performed to determine a nontoxic dose of FK-228 for renal cancer cells. The level of coxsackie and adenovirus receptor expression was determined by fluorescence activated cell scanning and/or reverse transcriptase-polymerase chain reaction in FK-228 treated renal cancer cells. The effect in vivo on adenoviral gene expression was investigated in athymic mice. RESULTS: In several human renal cancer specimens a loss of or decreased coxsackie and adenovirus receptor expression was detected by reverse transcriptase-polymerase chain reaction based analysis and immunohistochemistry. The nontoxic dose of FK-228 for renal carcinoma cells was 0.5 ng/ml. Treatment of cancer cells with 0.5 ng/ml FK-228 increased levels of coxsackie and adenovirus receptor RNA and acetylated histone H3. This increase was associated with an approximately 10-fold increase in adenoviral infection, as evidenced by increased transgene expression from a beta-galactosidase containing adenoviral vector. Intravenous administration of FK-228 enhanced coxsackie and adenovirus receptor expression in athymic mice. The combination of beta-galactosidase adenovirus and FK-228 was significantly more effective than adenovirus only in A498 cells 3 weeks after treatment in vivo. The combination of p21 adenovirus and FK-228 resulted in significant tumor inhibition in vitro and in vivo. CONCLUSIONS: In human renal cancer specimens a loss of or decrease in coxsackie and adenovirus receptor expression may be an early event in renal cancer progression. Pretreatment with FK-228 may increase tumor cell sensitivity to adenoviral gene therapy vectors.

Okimoto, K., J. Sakurai, et al. (2004). "A germ-line insertion in the Birt-Hogg-Dube (BHD) gene gives rise to the Nihon rat model of inherited renal cancer." Proc Natl Acad Sci U S A **101**(7): 2023-7.

A rat model of hereditary renal carcinoma (RC) was found in a rat colony of the Sprague-Dawley strain in Japan and named the "Nihon" rat. In heterozygotes, RCs, predominantly the clear cell type, develop from early preneoplastic lesions, which began to appear as early as 3 weeks of age, to adenocarcinomas by the age of 6 months. The Nihon rat is an example of a Mendelian dominantly inherited predisposition for development of RCs like the Eker (Tsc2 gene mutant) rat. We have previously shown that the Nihon mutation was tightly linked to genes that are located on the distal part of rat chromosome 10. The order of the genes is the Eker (Tsc2 gene (human 16p13.3)-Il3 gene-Nihon gene-Llgl1 locus-Myhse gene. We now describe a germ-line mutation in the Birt-Hogg-Dube gene (Bhd) (human 17p11.2) caused by the insertion of a single nucleotide in the Nihon rat, resulting in a frameshift and producing a stop codon 26 aa downstream. We found that the homozygous mutant condition was lethal at an early stage of fetal life in the rat. We detected a high frequency of loss of heterozygosity (LOH) in primary RCs (10/11) at the Bhd locus and found a point mutation (nonsense) in one LOH-negative case, fitting Knudson's "two-hit" model. The Nihon rat may therefore provide insights into a tumor-suppressor

gene that is related to renal carcinogenesis and an animal model of human BHD syndrome.

Ornstein, D. K., I. A. Lubensky, et al. (2000). "Prevalence of microscopic tumors in normal appearing renal parenchyma of patients with hereditary papillary renal cancer." J Urol **163**(2): 431-3.

PURPOSE: We describe the earliest renal lesions associated with hereditary papillary renal cancer and estimate the prevalence of microscopic papillary renal tumors. MATERIALS AND METHODS: Grossly normal tissue was obtained from 12 kidneys during renal surgery in 9 patients with hereditary papillary renal cancer. Tissue was examined microscopically and findings were compared to those previously reported to be associated with von Hippel-Lindau disease and sporadic renal cell carcinoma. RESULTS: A total of 92 microscopic papillary renal cell carcinoma lesions were identified on 46 of 88 slides (53%). No other lesions were identified. All tumors were solid and displayed the basophilic papillary histology characteristic of hereditary papillary renal cancer. Extrapolation of the data predicted the prevalence of 1,100 to 3,400 microscopic papillary tumors in a single kidney in a patient with hereditary papillary renal cancer. CONCLUSIONS: The basophilic papillary histology characteristic of clinically apparent renal tumors in patients with hereditary papillary renal cancer also characterizes the multiple microscopic lesions seen in the kidneys. These findings suggest that the earliest renal tumor in patients with an activating hereditary mutation of the met gene is papillary basophilic renal cancer. The large number of microscopic tumors in patients with hereditary papillary renal cancer was comparable to or greater than that seen in those with von Hippel-Lindau disease.

Osawa, A., Y. Sumiyama, et al. (2006). "Single case of renal cell carcinoma and endocrine pancreatic head cancer occurring with von Hippel-Lindau disease." J Hepatobiliary Pancreat Surg **13**(2): 174-80.

Von Hippel-Lindau (VHL) disease is an autosomal dominant genetic disease in which various neoplastic lesions occur in multiple organs. Reported here is a case of VHL disease with concurrent renal cell carcinoma and endocrine pancreatic cancer. The patient was a 43-year old woman. On this occasion, the patient had sought treatment from her local physician, complaining chiefly of yellowing of the skin and bulbar conjunctiva. Abdominal ultrasound and computed tomography scans revealed a mass in the right kidney and a mass in the pancreatic head. Peripheral blood genetic analysis revealed an Arg/stop heteroconjugative mutation in codon 113 in exon 1 of the VHL gene on the short arm of chromosome 3 (p25-26). After various tests were performed, the patient was diagnosed with right renal cell carcinoma, malignant tumor of the pancreatic head, and multiple pancreatic cysts accompanying von Hippel-Lindau disease. Right nephrectomy and pancreatoduodenectomy were performed. Based on the histopathological results, the patient was diagnosed with right renal cell carcinoma and highly differentiated endocrine pancreatic cancer. Immunohistologically, a large number of atypical cells were found to be positive for both anti-chromogranin and anti-synaptophysin antibodies in the endocrine tumor. Immunostaining for each type of gut hormone was also performed, but all results were negative. Based on the above findings, nonfunctioning, highly differentiating endocrine cancer was diagnosed. This is the first confirmed case of renal cell carcinoma and endocrine pancreatic cancer occurring concurrently with VHL. This is an important case, so it is presented here along with a short discussion of the literature.

Peter, C., J. T. Kielstein, et al. (2007). "A novel bioluminescent tumor model of human renal cancer cell lines: an in vitro and in vivo characterization." J Urol **177**(6): 2342-6.

PURPOSE: Bioluminescent imaging permits sensitive in vivo detection and quantification of cells engineered to emit light. We developed a bioluminescent human renal cancer cell line for in vitro and in vivo studies. MATERIAL AND METHODS: The 2 human renal cell carcinoma cell lines SN12-C and SN12-L1 were stably transfected to constitutively express luciferase using a retroviral shuttle. The bioluminescent signal was correlated with tumor cell numbers in vitro. Parental and transfected cells were compared by growth kinetics and histology. Tumor burden after heterotopic injection in immune deficient mice was monitored up to 39 days. The kinetics of the bioluminescent signal was evaluated for 1 to 60 minutes following luciferin injection. RESULTS: Bioengineered renal cancer cell lines stably expressed luciferase. The growth kinetics of the cells in vitro and the histology of tumors resulting from implantation of these cells were unaffected by retroviral transfection with the luciferase gene. As few as 1,000 cells could be reliably detected. The intensity of the bioluminescent signal correlated with the number of tumor cells in vitro. Photon emission in vivo and ex vivo correlated significantly with tumor weight at sacrifice. After intraperitoneal injection of luciferin there was a time dependent change in the intensity of the bioluminescent signal with maximum photon emission at 20 minutes (optimal 17 to 25). CONCLUSIONS: Luciferase transfected human renal

cancer lines allow reliable, rapid, noninvasive and longitudinal monitoring of tumor growth in vivo. The ability to assess tumor development in vivo with time is economical and effective compared to end point data experiments.

Piekielko-Witkowska, A., A. Master, et al. (2009). "Disturbed expression of type 1 iodothyronine deiodinase splice variants in human renal cancer." Thyroid **19**(10): 1105-13.

BACKGROUND: Alternative splicing, one of the sources of protein diversity, is often disturbed in cancer. Type 1 iodothyronine deiodinase (DIO1) catalyzes deiodination of thyroxine generating triiodothyronine, an important regulator of cell proliferation and differentiation. The expression of DIO1 is disturbed in different types of cancer. The aim of the study was to analyze the alternative splicing of DIO1 and its possible disturbance in renal cancer. METHODS: Using real-time PCR, we analyzed 19 tissue samples (T) of renal cancer and 19 matched control samples (C) of the opposite pole of the kidney, not infiltrated by tumor, and 6 control samples (N) (nonneoplastic kidney abnormalities). RESULTS: Cloning of DIO1 mRNA isoforms revealed 11 different transcripts, among them 7 new splice variants, not previously reported. The expression of all variants of DIO1 was dramatically (>90%) and significantly ($p < or = 0.0003$) lowered in samples T compared to control samples C. The ratio of mRNA isoforms encoding DIO1 protein variants possessing or lacking the active center was lowered in samples T compared with control samples C, suggesting disturbed alternative splicing of DIO1. The expression of mRNA of splicing factors SF2/ASF (splicing factor-2/alternative-splicing factor) and hnRNPA1 (heterogeneous ribonucleoprotein A1), regulating 5'-splice site selection, was significantly but not proportionally lowered in samples T compared to samples C. The mRNA ratio of splicing factors SF2/ASF and hnRNPA1 correlated with the ratio of mRNA isoforms encoding DIO1 protein variants possessing or lacking the active center in controls C but not in samples T. CONCLUSIONS: Our results show that the expression and alternative splicing of DIO1 mRNA is disturbed in renal cancer, possibly due to changes in expression of splicing factors SF2/ASF and hnRNPA1.

Pollard, P., N. Wortham, et al. (2005). "Evidence of increased microvessel density and activation of the hypoxia pathway in tumours from the hereditary leiomyomatosis and renal cell cancer syndrome." J Pathol **205**(1): 41-9.

The Mendelian tumour syndromes hereditary leiomyomatosis and renal cell cancer (HLRCC) and hereditary paragangliomatosis with phaeochromocytomas (HPGL) result from mutations in nuclear genes (FH and SDHB/C/D, respectively) that encode Krebs cycle enzymes. HPGL tumours are highly vascular and there is evidence that inactivation of SDH leads to activation of the hypoxia/angiogenesis pathway. In contrast, uterine leiomyomas are not generally regarded as particularly vascular lesions. In order to test the possibility that activation of the hypoxia/angiogenesis pathway contributes to tumourigenesis in HLRCC, increased vascularity and hypoxia pathway activation were searched for in HLRCC tumours. Microvessel density was markedly higher in uterine leiomyomas from HLRCC than in the surrounding myometrium; it was notable that sporadic uterine leiomyomas were actually less vascular than normal myometrium. In HLRCC tumours, there was increased expression of transcripts from the hypoxia-responsive genes vascular endothelial growth factor (VEGF) and BNIP3; sporadic uterine leiomyomas did not show these changes. All uterine leiomyomas showed decreased expression of thrombospondin 1. Although sporadic and HLRCC uterine leiomyomas appear to have identical morphology, their pathways of tumourigenesis may be fundamentally different. As is the case in HPGL, it is probable that failure of the Krebs cycle in HLRCC tumours causes inappropriate signalling that the cell is in a hypoxic state, leading to angiogenesis and perhaps directly to clonal expansion and tumour growth through some uncharacterized, cell-autonomous effect.

Queille, S., L. Luron, et al. (2007). "Analysis of skin cancer risk factors in immunosuppressed renal transplant patients shows high levels of UV-specific tandem CC to TT mutations of the p53 gene." Carcinogenesis **28**(3): 724-31.

Immunosuppressed renal transplant recipients (RTRs) are predisposed to non-melanoma skin cancers (NMSCs), predominantly squamous cell carcinomas (SCCs). We have analyzed skin lesions from RTRs with aggressive tumors for p53 gene modifications, the presence of Human Papillomas Virus (HPV) DNA in relation to the p53 codon 72 genotype and polymorphisms of the XPD repair gene. We detected 24 p53 mutations in 15/25 (60%) NMSCs, 1 deletion and 23 base substitutions, the majority (78%) being UV-specific C to T transitions at bipyrimidine sites. Importantly, 35% (6/17) are tandem mutations, including 4 UV signature CC to TT transitions possibly linked to modulated DNA repair caused by the immunosuppressive drug cyclosporin A (CsA). We found 8 p53 mutations in 7/17 (41%) precancerous actinic keratosis (AK), suggesting that p53 mutations are early events in RTR skin

carcinogenesis. Immunohistochemical analysis shows a good correlation between p53 accumulation and mutations. HPV DNA was detected in 78% of skin lesions (60% Basal Cell Carcinomas, 82%AK and 79% SCCs). Thus, immunosuppression has increased the risk of infections by HPVs, predominantly epidermodysplasia verruciformis, speculated to play a role in skin cancer development. No association is found between HPV status and p53 mutation. Moreover, p53 codon 72 or frequencies of three XPD genotypes of RTRs are comparable with control populations. The p53 mutation spectrum, presenting a high level of CC to TT mutations, shows that the UV component of sunlight is the major risk factor and modulated DNA repair by immunosuppressive drug treatment may be significant in the skin carcinogenesis of RTRs.

Radulovic, S. and S. K. Bjelogrlic (2007). "Sunitinib, sorafenib and mTOR inhibitors in renal cancer." J Buon **12 Suppl 1**: S151-62.

Understanding the alterations in cellular protein interactions and their relations to genetic mutations that cause renal cell carcinoma (RCC) provides a unique opportunity for the development of disease-specific therapy for patients with advanced forms of this disease. There is substantial evidence of an association between mutation on von Hippel-Lindau (VHL) gene and the earliest stages of tumorigenesis of RCC. The main consequence of VHL loss is the upregulation of downstream proangiogenic factors leading to highly vascular tumors. Overexpression of hypoxia inducible factor (HIF) is also caused by the mammalian target of rapamycin (mTOR), a key component of signaling pathways inside the cell, involved in cell proliferation. The inhibition of proangiogenic factors and mTOR was the main idea behind the development of new targeted agents in advanced RCC. Since December 2005, 3 targeted agents have been approved by the U.S. Food and Drug Administration (FDA) for the treatment of advanced RCC: sorafenib, sunitinib and temsirolimus. Sorafenib and sunitinib are synthetic, orally active agents shown to directly inhibit vascular endothelial growth factor receptors -2 and -3 (VEGFR-2, VEGFR-3) and platelet-derived growth factor receptor beta (PDGFR-beta), while temsirolimus is an mTOR inhibitor. Recent clinical studies form the basis for new guidelines for the treatment of advanced RCC: sorafenib should be used as a second-line treatment, sunitinib as the first-line therapy for good and intermediate-risk patients, and temsirolimus should be considered as first-line treatment for poor-risk patients. Future approaches to targeted therapy should focus on optimizing the use of current active drugs, exploring their combinations or investigating their sequential use. In addition, it is important to define the mechanisms of resistance on their use and to further investigate biomarkers and enhance treatment efficacy for the individual patients. The development of these targeted therapies represents an exciting step forward in the treatment of advanced RCC.

Ramsay, H. M., P. N. Harden, et al. (2001). "Polymorphisms in glutathione S-transferases are associated with altered risk of nonmelanoma skin cancer in renal transplant recipients: a preliminary analysis." J Invest Dermatol **117**(2): 251-5.

Non-melanoma skin cancer (NMSC) represents a significant cause of morbidity and mortality among renal transplant recipients, with tumors behaving more aggressively than those in nontransplant patients. Not all immunosuppressed patients develop NMSC, however, and in those that do, the rate of accrual and numbers of lesions vary considerably. Though ultraviolet light is critical, it is unlikely that this alone explains the observed phenotypic diversity, suggesting the possible involvement of genetic factors. Furthermore, although twin studies in nontransplant patients with NMSC suggest a low genetic component, several genes associated with susceptibility and outcome in these patients have been identified. Thus, having previously shown that polymorphism in members of the glutathione S-transferase (GST) supergene family is associated with altered NMSC risk in nontransplant patients, we examined allelism in GSTM1, GSTP1, GSTM3, and GSTT1 in 183 renal transplant recipients. GSTM1 null was associated with increased squamous cell carcinoma (SCC) risk ($p = 0.042$, OR = 3.1). This remained significant after correction for age, gender, and ultraviolet light exposure ($p = 0.012$, OR = 8.4) and was particularly strong in patients with higher ultraviolet light exposure (e.g., sunbathing score > 3, $p = 0.003$, OR = 11.5) and in smokers ($p = 0.021$, OR = 4.8). Analysis of the interaction between GSTM1 null and sunbathing score showed that the two factors were synergistic and individuals with both risk parameters demonstrated a shorter time from transplantation to development of the first SCC ($p = 0.012$, hazard ratio = 7.1). GSTP1*Ile homozygotes developed larger numbers of SCC ($p = 0.002$, rate ratio = 7.6), particularly those with lower ultraviolet light exposure and cigarette consumption. GSTM3 and GSTT1 also demonstrated significant associations, though some genotype frequencies were low. These preliminary data suggest that genetic factors mediating protection against oxidative stress are important in NMSC development in immunosuppressed patients and may be useful in identifying high-risk individuals.

Refae, M. A., N. Wong, et al. (2007). "Hereditary leiomyomatosis and renal cell cancer: an unusual and aggressive form of hereditary renal carcinoma." Nat Clin Pract Oncol **4**(4): 256-61.

BACKGROUND: A 17-year-old male presented with cervical adenopathy and a palpable left flank mass. After an initial biopsy of the neck mass, which revealed metastatic carcinoma, a left radical nephrectomy was performed as well as excision of a left supraclavicular lymph node. Subsequent inquiry revealed that the patient's father had died of metastatic renal cell carcinoma (RCC) at the age of 40 years, and that other family members had also developed skin and uterine leiomyomas. INVESTIGATIONS: Physical examination, CT scans of the chest, abdomen, and pelvis, lymph-node biopsy and genetic counseling, followed by genetic testing. DIAGNOSIS: Papillary type 2 RCC described in the context of hereditary leiomyomatosis and renal cell cancer (HLRCC), an autosomal dominant syndrome attributable to a mutation in the fumarate hydratase (FH) gene on chromosome 1. MANAGEMENT: Radical nephrectomy, immunotherapy, chemotherapy and repeat surgical debulking. Genetic counseling and testing for family members was also undertaken. Annual skin examination of the carriers and radiological evaluation of both kidneys with CT scan and/or MRI.

Rennel, E., S. Mellberg, et al. (2007). "Endocan is a VEGF-A and PI3K regulated gene with increased expression in human renal cancer." Exp Cell Res **313**(7): 1285-94.

An in vitro model of VEGF-A-induced angiogenesis was used to generate transcription profiles of human microvascular endothelial cells. Microarray analysis showed increased transcription of genes known to regulate angiogenesis, but also genes that previously have not been firmly associated with angiogenesis such as endocan, pinin, plakophilin, phosphodiesterase 4B and gelsolin. Increased endocan mRNA levels in response to VEGF-A in endothelial cells and in human renal cancer have previously been reported. We now show increased endocan protein levels in VEGF-A treated endothelial cells and in human renal clear cell carcinoma. Increased protein expression was observed both in tumor cells and in a subset of tumor vessels, while expression in normal kidney tissue was low. VEGF-A seemed to be a specific inducer of endocan transcription since FGF-2, PDGF-BB, HGF/SF and EGF did not alter expression levels. Inhibition of PI3K with LY294002 caused a 12-fold increase in endocan transcription suggesting a repressive function of PI3K. In contrast inhibition of Src or MEK, which are signaling pathways activated by VEGF-A, did not influence basal or VEGF-A-induced endocan levels. In conclusion our study shows that, among angiogenic growth factors, VEGF-A is a specific inducer of endocan transcription which is translated into increased protein levels in VEGF-A treated endothelial cells. Increased endocan protein expression in human renal cancer suggests a role in tumor growth.

Richard, S., C. Beroud, et al. (1998). "[Von Hippel-Lindau disease and renal cancer: 10 years of genetic progress. GEFVHL (French-Speaking Study Group on von Hippel-Lindau disease)]." Prog Urol **8**(3): 330-9.

Von Hippel-Lindau (VHL) disease is a genetic disease predisposing to the development of various tumours (haemangioblastomas of the neuraxis and retina, tumours of the membranous labyrinth, renal clear cell carcinomas or cysts, phaeochromocytomas, pancreatic cysts or tumours, epididymal cystadenomas), affecting one in 36,000 people. Renal cancer constitutes one of the main causes of death. The VHL gene, situated at 3p25-26, is a tumour suppressor gene which plays a major role in regulation of VEGF transcription and expression. The germ cell mutation can be identified in 70% of patients. Somatic mutations of the VHL gene are also responsible for sporadic clear cell carcinomas. In the urological setting, any patient presenting with "sporadic" bilateral clear cell renal cancer or detected at an early age, or bilateral epididymal cystadenomas, should be investigated for the presence of VHL disease.

Rini, B. I., S. Halabi, et al. (2004). "Cancer and Leukemia Group B 90206: A randomized phase III trial of interferon-alpha or interferon-alpha plus anti-vascular endothelial growth factor antibody (bevacizumab) in metastatic renal cell carcinoma." Clin Cancer Res **10**(8): 2584-6.

The majority of sporadic clear cell renal cell carcinoma (RCC) is characterized by loss of heterozygosity of the von Hippel-Lindau (VHL) tumor suppressor gene and somatic inactivation of the remaining VHL allele. The resulting VHL gene silencing leads to induction of hypoxia-regulated genes including vascular endothelial growth factor (VEGF). Thus, therapeutic inhibition of VEGF holds promise for treatment of this historically refractory malignancy. An antibody to VEGF (bevacizumab, Avastin) has demonstrated a significant prolongation of time to disease progression compared with placebo in patients with metastatic RCC. Interferon-alpha (IFN-alpha) is a standard initial cytokine therapy in RCC with a modest response rate and a survival advantage demonstrated in randomized trials. We hypothesized that the addition of anti-VEGF therapy

to IFN-alpha would prolong survival in untreated metastatic RCC patients. A Phase III trial is now being conducted randomizing untreated, metastatic clear cell RCC patients to IFN-alpha alone or IFN-alpha plus Avastin.

Saenz Lopez, P., F. Vazquez Alonso, et al. (2009). "[Polymorphisms in inflammatory response genes in metastatic renal cancer]." Actas Urol Esp **33**(5): 474-81.

Inflammation has been implicated as an etiological factor in different human cancers. Allelic variations in the genes implicated in inflammation are candidates as genetic determinants or markers of renal carcinoma risk. The present stud investigates whether polymorphisms of the genes that give rise to increases in the levels of proinflammatory cytokines and chemokines are associated with an increased risk of renal carcinoma. To this effect, a number of case-control studies were designed to assess the correlation between renal carcinoma and polymorphisms IL10-1082 A/G (rs 1800896), IL10-592 A/C (rs 1800872), IL10-819 C/T (rs 1800871), IL10-1082 A/G, IL4-590 C/T (rs 2243250), TNF-A-308 A/G (rs 1800629), RANTES-403 G/A (rs 2107538), IL1-A-889 C/T (rs 1800587), MCP-1 2518 G/A (rs 1024611), CTLA-4/+49 A/G (rs 231775) and CTLA-4 CT60 A/G (rs 3087243) in 127 renal carcinoma patients and in 176 healthy subjects. The results obtained in relation to cytokine polymorphism IL-10-1082 A/G indicate that AG heterozygosity status is the principal risk factor in relation to locally advanced or metastatic tumor stage and renal carcinoma. In the case of the molecule CTLA4, the results obtained in renal cancer reveal an association between the polymorphisms of the CTLA-4 gene and an increased risk of developing renal cell carcinoma. A high genotypic frequency of polymorphisms CTLA4/CT60-AA and CTLA4/A49G-AA is observed in patients with renal cell carcinoma versus the controls. An association has been established between polymorphism CTLA4/CT60 and tumor grade in patients with renal cell carcinoma. Logistic regression analysis has confirmed these data, demonstrating a high frequency of the AA genotype in patients with high-grade tumors. The results obtained support the hypothesis that different genetic factors implicated in the regulation of adaptive immune responses, stromal cell composition and local cytokine production levels may be crucial elements in the modification of the clinicopathological parameters of renal carcinoma.

Sanz-Ortega, J., C. Olivier, et al. (2009). "[Hereditary renal cancer]." Actas Urol Esp **33**(2): 127-33.

Kidney cancer is the tenth most common cause of cancer death. There are a growing number of genes known to be associated with an increased risk of specific types of kidney cancer. People with Von Hippel-Lindau syndrome have about a 40% risk of developing multiple bilateral clear cell kidney cancers. They can also develop retinal and brain hemangioblastoma, kidneys or pancreas cysts, pheochromocytoma and endolymphatic sac tumor. Four phenotypes with different renal cancer and pheocromocitoma risk have been described depending on the germline mutation. Hereditary papillary renal cell carcinoma syndrome has type 1 papillary renal cell carcinomas associated with protooncogene c-MET germline mutations. Birt-Hogg-Dube syndrome has FLCN gene mutations associated with fibrofolliculomas, lung cysts with a high risk for spontaneous pneumothorax, and a 15% to 30% risk of kidney cancer (most classified as chromophobe carcinoma, oncocytoma or oncocytic hybrid, but clear cell and papillary kidney cancers have also been reported). Histopathological findings such as oncocytosis and oncocytic hybrids are very unusual outside the syndrome. Hereditary leiomyomatosis and renal cell cancer syndrome shows mutations of Fumarate hydratase gene and cutaneous leiomyomata in 76% of affected individuals, uterine leiomyomata in 100% of females, and unilateral, solitary, and aggressive papillary renal cancer in 10 to 16% of patients. A specific histopathological change is eosinophilic prominent nucleoli with a perinucleolar halo. Tuberous sclerosis complex is one of the most prevalent (1/5.800) hereditary syndromes where renal disease is the second leading cause of death, associated with angiomyolipomas (70%), renal cysts, oncocytomas or clear cell cancer.

Sasaki, M., Y. Tanaka, et al. (2004). "Polymorphisms of the CYP1B1 gene as risk factors for human renal cell cancer." Clin Cancer Res **10**(6): 2015-9.

PURPOSE: CYP1B1 activates various environmental carcinogens in human tissues, including renal tissues. We hypothesize that certain polymorphisms of the CYP1B1 gene are risk factors for renal cell cancer. The rationale for this hypothesis is that chemical procarcinogenic compounds require metabolic activation by oxidative enzymes such as CYP1B1 to be transformed into potentially carcinogenic forms. To test this hypothesis, we investigated the genotypic distributions of six different loci on the CYP1B1 gene and their association with renal cell cancer. EXPERIMENTAL DESIGN: DNA from 211 cases of human renal cell cancer and 200 healthy controls was analyzed by sequence-specific PCR and direct DNA sequencing to determine the genotypic frequencies of six different polymorphic loci on the CYP1B1 gene. RESULTS: The results of this study demonstrate that the frequencies of

genotype 119T/T and genotype 432G/G were significantly higher in renal cell cancer patients compared with healthy normal controls. The relative risks were calculated as 3.01 and 2.17 for genotypes 119T/T and 432G/G, respectively, in renal cell carcinoma patients. These genotypic distributions were also significantly different between male and female patients. The relative risks of genotype 119T/T were calculated as 3.95 in males and 1.92 in females, and the relative risks of genotype 432G/G were calculated as 2.81 in males and 1.35 in females. CONCLUSIONS: The present study demonstrates for the first time that the polymorphisms at codons 119 and 432 may be risk factors for renal cancer, especially in the male population.

Sato, A., M. Oya, et al. (2006). "Survivin associates with cell proliferation in renal cancer cells: regulation of survivin expression by insulin-like growth factor-1, interferon-gamma and a novel NF-kappaB inhibitor." Int J Oncol **28**(4): 841-6.

Although survivin has been widely recognized as an attractive target for cancer therapy, the exact mechanism regarding the regulation of survivin and its effect on cell proliferation have yet to be clearly defined in renal cell carcinoma (RCC). We investigated herein the association between survivin expression and cell proliferation in a RCC cell line, KU19-20. In KU19-20 cells, cell proliferation and survivin expression were significantly induced by IGF-1, whereas they were inhibited by a novel NF-kappaB inhibitor dehydroxymethyl-epoxyquinomicin (DHMEQ) and IFN-gamma. The combination of DHMEQ and IFN-gamma inhibited cell proliferation synergistically with a pronounced attenuation of survivin expression. Furthermore, treatment with survivin-specific siRNA reduced expression of survivin and significantly inhibited cell proliferation. Survivin expression was thus associated with cell proliferation in KU19-20 cells. The regulation of survivin by IFN-gamma and/or an NF-kappaB inhibitor may therefore be a potential treatment modality for RCC.

Schmidt-Wolf, I. G., S. Finke, et al. (1999). "Phase I clinical study applying autologous immunological effector cells transfected with the interleukin-2 gene in patients with metastatic renal cancer, colorectal cancer and lymphoma." Br J Cancer **81**(6): 1009-16.

Natural killer-like T lymphocytes termed cytokine-induced killer (CIK) cells have been shown to eradicate established tumours in a severe combined immune deficient (SCID) mouse/human lymphoma model. Recently, we demonstrated that CIK cells transfected with cytokine genes possess an improved proliferation rate and a significantly higher cytotoxic activity as compared to non-transfected cells. Here, in a phase I clinical protocol, autologous CIK cells were generated from peripheral blood obtained by leukapheresis in patients with metastatic renal cell carcinoma, colorectal carcinoma and lymphoma. CIK cells were transfected with a plasmid containing the interleukin-2 (IL-2) gene via electroporation. Transfected cells generated IL-2 in the range of 330-1800 pg 10(-6) cells 24 h(-1) with a mean of 836 pg 10(-6) cells 24 h(-1). Ten patients received 1-5 intravenous infusions of IL-2-transfected CIK cells; five infusions with transfected CIK cells were given. In addition, the same patients received five infusions with untransfected CIK cells for control reasons. In three patients, WHO grade 2 fever was observed. Based on polymerase chain reaction of peripheral blood transfected cells could be detected for up to 2 weeks after infusion. There was a significant increase in serum levels of interferon gamma (IFN-gamma), granulocyte-macrophage colony-stimulating factor (GM-CSF) and transforming growth factor beta (TGF-beta) during treatment. Interestingly, there was also an increase in CD3+ lymphocytes in the blood of patients during therapy. In accordance, a partial increase in cytotoxic activity in peripheral blood lymphocytes (PBLs) was documented when patient samples before and after therapy were compared. Concerning clinical outcome, six patients remained in progressive disease, three patients showed no change by treatment, and one patient with lymphoma developed a complete response. In conclusion, we were able to demonstrate that CIK cells transfected with the IL-2 gene can be administered without major side-effects and are promising for future therapeutic trials.

Seki, N., A. D. Brooks, et al. (2002). "Tumor-specific CTL kill murine renal cancer cells using both perforin and Fas ligand-mediated lysis in vitro, but cause tumor regression in vivo in the absence of perforin." J Immunol **168**(7): 3484-92.

Kidney cancer is a devastating disease; however, biological therapies have achieved some limited success. The murine renal cancer Renca has been used as a model for developing new preclinical approaches to the treatment of renal cell carcinoma. Successful cytokine-based approaches require CD8(+) T cells, but the exact mechanisms by which T cells mediate therapeutic benefit have not been completely identified. After successful biological therapy of Renca in BALB/c mice, we generated CTLs in vitro using mixed lymphocyte tumor cultures. These CTL mediated tumor-specific H-2K(d)-restricted lysis and production of IFN-gamma, TNF-alpha, and Fas ligand (FasL) in response to Renca. CTL used both granule- and FasL-mediated mechanisms to lyse Renca, although granule-mediated killing was the

predominant lytic mechanism in vitro. The cytokines IFN-gamma and TNF-alpha increased the sensitivity of Renca cells to CTL lysis by both granule- and FasL-mediated death pathways. Adoptive transfer of these anti-Renca CTL into tumor-bearing mice cured most mice of established experimental pulmonary metastases, and successfully treated mice were immune to tumor rechallenge. Interestingly, we were able to establish Renca-specific CTL from mice gene targeted for perforin (pfp(-/-)) mice. Although these pfp(-/-) CTL showed reduced cytotoxic activity against Renca, their IFN-gamma production in the presence of Renca targets was equivalent to that of wild-type CTL, and adoptive transfer of pfp(-/-) CTL was as efficient as wild-type CTL in causing regression of established Renca pulmonary metastases. Therefore, although granule-mediated killing is of paramount importance for CTL-mediated lysis in vitro, some major in vivo effector mechanisms clearly are independent of perforin.

Shears, L., L. Plowright, et al. (2008). "Disrupting the interaction between HOX and PBX causes necrotic and apoptotic cell death in the renal cancer lines CaKi-2 and 769-P." J Urol **180**(5): 2196-201.

PURPOSE: The HOX genes are a family of homeodomain containing transcription factors that determine embryonic tissue identity and also have regulatory and oncogenic roles in adult cells. We quantified the expression of HOX genes in normal kidney tissue, primary tumors and derived cell lines, and examined their role in renal cancer cell survival. MATERIALS AND METHODS: Quantitative polymerase chain reaction was used to evaluate HOX gene expression in cells and tissues. HOX gene function was disrupted using a peptide that blocks the interaction between HOX proteins and their PBX cofactor. Apoptosis was assessed by annexin/propidium iodide staining and direct measurement of caspase activity. RESULTS: Primary renal tumors and derived cell lines showed abnormal HOX gene expression. Furthermore, blocking HOX activity by targeting the interaction between HOX and its cofactor PBX caused apoptotic and necrotic cell death in the renal cancer cell lines CaKi-2 and 769-P, while sparing normal adult kidney cells. CONCLUSIONS: Our findings suggest that the HOX/PBX dimer is a potential therapeutic target in renal cancer.

Shinojima, T., M. Oya, et al. (2007). "Renal cancer cells lacking hypoxia inducible factor (HIF)-1alpha expression maintain vascular endothelial growth factor expression through HIF-2alpha." Carcinogenesis **28**(3): 529-36.

Recent efforts have been aimed at targeting the hypoxia inducible factor (HIF)-mediated hypoxia-induced gene pathway for renal cell carcinomas (RCC) therapy. Among the various genes induced by HIF, vascular endothelial growth factor (VEGF) is one of the critical mediators in angiogenesis, tumor growth and metastasis. To date, however, limited information is available on the functional differences regarding VEGF transcription between the HIF subunits, namely HIF-1alpha and HIF-2alpha. To investigate the HIF-1alpha and HIF-2alpha-dependent effect on VEGF gene induction in RCC, a panel of human RCC cell lines was analyzed. We found that a loss of HIF-1alpha protein expression was a common event in RCC cell lines, which was associated not only with truncated HIF-1alpha mRNA transcripts but also with transcriptional silencing. Since the CpG rich promoter region of the HIF-1alpha gene contained a similar frequency of methylated CpG dinucleotides in RCC cell lines, a complex and non-uniform mechanism may be involved in this phenomenon. In these HIF-1alpha defective cell lines, the knockdown of the HIF-2alpha gene demonstrated that HIF-2alpha regulated the VEGF production, irrespective of the VHL gene mutation status. In contrast, HIF-1alpha played a predominant role in VEGF secretion in the cells expressing both wild-type HIF-1alpha and HIF-2alpha proteins. HIF-1alpha may therefore represent an important target molecule for RCC therapy; however, HIF-2alpha should be targeted in HIF-1alpha defective renal cancer cells.

Shioi, K., A. Komiya, et al. (2006). "Vascular cell adhesion molecule 1 predicts cancer-free survival in clear cell renal carcinoma patients." Clin Cancer Res **12**(24): 7339-46.

PURPOSE: Vascular cell adhesion molecule 1 (VCAM1) is a cell surface glycoprotein implicated in various pathophysiologic conditions. We measured VCAM1 expression levels in tumor tissues and evaluated its significance and prognostic use in renal cell carcinoma (RCC). EXPERIMENTAL DESIGN: We used real-time quantitative PCR to examine the VCAM1 expression levels of a total of 485 sporadic renal tumors, including 429 clear cell, 21 papillary, 17 chromophobe, 11 oncocytomas, and 7 collecting duct carcinomas. We retrospectively examined the relationship of this expression to various clinicopathologic variables and the von Hippel-Lindau alteration status. We evaluated its significance with respect to patient survival rates using the Cox regression model combined with the split-sample method. RESULTS: Compared with normal kidney samples (n = 43), VCAM1 was significantly up-regulated in clear cell RCC and papillary RCC, whereas it was down-regulated in chromophobe RCC

and oncocytoma. In clear cell RCC, VCAM1 expression levels were apparently high in patients asymptomatic at presentation and in patients with small tumor size, low-stage, low-grade, microvascular invasion-negative, and von Hippel-Lindau alteration-positive tumors. Univariate analyses showed that VCAM1 high expression is strongly associated with better outcomes in clear cell and papillary RCCs. Further, Cox multivariate analysis models combined with the split-sample method revealed that this association is significant only in cancer-free survival for patients with clear cell RCC after curative surgical resection. CONCLUSIONS: VCAM1 expression levels were found to be histologically subtype specific in renal tumors. Determination of the VCAM1 expression level as a biomarker can provide useful prognostic information for patients with clear cell RCC.

Skubitz, K. M. and A. P. Skubitz (2002). "Differential gene expression in renal-cell cancer." J Lab Clin Med **140**(1): 52-64.

Renal-cell carcinoma (RCC) is an important cause of morbidity and mortality, and its incidence has been increasing. Malignant transformation is thought to be associated with changes in the expression of several genes, and this alteration in gene expression is believed to be critical to the development of the malignant phenotype. In this study, the expression of about 60,000 genes/expressed sequence tags in clear-cell RCC, normal kidney, and a set of diseased nonmalignant kidneys was determined with the use of the Affymetrix microarray technique, and differences in gene expression were analyzed. Many genes were found to be differentially expressed in these two sample sets. The genes that were expressed greater than four times more in RCC, those expressed only in RCC, and those expressed greater than two times more in RCC and also expressed in a limited number of other tissues were analyzed for their expression in a variety of other normal and diseased tissues. Some of the genes identified were overexpressed only in RCC among the tissues examined, and some were overexpressed in several other malignant tissues in addition to RCC. Other genes were overexpressed in RCC compared with normal kidney but were also overexpressed in diseased nonmalignant kidney or a variety of other normal tissues. All of the RCC samples could be clustered together, separate from the normal and diseased kidney samples, with the use of the Eisen clustering technique and a set of 50 genes. The observed changes in gene expression in RCC should help further the understanding of the biology of RCC and may be useful in diagnosis, treatment, and imaging.

Smith, K., L. Gunaratnam, et al. (2005). "Silencing of epidermal growth factor receptor suppresses hypoxia-inducible factor-2-driven VHL-/- renal cancer." Cancer Res **65**(12): 5221-30.

Inactivating mutations in the von Hippel-Lindau (VHL) tumor suppressor gene are associated with clear cell renal cell carcinoma (VHL-/- RCC), the most frequent malignancy of the human kidney. The VHL protein targets the alpha subunits of hypoxia-inducible factor (HIF) transcription factor for ubiquitination and degradation. VHL-/- RCC cells fail to degrade HIF resulting in the constitutive activation of its target genes, a process that is required for tumorigenesis. We recently reported that HIF activates the transforming growth factor-alpha/epidermal growth factor receptor (TGF-alpha/EGFR) pathway in VHL-defective RCC cells. Here, we show that short hairpin RNA (shRNA)-mediated inhibition of EGFR is sufficient to abolish HIF-dependent tumorigenesis in multiple VHL-/- RCC cell lines. The 2alpha form of HIF (HIF-2alpha), but not HIF-1alpha, drives in vitro and in vivo tumorigenesis of VHL-/- RCC cells by specifically activating the TGF-alpha/EGFR pathway. Transient incubation of VHL-/- RCC cell lines with small interfering RNA directed against EGFR prevents autonomous growth in two-dimensional culture as well as the ability of these cells to form dense spheroids in a three-dimensional in vitro tumor assay. Stable expression of shRNA against EGFR does not alter characteristics associated with VHL loss including constitutive production of HIF targets and defects in fibronectin deposition. In spite of this, silencing of EGFR efficiently abolishes in vivo tumor growth of VHL loss RCC cells. These data identify EGFR as a critical determinant of HIF-2alpha-dependent tumorigenesis and show at the molecular level that EGFR remains a credible target for therapeutic strategies against VHL-/- renal carcinoma.

Smith, W. M., X. P. Zhou, et al. (2001). "Opposite association of two PPARG variants with cancer: overrepresentation of H449H in endometrial carcinoma cases and underrepresentation of P12A in renal cell carcinoma cases." Hum Genet **109**(2): 146-51.

Peroxisome proliferator activated receptor gamma (PPARgamma) is a nuclear hormone receptor that has been shown to regulate differentiation and cell growth. Studies of the differentiative effects of PPARgamma agonists on several cancer cell lines led to the hypothesis that dysfunction of PPARgamma contributes to tumorigenesis. These functional observations were strengthened by genetic evidence: somatic loss-of-function mutations in PPARG, encoding PPARgamma, in sporadic colorectal carcinomas and somatic translocation of PAX8 and

PPARG in follicular thyroid carcinoma. Recently overrepresentation of the H449H variant was found in a cohort of American patients with glioblastoma multiforme. The glioblastoma multiforme data suggest that PPARG contributes common, low-penetrance alleles for cancer susceptibility. To test this hypothesis in a broader range of cancers we examined a series of carcinomas of the cervix, endometrium, ovary, prostate, and kidney for germline sequence variation in PPARG. In addition to the two common sequence variants, P12A and H449H, there were five other sequence variants. P12A alleles were underrepresented in renal cell carcinoma patients compared to country-of-origin race-matched controls (3.75% vs. 12.1%, P<0.04). In contrast, the H449H variant was overrepresented in individuals with endometrial carcinoma compared to controls (14.4% vs. 6.25%, P<0.02). These observations lend genetic evidence consistent with our hypothesis that PPARG serves as a common, low-penetrance susceptibility gene for cancers of several types, especially those epidemiologically associated with obesity and fat intake.

Smits, K. M., L. J. Schouten, et al. (2008). "Genetic and epigenetic alterations in the von hippel-lindau gene: the influence on renal cancer prognosis." Clin Cancer Res **14**(3): 782-7.

BACKGROUND: Inactivation of the von Hippel-Lindau (VHL) gene is considered as an early event in renal cancer tumorigenesis. The prognostic relevance of these changes, however, is not clear and previous results are contradictory. We have evaluated the influence of (epi)genetic alterations in VHL on cause-specific survival in clear-cell renal cell cancer (ccRCC) in a large, population-based group of cases. METHODS: One hundred and eighty-five cases of ccRCC, identified in the Netherlands Cohort Study on diet and cancer diagnosed in the period 1986 to 1997, were included in the analyses. Mortality information until December 2005, including causes of death, were obtained for all cases through linkage with the Central Bureau of Statistics. VHL mutations were determined with PCR single-strand conformational polymorphism and direct sequencing. VHL methylation was determined with methylation-specific PCR. Kaplan-Meier analyses and Cox proportional hazards models were used to assess associations between VHL alterations and cause-specific mortality. RESULTS: Median follow-up in our population was 6 years. The frequency of loss of function mutations and methylation, separately or combined, did not differ statistically significant between different cancer stages or between tumors with different sizes. We observed no influence of loss of function mutations or methylation of the VHL gene on cause-specific mortality (hazard ratio, 1.08; 95% confidence interval, 0.69-1.68, P = 0.735) as compared with patients with a wild-type or silent mutation in VHL. DISCUSSION: Our results indicate that (epi)genetic alterations in the VHL gene do not have prognostic value in ccRCC.

Smits, K. M., L. J. Schouten, et al. (2008). "Polymorphisms in genes related to activation or detoxification of carcinogens might interact with smoking to increase renal cancer risk: results from The Netherlands Cohort Study on diet and cancer." World J Urol **26**(1): 103-10.

Metabolic gene polymorphisms have previously been suggested as risk factors for renal cell carcinoma (RCC). These polymorphisms are involved in activation or detoxification of carcinogens in cigarette smoke which is another RCC risk factor. We evaluated gene-environment interactions between CYP1A1, GSTmicro1 and smoking in a large population-based RCC case group. The Netherlands Cohort Study on diet and cancer (NLCS) comprises 120,852 persons who completed a questionnaire on smoking and other risk factors at baseline. After 11.3 years of follow-up, 337 incident RCC cases were identified. DNA was collected for 245 cases. In a case-only analysis, interaction-odds ratios (OR) and 95% confidence intervals (95% CI) were calculated using logistic regression. We observed a moderate, not statistically significant, interaction between current smoking and CYP1A1*2C (OR 1.42; 95% CI 0.70-2.89) and GSTmicro1 null (OR 1.35; 95% CI 0.65-2.79). For current smokers with both a variant (heterozygous or homozygous) in CYP1A1 and GSTmicro1 null, risk was also increased (OR 1.63; 95% CI 0.63-4.24). No interaction was observed between ever smokers, smoking duration (increments of 10 smoking years) or amount (increments of 5 cigarettes/day) and CYP1A or GSTmicro1. Our results show a modest trend towards a statistically significant gene-environment interaction between CYP1A1, GSTmicro1 and smoking in RCC. This could indicate that RCC risk among smokers might be more increased with the CYP1A1*2C genotype, GSTmicro1 null, or both a CYP1A1 variant and GSTmicro1 null.

Smyth, A., H. M. Reid, et al. (2007). "Modifications of the radiosensitivity of a renal cancer cell line as a consequence of stable TIMP-1 overexpression." Int J Radiat Biol **83**(1): 13-25.

PURPOSE: To investigate the potential effects of stable tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) overexpression on DNA damage and cell killing following low-dose gamma-radiation and whether this up-regulation interfered with the activation of the matrix

metalloproteinase -2 (MMP-2) and -9 (MMP-9) in a highly metastatic renal carcinoma cell line. MATERIALS AND METHODS: Stable transfections were carried out using the cytomegalovirus expression plasmid pRc/CMV carrying TIMP-1 cDNA and LIPOFECTAMINE reagent. TIMP-1 expression in selected clones was determined by reverse transcriptase-polymerase chain reaction (RT-PCR) and Western blot analysis. Exponentially growing Caki-1 cells were treated with sub lethal doses of ionizing radiation (0- 10Gy) either alone or following stable TIMP-1 transfection. DNA damage was assessed by the Alkaline Comet Assay and cell survival was determined by a clonogenic assay. Caki-1 cell cycle alterations following TIMP-1 transfection were assessed by fluorescence activated cell sorting (FACS) analysis of propidium iodide (PI)-stained cells. The interactions between TIMP-1 and MMP-2 and MMP-9 were analysed 24 hours post-irradiation by means of gelatin zymography. RESULTS: Three clones with varying degrees of TIMP-1 expression were selected and used for further analysis. TIMP-1 transfected Caki-1 cells displayed significantly higher mean tail moment values ($p < 0.05$) following irradiation at doses between 5 and 10 Gy relative to that seen with radiation alone. The TIMP-1 radiosensitizing effect was accompanied by large decreases in the survival fraction of the parental Caki-1 cell line and significant increases in the alpha-parameter of the linear-quadratic fit. These effects were directly correlated to the degree of TIMP-1 gene expression detected in the selected clones. Interestingly, elevated levels of TIMP-2 protein were detected in the three TIMP-1 clones compared to TIMP-2 levels present in Caki-l cells. The three clones also displayed marked phenotypic alterations relative to their parental cell line. Significant increases in the percentage of cells arrested in the G2/M phase of the cell cycle were detected in the three clones under normal growth conditions and reduced serum conditions ($p < 0.05$). When the TIMP-1 clones were assessed for their MMP-2 activity, a marked decrease in the MMP-2 mean protein levels was detected in clone T1-3 following irradiation at doses between 2 and 6 Grays (Gy) ($p < 0.01$) and clone T1-2 at 2- 5Gy ($p < 0.05$). MMP-9 activity was differentially affected by ionizing radiation in the three TIMP-1 clones. T1-3 and TI-2 displayed significantly reduced MMP-9 levels at various dose points whereas T1-1 exhibited elevated levels of MMP-9 activity at higher doses of treatment ($p < 0.05$). CONCLUSION: These results demonstrate a dual role for the TIMP-1 overexpression in this renal carcinoma cell line, both as radiosensitizing agents and effectors of MMP-2 and MMP-9 activity.

Sommerer, C., W. Hartschuh, et al. (2008). "Pharmacodynamic immune monitoring of NFAT-regulated genes predicts skin cancer in elderly long-term renal transplant recipients." Clin Transplant **22**(5): 549-54.

INTRODUCTION: Among elderly allograft recipients non-melanoma skin cancer (NMSC) is the most common malignancy. We have previously shown that malignancies are associated with a higher intensity of ciclosporin A (CsA)-induced immunosuppression. METHOD: Fifty-five long-term elderly renal transplant patients with a stable transplant function had regular skin examinations. The expression of the nuclear factor of activated T cells (NFAT)-regulated genes (interleukin-2, granulocyte-macrophage colony stimulating-factor, interferon-gamma) was determined by real-time PCR at CsA trough levels and two h after oral intake. RESULTS: The CsA dose was 2.0 mg/kg (0.95-3.50), with CsA trough level (C0) level 97 microg/L (33-157) and CsA two-h level (C2) 538 microg/L (350-1228). NMSC was diagnosed in 14/55 patients (25.4%). A total of 85.7% of allograft recipients with NMSC were male ($p < 0.005$). Age, time after transplantation, CsA dose, CsA C0 and C2 level were comparable in both groups. NFAT-regulated gene expression was signficantly lower in patients with skin cancer compared with patients without skin cancer [4.94% (0.91-13.4) vs. 11.6% (3.3-40.8), $p < 0.001$). CONCLUSION: The unproportional high incidence of NMSC in elderly long-term kidney-transplanted patients correlates with a lower NFAT-regulated gene expression which is a surrogate biomarker for a higher degree of functional immunosuppression. Further studies are required to determine whether the reduction of CsA with an increased NFAT-regulated gene expression is associated with a lower NMSC incidence.

Sosman, J. A., I. Puzanov, et al. (2007). "Opportunities and obstacles to combination targeted therapy in renal cell cancer." Clin Cancer Res **13**(2 Pt 2): 764s-769s.

The treatment of advanced renal cell carcinoma (RCC) has undergone a major change with the development of potent angiogenesis inhibitors and targeted agents. Several multitargeted tyrosine kinase inhibitors, sorafenib and sunitinib, have already been approved for the treatment of advanced RCC. Temsirolimus (CCI-779), a mammalian target of rapamycin inhibitor, has shown a survival advantage over IFN in advanced, poor-prognosis RCC patients. Bevacizumab, an antibody targeting vascular endothelial growth factor (VEGF) A, has also shown promising clinical activity. Benefits attributable to these agents have been recognized by high objective response rates (sunitinib), significant increases in

progression-free survival (sunitinib, sorafenib and bevacizumab), or improved overall survival (temsirolimus). These agents mediate much of their effect through inhibition of the hypoxia-inducible factor (HIF)-VEGF-VEGF receptor axis. Their inhibitory activity for the signaling of platelet-derived growth factor (PDGF) receptor beta or kinases like c-Raf may contribute to the antitumor effects of the multitargeted kinase inhibitors. Nevertheless, all four single agents rarely, if ever, induce complete responses and, at present, all patients develop resistance and, ultimately, progress during therapy. A critical need exists to develop strategies that may increase the degree of the antitumor effects with the hope of inducing more complete responses impeding the onset of or elimination of refractory disease. Combinations of these and other targeted agents may overcome the resistance that develops with single-agent therapy and could be incorporated either as part of initial therapy or later when disease resistance develops. Approaches aimed at combining these agents can be based on the genetics and biology of clear cell RCC. von Hippel-Lindau loss leads to an increase in cellular levels of HIF (HIF-1alpha or HIF-2alpha) leading to increased expression of a number of hypoxia-regulated genes critical to cancer progression. Combinations of targeted agents may block several of these mediators (VEGF, epidermal growth factor receptor, and PDGF), so-called horizontal blockade. Blockade could also take place at two levels of the pathways (vertical blockade), either at HIF and VEGF or at VEGF and VEGF receptor signaling. Many of the above strategies are ongoing and will require careful phase 1 determination of toxicity and even more rigorous phase 2 analysis before moving onto phase 3 trials.

Stewart, L., G. M. Glenn, et al. (2008). "Association of germline mutations in the fumarate hydratase gene and uterine fibroids in women with hereditary leiomyomatosis and renal cell cancer." Arch Dermatol **144**(12): 1584-92.

OBJECTIVE: To investigate the risk of uterine fibroids and other reproductive risk factors in women with hereditary leiomyomatosis and renal cell cancer (HLRCC). DESIGN: Case-control study. SETTING: National Institutes of Health, Rockville, Maryland. Patients A family-based case-control study was conducted between July 1, 2004, and June 30, 2006, including 105 women from families with HLRCC ascertained throughout North America. A telephone interview was conducted with all participants using a standardized questionnaire that elicited information about their menstrual, pregnancy, uterine fibroid, and hormonal contraceptive use history. Diagnosis of uterine fibroids was confirmed by pathologic diagnosis and by medical record review. DNA was extracted from blood samples and was screened for germline mutations in the fumarate hydratase (FH) gene. MAIN OUTCOME MEASURES: FH germline mutation status, presence of uterine fibroids, age at diagnosis, and symptoms and treatment of uterine fibroids. RESULTS: Of 105 women, 77 reported a history of uterine fibroids. Regardless of uterine fibroid status, 75 of 105 women had a germline mutation in FH (FH(mut) positive). The risk of uterine fibroids in FH(mut)-positive women was statistically significantly increased compared with that in FH(mut)-negative women (odds ratio [OR], 7.6; 95% confidence interval [CI], 2.9-20.0), as it was among women clinically affected with HLRCC compared with those clinically unaffected with HLRCC (8.6; 3.1-24.0). The median age at uterine fibroid diagnosis for FH(mut)-positive women (28 years) was significantly younger than that for FH(mut)-negative women (38 years) (P =.03). Women with a germline mutation in FH or clinically affected with HLRCC reported younger age at menarche (P < .004) compared with FH(mut)-negative women (P = .02) or women who were clinically unaffected with HLRCC. Women with HLRCC were more likely to have had treatment for uterine fibroids (OR, 4.6; 95% CI, 1.4-15.8), including hysterectomy (P =.02) at an earlier age compared with women who were clinically unaffected with HLRCC. CONCLUSIONS: This study provides the first evidence (to our knowledge) that women with germline mutations in FH and with clinical HLRCC have an increased risk of developing uterine fibroids. These women also have a younger age at uterine fibroid diagnosis and are more likely to have treatment for uterine fibroids at a younger age than women without HLRCC in their families.

Sudarshan, S., W. M. Linehan, et al. (2007). "HIF and fumarate hydratase in renal cancer." Br J Cancer **96**(3): 403-7.

Hereditary leiomyomatosis and renal cell cancer is a recently described hereditary cancer syndrome in which affected individuals are predisposed to the development of leiomyomas of the skin and uterus. In addition, this clinical entity also can result in the development of biologically aggressive kidney cancer. Affected individuals harbour a germline mutation of the fumarate hydratase (FH) gene, which encodes an enzyme that catalyses conversion of fumarate to malate in the Kreb's cycle. Thus far, proposed mechanisms for carcinogeneis associated with this syndrome include aberrant apoptosis, oxidative stress, and pseudohypoxic drive. At this time, the majority of accumulating data support a role for pseudohypoxic drive in tumour development. The link between FH mutation and

pseudohypoxic drive may reside in the biochemical alterations resulting from diminished/absent FH activity. These biochemical derangements may interfere with oxygen homeostasis and result in a cellular environment conducive to tumour formation.

Sudarshan, S., P. A. Pinto, et al. (2007). "Mechanisms of disease: hereditary leiomyomatosis and renal cell cancer--a distinct form of hereditary kidney cancer." Nat Clin Pract Urol **4**(2): 104-10.

Renal cell carcinoma (RCC) represents a group of diseases linked by their primary site of origin, the kidney. Studies of families with a genetic predisposition to the development of kidney cancer have revealed that multiple genes are involved in the molecular pathogenesis of RCC. Germline mutations in a gene that encodes a Krebs cycle enzyme have been found to result in a distinct clinical entity referred to as hereditary leiomyomatosis and renal cell cancer (HLRCC). HLRCC is inherited in an autosomal-dominant fashion. Affected individuals in HLRCC families are at risk for the development of leiomyomas of the skin and uterus as well as renal cancers. HLRCC-associated kidney tumors are often biologically aggressive. Linkage analysis has identified germline alterations in the fumarate hydratase (FH) gene associated with HLRCC. While the mechanisms of molecular carcinogenesis are not entirely understood, several lines of evidence derived from clinical and basic research suggest that pseudohypoxia might drive cellular transformation. The role of FH mutations in sporadic tumors seems to be limited. Nevertheless, continued investigation of HLRCC should provide further insight into the mechanisms of kidney cancer development, and could potentially identify targets for new therapeutic approaches to RCC.

Sudarshan, S., C. Sourbier, et al. (2009). "Fumarate hydratase deficiency in renal cancer induces glycolytic addiction and hypoxia-inducible transcription factor 1alpha stabilization by glucose-dependent generation of reactive oxygen species." Mol Cell Biol **29**(15): 4080-90.

Hereditary leiomyomatosis and renal cell cancer (HLRCC) is an inherited cancer syndrome linked to biallelic inactivation of the gene encoding the tricarboxylic acid cycle enzyme fumarate hydratase (FH). Individuals with HLRCC are at risk to develop cutaneous and uterine leiomyomas and an aggressive form of kidney cancer. Pseudohypoxic drive-the aberrant activation of cellular hypoxia response pathways despite normal oxygen tension-is considered to be a likely mechanism underlying the etiology of this tumor. Pseudohypoxia requires the oxygen-independent stabilization of the alpha subunit of the hypoxia-inducible transcription factor (HIF-1alpha). Under normoxic conditions, proline hydroxylation of HIF-1alpha permits VHL recognition and subsequent targeting for proteasomal degradation. Here, we demonstrate that inactivating mutations of FH in an HLRCC-derived cell line result in glucose-mediated generation of cellular reactive oxygen species (ROS) and ROS-dependent HIF-1alpha stabilization. Additionally, we demonstrate that stable knockdown of FH in immortalized renal epithelial cells results in ROS-dependent HIF-1alpha stabilization. These data reveal that the obligate glycolytic switch present in HLRCC is critical to HIF stabilization via ROS generation.

Tamura, T., T. Nishi, et al. (2001). "Intratumoral delivery of interleukin 12 expression plasmids with in vivo electroporation is effective for colon and renal cancer." Hum Gene Ther **12**(10): 1265-76.

We report on an antitumor treatment involving electrogene therapy (EGT), a newly developed in vivo gene transfer method using electroporation. We carried out in vivo EGT in a subcutaneous model of CT26 colon carcinoma cells, using plasmid DNAs encoding interleukin 12 (IL-12) subunits. For this purpose, we developed two IL-12 expression systems: a cotransfer system using a plasmid encoding the IL-12 p40 subunit and a plasmid encoding the IL-12 p35 subunit, and a single-vector system using a plasmid expressing a p40-p35 fusion protein. Both transfer systems significantly inhibited the growth of CT26 tumor. Immunohistochemical analysis of IL-12 EGT-treated tumors revealed enhanced infiltration of CD8(+) cells into the tumor tissue, while reverse transcriptase-polymerase chain reaction confirmed the increased expression of interferon gamma within treated tumors. The same IL-12 EGT applied to the nude mouse model was not effective, suggesting the critical role of T cell infiltration in this treatment. The inhibitory effects revealed in experiments in which previously treated mice were rechallenged with a second inoculation of CT26 tumor cells suggested that IL-12 EGT may also establish partial systemic antitumor immunity. The growth of IL-12 EGT-treated Renca tumors, a renal cell carcinoma, was also significantly inhibited. These findings suggest that EGT of the IL-12 gene has the potential to be an effective anticancer gene therapy.

Tanaka, Y., H. Hirata, et al. (2007). "Polymorphisms of catechol-O-methyltransferase in men with renal cell cancer." Cancer Epidemiol Biomarkers Prev **16**(1): 92-7.

The estrogen metabolite, 4-hydroxy-estrogen, has been shown to play a role in malignant transformation of male kidneys. To counteract the

effects of this catechol-estrogen, the catechol-O-methyltransferase (COMT) enzyme is capable of neutralizing the genotoxic effects of this compound. A polymorphic variant of COMT has been shown to have a reduced enzyme activity, and thus, we hypothesize that single nucleotide polymorphisms of the COMT gene can be a risk factor for renal cell cancer (RCC). To determine this hypothesis, a study of a Japanese male population was used and the genetic distributions of COMT polymorphisms at codons 62 (C-->T), 72 (G-->T), and 158 (G-->A) were analyzed in 157 normal healthy subjects and 123 sporadic RCC (clear cell type) samples by using a sequence-specific PCR technique. These experiments show that the variant genotype (P = 0.025) and allele (P = 0.011) at codon 62 is a risk factor for RCC. The odds ratio and 95% confidence interval for cancer were 3.16 and 1.29 to 7.73, respectively, for the T/T genotype as compared with wild-type. No associations for renal cancer were found at either codons 72 or 158 in this Japanese male population. However, codons 62 and 158 were observed to be in linkage disequilibrium, and haplotype analysis shows the combined forms of T-A, T-G, and C-A to be associated with RCC as compared with C-G (P < 0.001). When evaluating the risk of COMT polymorphisms with grade of cancer, no associations were observed for any of the genotypes. This study is the first to report COMT polymorphism to be associated with RCC. These results are important in understanding the role of COMT polymorphisms in the pathogenesis of RCC.

Tani, K., M. Azuma, et al. (2004). "Phase I study of autologous tumor vaccines transduced with the GM-CSF gene in four patients with stage IV renal cell cancer in Japan: clinical and immunological findings." Mol Ther **10**(4): 799-816.

We produced lethally irradiated retrovirally GM-CSF-transduced autologous renal tumor cell vaccines (GVAX) from six Japanese patients with stage IV renal cell cancer (RCC). Four patients received GVAX ranging from 1.4 x 10(8) to 3.7 x 10(8) cells on 6-17 occasions. Throughout a total of 48 vaccinations, there were no severe adverse events. After vaccination, DTH skin tests became positive to autologous RCC (auto-RCC) in all patients. The vaccination sites showed significant infiltration by CD4(+) T cells, eosinophils, and HLA-DR-positive cells. The kinetic analyses of cellular immune responses using peripheral blood lymphocytes revealed an enhanced proliferative response against auto-RCC in four patients, and cytotoxicity against auto-RCC was augmented in three patients. T cell receptor beta-chain analysis revealed oligoclonal expansion of T cells in the peripheral blood, skin biopsy specimens from DTH sites, and tumors. Western blot analysis demonstrated the induction of a humoral immune response against auto-RCC. Two of the four patients are currently alive 58 and 40 months after the initial vaccination with low-dose interleukin-2. Our results suggest that GVAX substantially enhanced the antitumor cellular and humoral immune responses, which might have contributed to the relatively long survival times of our patients in the present study.

Tani, K., Y. Nakazaki, et al. (2000). "Progress reports on immune gene therapy for stage IV renal cell cancer using lethally irradiated granulocyte-macrophage colony-stimulating factor-transduced autologous renal cancer cells." Cancer Chemother Pharmacol **46 Suppl**: S73-6.

There is no effective treatment for patients with stage IV renal cell cancer (RCC), although the introduction of new therapy is imminent. Cancer gene therapy is currently considered to be one of the most promising therapeutic modalities in the field of cancer treatment. Based on the results of animal studies, vaccination using autologous granulocyte-macrophage colony-stimulating factor-transduced renal cancer cells appears promising. Before initiating a clinical study using an ex vivo gene-transduced autologous cell vaccine-based immunogene therapy for RCC in Japan, in 1992 we initially planned a Japanese version of a clinical protocol in collaboration with a US group. In 1993, the original protocol was refined. We performed five preclinical qualification studies using RCC nephrectomy specimens from patients in 1997, and the results showed that preparation of RCC cells for autologous vaccines at the Clinical Cell Technology Facility, Research Hospital of the Institute of Medical Science, University of Tokyo, was feasible. Subsequently in August 1998, the Ministry of Health and Welfare and the Ministry of Education, Science, Culture, and Sport approved our clinical protocol. We have recruited two patients with stage IV RCC to our study so far. Here we report the background to the initiation of cancer gene therapy in Japan.

Thrash-Bingham, C. A. and K. D. Tartof (1999). "aHIF: a natural antisense transcript overexpressed in human renal cancer and during hypoxia." J Natl Cancer Inst **91**(2): 143-51.

BACKGROUND: Nonpapillary renal carcinoma is the predominant form of human kidney cancer and represents a distinct disease entity, morphologically and molecularly, from papillary renal carcinoma. We have discovered a natural antisense transcript that is complementary to the 3' untranslated region of hypoxia inducible factor alpha (HIF1alpha)

messenger RNA (mRNA) and is strikingly overexpressed specifically in nonpapillary kidney cancer. HIF1alpha encodes a protein that is known to have two important functions: 1) to act as a transcription factor for hypoxia inducible genes and 2) to stabilize p53 protein during hypoxia. Because of the importance of HIF1alpha, we have characterized this natural antisense transcript, which we have named "aHIF." METHODS: Differential display, reverse transcription-polymerase chain reaction, ribonuclease protection, and DNA-sequencing methods were used in our analysis. RESULTS AND CONCLUSIONS: We show the following: 1) aHIF is a natural antisense transcript derived from HIF1alpha gene sequences encoding the 3' untranslated region of HIF1alpha mRNA; 2) aHIF is specifically overexpressed in all nonpapillary clear-cell renal carcinomas examined, but not in the papillary renal carcinomas examined; 3) aHIF is overexpressed in an established nonpapillary renal carcinoma cell line under both normoxic (i.e., normal aerobic) and hypoxic conditions; and 4) although aHIF is not further induced by hypoxia in nonpapillary disease, it can be induced in lymphocytes where there is a concomitant decrease in HIF1alpha mRNA. To our knowledge, this is the first case of overexpression of a natural antisense transcript exclusively associated with a specific human malignant disease.

Tolle, A., M. Jung, et al. (2009). "Brain-type and liver-type fatty acid-binding proteins: new tumor markers for renal cancer?" BMC Cancer **9**: 248.

BACKGROUND: Renal cell carcinoma (RCC) is the most common renal neoplasm. Cancer tissue is often characterized by altered energy regulation. Fatty acid-binding proteins (FABP) are involved in the intracellular transport of fatty acids (FA). We examined the level of brain-type (B) and liver-type (L) FABP mRNA and the protein expression profiles of both FABPs in renal cell carcinoma. METHODS: Paired tissue samples of cancerous and noncancerous kidney parts were investigated. Quantitative RT-PCR, immunohistochemistry and western blotting were used to determine B- and L-FABP in tumor and normal tissues. The tissue microarray (TMA) contained 272 clinico-pathologically characterized renal cell carcinomas of the clear cell, papillary and chromophobe subtype. SPSS 17.0 was used to apply crosstables (chi2-test), correlations and survival analyses. RESULTS: B-FABP mRNA was significantly up-regulated in renal cell carcinoma. In normal tissue B-FABP mRNA was very low or often not detectable. RCC with a high tumor grading (G3 + G4) showed significantly lower B-FABP mRNA compared with those with a low grading (G1 + G2). Western blotting analysis detected B-FABP in 78% of the cases with a very strong band but in the corresponding normal tissue it was weak or not detectable. L-FABP showed an inverse relationship for mRNA quantification and western blotting. A strong B-FABP staining was present in 52% of the tumor tissues contained in the TMA. In normal renal tissue, L-FABP showed a moderate to strong immunoreactivity in proximal tubuli. L-FABP was expressed at lower rates compared with the normal tissues in 30.5% of all tumors. There was no correlation between patient survival times and the staining intensity of both FABPs. CONCLUSION: While B-FABP is over expressed in renal cell carcinoma in comparison to normal renal tissues L-FABP appears to be reduced in tumor tissue. Although the expression behavior was not related to the survival outcome of the RCC patients, it can be assumed that these changes indicate fundamental alterations in the fatty metabolism in the RCC carcinogenesis. Further studies should identify the role of both FABPs in carcinogenesis, progression and with regard to a potential target in RCC.

Toloczko-Grabarek, A., A. Sikorski, et al. (2005). "Nuclear Pedigree Criteria for the Identification of Individuals Suspected to be at Risk of an Inherited Predisposition to Renal Cancer." Hered Cancer Clin Pract **3**(3): 129-34.

Renal clear cell carcinomas represent about 3% of all visceral cancers and account for approximately 85% of renal cancers in adults. Environmental and genetic factors are involved in the development of renal cancer. Although to date there are 19 hereditary syndromes described in which renal cell cancer may occur, only four syndromes with an unequivocal genetic predisposition to renal cell carcinoma have been identified: VHL syndrome (mutations in the VHL gene), hereditary clear cell carcinoma (translocations t(3:8), t(2:3)), hereditary papillary carcinoma (mutations in the MET protooncogene) and tuberous sclerosis (mutations in the TSC1 and TSC2 genes). Little is known genetically about the other forms of familial renal cell cancer. Since there is a growing awareness about the necessity of early intervention, clinical criteria have been developed that aid in the identification of hereditary forms of renal cancer. The aim of the current study was to identify minimal inclusion criteria so that nuclear pedigree families can be ascertained for risk assessment and/or kidney tumour screening. The results reveal that inclusion features described herein, such as (a) renal clear cell cancer diagnosed before 55 years of age, and (b) renal clear cell cancer and gastric cancer or lung cancer among

first degree relatives, are useful in identifying suspected hereditary clear cell renal cancer patients.

Tomlinson, I. P., N. A. Alam, et al. (2002). "Germline mutations in FH predispose to dominantly inherited uterine fibroids, skin leiomyomata and papillary renal cell cancer." Nat Genet **30**(4): 406-10.

Uterine leiomyomata (fibroids) are common and clinically important tumors, but little is known about their etiology and pathogenesis. We previously mapped a gene that predisposes to multiple fibroids, cutaneous leiomyomata and renal cell carcinoma to chromosome 1q42.3-q43 (refs 4-6). Here we show, through a combination of mapping critical recombinants, identifying individuals with germline mutations and screening known and predicted transcripts, that this gene encodes fumarate hydratase, an enzyme of the tricarboxylic acid cycle. Leiomyomatosis-associated mutations are predicted to result in absent or truncated protein, or substitutions or deletions of highly conserved amino acids. Activity of fumarate hydratase is reduced in lymphoblastoid cells from individuals with leiomyomatosis. This enzyme acts as a tumor suppressor in familial leiomyomata, and its measured activity is very low or absent in tumors from individuals with leiomyomatosis. Mutations in FH also occur in the recessive condition fumarate hydratase deficiency, and some parents of people with this condition are susceptible to leiomyomata. Thus, heterozygous and homozygous or compound heterozygous mutants have very different clinical phenotypes. Our results provide clues to the pathogenesis of fibroids and emphasize the importance of mutations of housekeeping and mitochondrial proteins in the pathogenesis of common types of tumor.

Toro, J. R., M. L. Nickerson, et al. (2003). "Mutations in the fumarate hydratase gene cause hereditary leiomyomatosis and renal cell cancer in families in North America." Am J Hum Genet **73**(1): 95-106.

Hereditary leiomyomatosis and renal cell cancer (HLRCC) is an autosomal dominant disorder characterized by smooth-muscle tumors of the skin and uterus and/or renal cancer. Although the identification of germline mutations in the fumarate hydratase (FH) gene in European families supports it as the susceptibility gene for HLRCC, its role in families in North America has not been studied. We screened for germline mutations in FH in 35 families with cutaneous leiomyomas. Sequence analysis revealed mutations in FH in 31 families (89%). Twenty different mutations in FH were identified, of which 18 were novel. Of these 20 mutations, 2 were insertions, 5 were small deletions that caused frameshifts leading to premature truncation of the protein, and 13 were missense mutations. Eleven unrelated families shared a common mutation: R190H. Eighty-one individuals (47 women and 34 men) had cutaneous leiomyomas. Ninety-eight percent (46/47) of women with cutaneous leiomyomas also had uterine leiomyomas. Eighty-nine percent (41/46) of women with cutaneous and uterine leiomyomas had a total hysterectomy, 44% at age < or =30 years. We identified 13 individuals in 5 families with unilateral and solitary renal tumors. Seven individuals from four families had papillary type II renal cell carcinoma, and another individual from one of these families had collecting duct carcinoma of the kidney. The present study shows that mutations in FH are associated with HLRCC in North America. HLRCC is associated with clinically significant uterine fibroids and aggressive renal tumors. The present study also expands the histologic spectrum of renal tumors and FH mutations associated with HLRCC.

Toschi, A., J. Edelstein, et al. (2008). "HIF alpha expression in VHL-deficient renal cancer cells is dependent on phospholipase D." Oncogene **27**(19): 2746-53.

Loss of the von Hippel-Lindau (VHL) tumor suppressor gene contributes to proliferative disorders including renal cell carcinoma. The consequence of VHL loss is increased levels of hypoxia-inducible factor-alpha (HIFalpha), which is targeted for proteolytic degradation by the VHL gene product pVHL. HIF is a transcription factor that increases the expression of factors critical for tumorigenesis in renal cell carcinoma. We report here another regulatory component of HIFalpha expression in renal cancer cells. Phospholipase D (PLD), which is commonly elevated in renal and other cancers, is required for elevated levels of both HIF1alpha and HIF2alpha in VHL-deficient renal cancer cells. The induction of both HIF1alpha and HIF2alpha by hypoxic mimetic conditions was also dependent on PLD in renal cancer cells with restored pVHL expression. The effect of PLD activity upon HIFalpha expression was at the level of translation. PLD activity also provides a survival signal that suppresses apoptosis induced by serum deprivation in the renal cancer cells. Suppression of HIF2alpha has been shown to reverse tumorigenesis with renal cancer cells. The finding here that HIF2alpha expression is dependent on PLD in renal cancer cells suggests that targeting PLD signals may represent an alternative therapeutic strategy for targeting HIF2alpha in renal cancers where HIF2alpha is critical for tumorigenesis and elevated PLD activity is common.

Trigo, J. M. and J. Bellmunt (2008). "[Current strategies in the treatment of renal-cell cancer: targeted therapies]." Med Clin (Barc) **130**(10): 380-92.

Renal-cell carcinoma represents 95% of all renal tumours. The Von Hippel-Lindau (VHL) tumor-suppressor gene is mutated or silenced in most clear cell renal carcinomas. pVHL loss results in the stabilization of the heterodimeric transcription factor hypoxia-inducible factor (HIF) and enhanced transactivation of HIF target genes. HIF itself has been difficult to inhibit with drug-like molecules although a number of agents that indirectly inhibit HIF, including mTOR (mammalian target of rapamycin) inhibitors, have been identified. Moreover, a number of drugs have been developed that target HIF-responsive gene products, such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF), implicated in tumor angiogenesis. Many of these targeted therapies, especially sunitinib, have demonstrated significant activity in kidney cancer clinical trials and represent a substantive advance in the treatment of this disease.

Trinder, P., U. Seitzer, et al. (1999). "Constitutive and IFN-gamma regulated expression of IL-7 and IL-15 in human renal cell cancer." Int J Oncol **14**(1): 23-31.

Although not structurally related, the pleiotropic cytokines interleukin-7 (IL-7) and interleukin-15 (IL-15) share a variety of biological functions including stimulation and maintenance of cellular immune responses. Cytokines, such as IL-7 or IL-15, elaborated by cells in situ, e.g. cancer cells, may be involved in shaping the quality of anti-tumor directed immune responses. We have analysed the constitutive and IFN-gamma-inducible expression of IL-15 or IL-7 mRNA, protein expression, and protein secretion in human tumor cell lines of distinct origin. IL-15 mRNA expression was detected in renal cell carcinoma (RCC), small cell lung carcinoma (SCLC), glioblastoma, neuroblastoma, mesothelioma cells and in EBV-transformed B-lymphocytes. IL-7-specific transcripts could be detected in colorectal cancer and in renal cell cancer cell lines. Immunohistochemical analysis demonstrated cytosolic IL-15 protein expression in renal cell cancer cells without apparent IL-15 protein secretion in vitro. Time kinetic analyses revealed that IFN-gamma mediated increase of IL-15 mRNA expression was transcriptionally regulated and dependent on de novo protein synthesis. However, enhanced IL-15 mRNA expression did not lead to effective protein secretion. In contrast, IL-7 mRNA expression in renal cell cancer or in colorectal cancer was associated with effective protein secretion which could be augmented by IFNgamma-treatment. These data suggest that both IL-7 and IL-15 mRNA are expressed in renal cell cancer, but exclusively IL-7 may be elaborated by tumor cells in situ. IL-15 regulation appears to be tightly controlled both at the transcriptional and post-transcriptional level. Appropriate stimuli leading to effective IL-15 secretion from tumor cells may aid in modulating cellular immune responses directed against cancer.

Tsao, C. C., B. T. Teh, et al. (2008). "Inhibition of Mxi1 suppresses HIF-2alpha-dependent renal cancer tumorigenesis." Cancer Biol Ther **7**(10): 1619-27.

In clear cell renal cancers, the primary molecular defect is inactivation of the von Hippel-Lindau (VHL) gene. Loss of pVHL, the VHL gene product, leads to constitutive activation of hypoxia-inducible factor (HIF) signaling. While downregulation of HIF suppresses tumor formation by pVHL-defective renal carcinoma cells, the relative contribution of individual HIF regulated genes to HIF-dependent tumorigenesis remains under investigation. Mxi1, a c-Myc antagonist, is a HIF target gene that inhibits mitochondrial biogenesis, reprograms cellular energy metabolism, and protects cells from c-Myc-dependent apoptosis in vitro. In the present study we show that Mxi1 is overexpressed in primary human clear cell kidney cancers. Inhibition of Mxi1 in pVHL-defective kidney cancer cells using shRNA alters their cell cycle parameters, inhibits their ability to invade matrigel, and suppresses their ability to form tumors in vivo. Compared to Mxi1-proficient tumors, Mxi1-deficient tumors display reduced cellular proliferation. These results establish Mxi1 as an important downstream target of HIF that contributes to pVHL-deficient renal cancer tumorigenesis.

Turner, K. J., J. W. Moore, et al. (2002). "Expression of hypoxia-inducible factors in human renal cancer: relationship to angiogenesis and to the von Hippel-Lindau gene mutation." Cancer Res **62**(10): 2957-61.

The von Hippel-Lindau tumor suppressor protein acts as the substrate recognition component of a ubiquitin E3 ligase that targets hypoxia-inducible factor (HIF)-alpha subunits for proteolysis. Stabilization of HIF-alpha subunits has been described in VHL-defective cell lines, leading to HIF activation and up-regulation of hypoxia-inducible mRNAs. Mutations of the von Hippel-Lindau tumor suppressor protein are found in most clear cell renal cell carcinomas (CC-RCCs) but not other renal tumors, raising a question about the importance of activation of the HIF pathway in CC-RCC development. To address this question, we have examined the expression of HIF-alpha subunits in 45 primary renal tumors and related this to tumor subtype, the presence of VHL mutations, and measures of angiogenesis. We show that HIF-alpha is up-regulated in the majority of CC-RCCs, and that the pattern of expression is biased

toward the HIF-2alpha isoform. Expression of HIF-alpha proteins was associated significantly with up-regulation of VEGF mRNA and protein and increased microvessel density. Up-regulation of HIF-alpha in CC-RCC was found to involve increased mRNA as well as protein expression, suggesting that both VHL-dependent and VHL-independent mechanisms are involved. These results suggest that activation of the HIF pathway is functionally important in CC-RCC development and might provide a new therapeutic target.

Ueki, T., T. Takeuchi, et al. (2001). "Silencing of the caspase-1 gene occurs in murine and human renal cancer cells and causes solid tumor growth in vivo." Int J Cancer **91**(5): 673-9.

Renal cell cancer is a unique solid tumor that occasionally shows spontaneous regression even at an advanced stage, of which the underlying mechanism is not well understood. To investigate a potential role of the pro-apoptotic molecule caspase-1 in the growth regulation of renal cell cancer, we created transfectants expressing exogenous caspase-1 from a murine renal cancer cell line, Renca. Overexpression of caspase-1 did not affect the growth of Renca cells in vitro at the exponential phase but induced apoptotic cell death at 50% to 75% confluence, whereas control cells underwent apoptosis only after reaching 100% confluence. When implanted into the flank of a syngeneic BALB/c mouse, caspase-1-overexpressing Renca cells did not effectively establish growth as a solid tumor, forming a measurable tumor in only 7 of 11 (64%) animals, whereas control cells formed a tumor in 6 of 6 (100%) animals. The growth of tumors from caspase-1-overexpressing cells slowed down markedly after the tumors reached 5 to 10 mm in diameter, and histological examination of such tumors revealed numerous apoptotic cells positively stained by TUNEL assay. Interestingly, endogenous caspase-1 was not detected in the tumors from control cells, which re-expressed caspase-1 when they were re-cultured and exposed to a demethylation reagent, 5-aza-2'-deoxycytidine. Furthermore, treatment of a human renal cancer cell line, ACHN, with 5-aza-2'-deoxycytidine also caused recovery of caspase-1 expression, which was not detected before treatment. These data suggest that silencing of caspase-1 through DNA methylation may be involved in the oncogenesis of some renal cell cancers growing as a solid tumor.

Uemura, H. (1999). "[Molecular detection of circulating cancer cells in patients with renal cell carcinoma]." Hinyokika Kiyo **45**(8): 571-5.

We have developed a highly sensitive technique to detect circulating renal cell carcinoma (RCC) cells in the blood using the reverse transcriptase-polymerase chain reaction (RT-PCR) with primers specific for the MN/CA9 gene. RT-PCR analysis of RCC specimens resulted in the clear detection of MN/CA9 mRNA signal in 93%. In contrast, no expression of MN/CA9 was observed in normal kidney specimens. Highly sensitive RT-PCR analysis of blood samples from RCC patients revealed the presence of circulating MN-positive cancer cells in the blood. Fifty samples obtained from the patients with RCC and 31 samples from healthy donors were investigated. The sensitivity and specificity of this RT-PCR analysis were 72% and 78%, respectively. These findings suggest that the MN antigen may be a potential diagnostic biomarker for early detection of RCC.

Unwin, R. D., R. A. Craven, et al. (2003). "Proteomic changes in renal cancer and co-ordinate demonstration of both the glycolytic and mitochondrial aspects of the Warburg effect." Proteomics **3**(8): 1620-32.

Renal cell carcinoma (RCC) is the tenth most common cancer although the incidence is increasing. The main clinical problems stem from the relatively late presentation of many patients due to the often asymptomatic nature of the illness, and the relative insensitivity of metastatic disease to conventional chemotherapy and radiotherapy. Despite increasing knowledge of some of the genetic changes underlying sporadic renal cancer such as those involving the Von Hippel Lindau (VHL) gene, many of the underlying pathophysiological changes are ill-defined and there remains a need for the identification of disease markers for use in diagnosis and prognosis or as potential therapeutic targets. This study has used a proteomic approach, based on two-dimensional gel electrophoresis and mass spectrometry, to compare the protein profiles of conventional RCC tissue with patient-matched normal kidney cortex. Sequencing of 32 protein spots with significantly increased expression in RCC samples (>/= 4/6 patients) and 41 proteins whose levels decreased (6/6 patients) confirmed several previously known RCC-associated changes such as increases in Mn-superoxide dismutase, lactate dehydrogenase-A, aldolase A and C, pyruvate kinase M2, and thymidine phosphorylase. Additionally, several previously unknown changes were identified, including increased expression of three members of the annexin family and increased levels of the actin depolymerisation factor cofilin. The Warburg effect was also demonstrated with the identification of increases in proteins involved in the majority of steps in the glycolytic pathway and decreases in the gluconeogenic reactions, together with a parallel decrease in several mitochondrial enzymes. A number of the alterations seen were further confirmed in additional samples by

immunohistochemistry, Western blotting, and laser capture microdissection.

Vaziri, S. A., D. R. Grabowski, et al. (2009). "Inhibition of proteasome activity by bortezomib in renal cancer cells is p53 dependent and VHL independent." Anticancer Res **29**(8): 2961-9.

BACKGROUND: Antiproliferative effects of proteasome inhibitors are suggested to be primarily due to effects on nuclear factor-kappaB (NF-kappaB)-dependent pathways and the induction of apoptosis. The objective of this study was to elucidate the mechanistic basis for the antiproliferative effects of the proteasome inhibitor, bortezomib, in human clear cell renal cell cancer cells (CCRCC). MATERIALS AND METHODS: von Hippel Lindau (VHL) mutation/methylation status and cytotoxic response to bortezomib was determined in a panel of CCRCC cell lines. Effects on target protein/gene expression and the role of p53 in bortezomib-mediated cytotoxicity, inhibition of proteasome activity, survivin transcript and protein expression as well as induction of p21 expression was determined in CCRCC that differed in their intrinsic sensitivity to bortezomib. RESULTS: VHL status was not associated with cytotoxic response to bortezomib treatment. Cytotoxicity in cell lines that differed in intrinsic sensitivity to bortezomib correlated with sustained inhibition of proteasome activity, survivin expression and induction of p21 expression. Stable down-regulation of p53 expression by siRNA led to attenuation of bortezomib effects, survivin down-regulation and p21 induction, suggesting that cellular effects are p53-dependent. CONCLUSION: These results demonstrate that the antiproliferative effects of bortezomib in CCRCC cells are VHL independent and dependent on pathways regulated by p53.

Velickovic, M., B. Delahunt, et al. (2001). "VHL and FHIT locus loss of heterozygosity is common in all renal cancer morphotypes but differs in pattern and prognostic significance." Cancer Res **61**(12): 4815-9.

Deletions involving 3p are believed to be typical for conventional (clear cell) renal cell carcinoma (cRCC), with confirmed and suspected targets being the VHL and FHIT tumor suppressor genes, respectively. By contrast, 3p deletions are felt to be rare in papillary RCC (pRCC) and chromophobe RCC (chRCC); however, this belief is based on relatively scant data. In particular, 3p14.2 deletions, possibly resulting in FHIT inactivation, have been rarely studied in pRCC or chRCC even though they may be relevant in early renal tumorigenesis. We therefore examined 3p deletion rates and patterns in pRCC and chRCC with particular attention to 3p14.2. We examined 16 chRCCs and 27 pRCCs for loss of heterozygosity (LOH) at 3p25-26 and 3p14.2 using 13 well-mapped microsatellite markers. Those pRCC with LOH at 3p25-26 were also screened for VHL gene mutations. The results were correlated with tumor histology and patient outcome and compared with data we had obtained previously on cRCC. We found similar overall 3p LOH rates in pRCC (59%), chRCC (86.6%), and cRCC (75.8%). In pRCC and chRCC, LOH at 3p25-26 was more common than at 3p14.2, whereas the converse was true for cRCC. In the pRCC with 3p25-26 LOH, we confirmed that this was not associated with mutations of the VHL gene. At 3p14.2, LOH rates of pRCC were lower than those of cRCC and chRCC ($p<0.02$). All morphotypes showed a predominately interstitial LOH pattern, which was most pronounced in the 3p14.2 region in cRCC. 3p LOH in chRCC was associated with improved patient outcome, mirroring our previous cRCC data. We conclude that 3p LOH is a universal phenomenon in RCC, but has different underlying mechanisms, molecular targets, and implications in the different morphotypes, although FHIT inactivation may play a role in both cRCC and chRCC tumorigenesis.

Wang, E., R. Lichtenfels, et al. (2004). "Ontogeny and oncogenesis balance the transcriptional profile of renal cell cancer." Cancer Res **64**(20): 7279-87.

Global transcript analysis is increasingly used to describe cancer taxonomies beyond the microscopic reach of the eye. Diagnostic and prognostic portraits are formulated by ranking cancers according to transcriptional proximity. However, the role that distinct biological factors play in defining these portraits remains undefined. It is likely that the transcriptional repertoire of cancers depends, on one hand, on the anamnestic retention of their ontogenesis and, on the other, on the emergence of novel expression patterns related to oncogenesis. We compared the transcriptional profile of primary renal cell cancers (RCCs) with that of normal kidney tissue and several epithelial cancers of nonrenal origin to weigh the contribution that ontogeny and oncogenesis make in molding their genetic profile. Unsupervised global transcript analysis demonstrated that RCCs retain transcriptional signatures related to their ontogeny and cluster close to normal renal epithelium. When renal lineage-associated genes are removed from the analysis and cancer-specific genes are analyzed, RCCs segregate with other cancers with limited lineage specificity underlying a predominance of the oncogenic process over lineage specificity. However, a RCC-specific set of oncogenesis-related genes was identified and surprisingly shared by sarcomas. In summary, the transcriptional portrait of primary RCCs is largely dominated by ontogeny.

Genes responsible for lineage specificity may represent poor molecular targets for immune or drug therapy. Most genes associated with oncogenesis are shared with other cancers and may represent better therapeutic targets. Finally, a small subset of genes is associated with lineage-specific oncogenesis, and these may provide information regarding the biological behavior of RCCs and facilitate diagnostic classification of RCCs.

Weber, A., I. Kristiansen, et al. (2008). "The FUSE binding proteins FBP1 and FBP3 are potential c-myc regulators in renal, but not in prostate and bladder cancer." BMC Cancer **8**: 369.

BACKGROUND: The three far-upstream element (FUSE) binding proteins (FBP1, FBP2, and FBP3) belong to an ancient family of single-stranded DNA binding proteins which are required for proper regulation of the c-myc proto-oncogene. Whereas it is known that c-myc alterations play a completely different role in various carcinomas of the urogenital tract, the relevance of FBPs is unclear. METHODS: FBP1, FBP3 and c-myc expression was studied in 105 renal cell, 95 prostate and 112 urinary bladder carcinomas by immunohistochemistry using tissue microarrays. RESULTS: High rates of FBP1 and FBP3 expression were observed in all cancer types. There was a concomitant up-regulation of FBP1 and FBP3 in renal cell and prostate carcinomas ($p < 0.001$ both). C-myc expression was detectable in 21% of prostate, 30% of renal and 34% of urothelial carcinomas. Interestingly, strong FBP1 and FBP3 expression was associated with c-myc up-regulation in clear cell renal cell carcinomas ($p < 0.001$ and 0.09 resp.), but not in bladder or prostate cancer. CONCLUSION: The correlation between FBP1/FBP3, c-myc and high proliferation rate in renal cell carcinoma provides strong in vivo support for the suggested role of FBP1 and FBP3 as activators of c-myc. The frequent up-regulation of FBP1 and FBP3 in urothelial and prostate carcinoma suggests that FBPs also have an important function in gene regulation of these tumors.

Wei, M. H., O. Toure, et al. (2006). "Novel mutations in FH and expansion of the spectrum of phenotypes expressed in families with hereditary leiomyomatosis and renal cell cancer." J Med Genet **43**(1): 18-27.

BACKGROUND: Hereditary leiomyomatosis and renal cell cancer (HLRCC; OMIM 605839) is the predisposition to develop smooth muscle tumours of the skin and uterus and/or renal cancer and is associated with mutations in the fumarate hydratase gene (FH). Here we characterise the clinical and genetic features of 21 new families and present the first report of two African-American families with HLRCC. METHODS: Using direct sequencing analysis we identified FH germline mutations in 100% (21/21) of new families with HLRCC. RESULTS: We identified 14 germline FH mutations (10 missense, one insertion, two nonsense, and one splice site) located along the entire length of the coding region. Nine of these were novel, with six missense (L89S, R117G, R190C, A342D, S376P, Q396P), one nonsense (S102X), one insertion (111insA), and one splice site (138+1G>C) mutation. Four unrelated families had the R58X mutation and five unrelated families the R190H mutation. Of families with HLRCC, 62% (13/21) had renal cancer and 76% (16/21) cutaneous leiomyomas. Of women FH mutation carriers from 16 families, 100% (22/22) had uterine fibroids. Our study shows that expression of cutaneous manifestations in HLRCC ranges from absent to mild to severe cutaneous leiomyomas. FH mutations were associated with a spectrum of renal tumours. No genotype-phenotype correlations were identified. CONCLUSIONS: In combination with our previous report, we identify 31 different germline FH mutations in 56 families with HLRCC (20 missense, eight frameshifts, two nonsense, and one splice site). Our FH mutation detection rate is 93% (52/56) in families suspected of HLRCC.

Wiesenhutter, B., S. Selinski, et al. (2007). "Re-assessment of the influence of polymorphisms of phase-II metabolic enzymes on renal cell cancer risk of trichloroethylene-exposed workers." Int Arch Occup Environ Health **81**(2): 247-51.

PROBLEM: Individual differences in susceptibility to trichloroethylene-induced nephrocarcinogenicity may be conferred by genetic polymorphisms of glutathione S-transferases (GST), because enzymes of this group are pivotal for the metabolic activation of trichloroethylene. Because of a potential involvement of N-acetylation in the detoxication of reactive trichloroethylene metabolite(s) to N-acetyl-cysteine derivatives, polymorphisms of the NAT2 gene may also be relevant. METHODS: The primary collective used for a re-investigation of these questions was that of a hospital-based case-control study by Bruning et al. (Am J Ind Med 43:274-285, 2003) of 134 renal cell cancer cases (20 cases exposed to trichloroethylene) and 401 matched controls. Genetic polymorphisms of GSTT1, GSTM1, GSTP1 and NAT2 were studied. Additional control collectives of non-diseased persons were used for comparison of allele frequencies. RESULTS: No genetic influences on the development of renal cancer due to trichloroethylene were apparent, related to the deletion polymorphisms of GSTT1 and GSTM1, as well as to the NAT2 rapid/slow acetylator states. However, renal cell cancer cases displayed a

somewhat higher proportion of the homozygous GSTP1 313A wild type (GSTP1*A), although this was not statistically significant (chi(2) test: P=0.1071, when using only the original controls of Bruning et al. (2003); P=0.0781 with inclusion of the additional controls). CONCLUSION: The re-investigation does not confirm the working hypothesis of an influence of the deletion polymorphisms of the glutathione S-transferases GSTT1 and GSTM1 on renal cell cancer development due to high occupational exposures to trichloroethylene.

Winquist, E., J. Knox, et al. (2006). "Phase II trial of DNA methyltransferase 1 inhibition with the antisense oligonucleotide MG98 in patients with metastatic renal carcinoma: a National Cancer Institute of Canada Clinical Trials Group investigational new drug study." Invest New Drugs **24**(2): 159-67.

DNA methyltransferases (DNMTs) methylate DNA, promoting local chromatin condensation and consequent repression of gene expression. The purpose of this two-stage phase II trial was to assess the antitumor activity of MG98, a second generation antisense oligodeoxynucleotide inhibitor of human DNMT 1, in patients with metastatic renal carcinoma (MRC). Untreated adult patients with measurable MRC were treated with MG98 at a dose of 360 mg/m2 via 2-h iv infusion twice weekly for three consecutive weeks out of four. The primary endpoint was objective response or absence of progression for at least eight weeks. Pharmacokinetics and DNMT1 mRNA levels in peripheral blood mononuclear cells (PBMCs) were also analyzed at pre-specified intervals. Seventeen eligible patients received a median of two cycles of treatment (range, 1-7), and no objective responses were seen. Nine patients had progressive disease, six had stable disease, and the study was stopped after the first stage. The most common symptomatic toxicities were rigors, fatigue, fever, and nausea. Hematological toxicity was mild. Seven patients treated with prior nephrectomy had grade 3 or 4 elevations in hepatic transaminases. Significantly higher Cmax and AUC(0-->inf) values were observed in these patients. No conclusive pattern of decreased DNMT1 activity in PBMCs was detected post MG98 treatment. The lack of objective responses observed may be explained by a lack of target effect or the choice of tumor type. Transaminitis was observed in patients with prior nephrectomy and appeared to be associated with altered drug exposure in these patients.

Wysocki, P. J., J. Zolnierek, et al. (2008). "Targeted therapy of renal cell cancer." Curr Opin Investig Drugs **9**(6): 570-5.

Rapid development of treatment strategies for renal cell cancer (RCC) has occurred in recent years. Elucidation of the crucial role of the Von Hippel-Lindau (VHL) tumor suppressor gene in upregulating growth factors associated with angiogenesis has provided new insight into RCC biology and has identified specific targets for novel therapeutic strategies. For almost two decades, cytokine-based immunotherapy has remained a treatment of choice in advanced RCC patients. However, it has provided only modest improvement in clinical outcome and has been associated with severe toxicity. With the advent of novel therapies directly targeting the VEGF molecule or VEGF receptor signal transduction pathway, the clinical outcome in high-risk, advanced RCC has significantly improved. In phase III clinical trials, novel targeted agents - temsirolimus, sorafenib, sunitinib and bevacizumab - significantly prolonged progression-free survival of patients with metastatic RCC and, crucially, temsirolimus also prolonged overall survival in patients with high-risk disease. Despite the obvious clinical efficacy of novel targeted therapies in the treatment of RCC, many unanswered questions still remain; in particular, the efficacy of targeted agents in patients with low-risk RCC, the optimal sequence and combination of therapies for first-, second-, or third-line treatment, and the efficacy of this strategy in adjuvant settings.

Zellweger, T., H. Miyake, et al. (2001). "Chemosensitization of human renal cell cancer using antisense oligonucleotides targeting the antiapoptotic gene clusterin." Neoplasia **3**(4): 360-7.

BACKGROUND: Renal cell cancer (RCC) is a chemoresistant disease with no active chemotherapeutic agent achieving objective response rates higher than 15%. Clusterin is a cell survival gene that increases in human renal tubular epithelial cells after various states of injury and disease. Downregulation of clusterin, using antisense oligonucleotides (ASO), has recently been shown to increase chemosensitivity in several prostate cancer models. The objectives in this study were to evaluate clusterin expression levels in human RCC and normal kidney tissue, and to test whether clusterin ASO could also enhance chemosensitivity in human RCC Caki-2 cells both in vitro and in vivo. METHODS: Immunohistochemical staining was used to characterize clusterin expression in 67 RCC and normal kidney tissues obtained from radical nephrectomy specimens. Northern blot analysis was used to assess changes in clusterin mRNA expression after ASO and paclitaxel treatment. The effects of combined clusterin ASO and paclitaxel treatment on Caki-2 cell growth was examined using an MTT assay. Athymic mice bearing Caki-2 tumors were

treated with clusterin ASO alone, clusterin ASO plus paclitaxel, and mismatch control oligonucleotides plus paclitaxel, over a period of 28 days with measurement of tumor volumes once weekly over 8 weeks. RESULTS: Immunohistochemistry of normal and malignant kidney tissue sections of 67 patients demonstrated positive clusterin staining for almost all RCC (98%) and an overexpression, compared to normal tissue, in a majority of RCC (69%). Clusterin ASO, but not mismatch control oligonucleotides, decreased clusterin mRNA expression in Caki-2 cells in a dose-dependent and sequence-specific manner. Pretreatment of Caki-2 cells with clusterin ASO significantly enhanced chemosensitivity to paclitaxel in vitro. Characteristic apoptotic DNA laddering was observed after combined treatment with ASO plus paclitaxel, but not with either agent alone. In vivo administration of clusterin ASO plus paclitaxel acted synergistically to increase apoptosis and significantly delay Caki-2 tumor growth, compared to mismatch control oligonucleotide plus paclitaxel. In addition, TUNEL staining revealed increased apoptotic cells in tumors treated with clusterin ASO plus paclitaxel compared to treatment with either clusterin ASO or paclitaxel alone. CONCLUSION: These findings confirm that the use of clusterin ASO may be a feasible strategy to enhance chemosensitivity for patients with advanced RCC.

Zivkovic, S., M. Kostov, et al. (2008). "[Histopathological characteristics and coexpression of p53 and p16(INK4a) proteins in renal cancer]." Vojnosanit Pregl **65**(11): 820-4.

BACKGROUND/AIM: Renal carcinoma represents histologically heterogeneous group of malignant tumors, with various clinical aggressiveness. The frequency of p53 mutation in primal renal carcinoma is rare, although there are information about its heterogeneous accumulation. The loss of protein p16 expression in primal renal carcinoma is detected in 20-30% of the cases. The aim of this paper was to determine frequency of mutated protein p53 and expression of protein p16(INK4a) in renal carcinoma, to analyze their correlative relation and relation with the examined clinicopathological parameters. METHODS: The examination included 12 patients (66.7% men, 33.3% women), with patohistologically verified renal carcinoma. Expression of mutated form of protein p53 and protein p16 was determined in tissue samples, by immunohistochemical analysis using of mice monoclonical antibodies produced by DAKO, Denmark RESULTS: In 9 (75%) of the cases was detected mutated protein p53, of whom 66.6% had higher histological gradus of tumor (G3-4) and higher pathological stadium of the disease (pT3a-b) at the same time. In 7 (58.3%) and 5 (41.7%) of the cases expression of protein p16, the loss of expression of protein p16 were detected respectively. A statistically significant positive correlation was determined between pathological stadium of disease (TNM) and the degree of tumor differentiation (G) ($p = 0.834$; $p < 0.001$), as well as between TNM and mitotic index ($p = 0.622$; $p = 0.031$). CONCLUSION: A mutated form of protein p53 exists in 75% of the cases with the renal carcinoma and 66.6% of then have higher histological gradus of tumor and higher stadium of tumor disease at the same time. Coexpression of mutated protein p53 and protein p16(INK4a) in renal carcinoma is not statistically significant and it is not in correlation with clinicopathological parameters. Immunohistochemical analysis of mutated protein p53 in renal carcinoma can have predictive significance.

References

1. Alam, N. A., A. J. Rowan, et al. (2003). "Genetic and functional analyses of FH mutations in multiple cutaneous and uterine leiomyomatosis, hereditary leiomyomatosis and renal cancer, and fumarate hydratase deficiency." Hum Mol Genet **12**(11): 1241-52.
2. Alam, N. A., S. Olpin, et al. (2005). "Fumarate hydratase mutations and predisposition to cutaneous leiomyomas, uterine leiomyomas and renal cancer." Br J Dermatol **153**(1): 11-7.
3. Allory, Y., Y. Matsuoka, et al. (2005). "The L1 cell adhesion molecule is induced in renal cancer cells and correlates with metastasis in clear cell carcinomas." Clin Cancer Res **11**(3): 1190-7.
4. Arya, M., D. Chao, et al. (2004). "Allogeneic hematopoietic stem-cell transplantation: the next generation of therapy for metastatic renal cell cancer." Nat Clin Pract Oncol **1**(1): 32-8.
5. Ashida, S., H. Okuda, et al. (2000). "Detection of circulating cancer cells with von hippel-lindau gene mutation in peripheral blood of patients with renal cell carcinoma." Clin Cancer Res **6**(10): 3817-22.
6. Atkins, M., M. Regan, et al. (2005). "Carbonic anhydrase IX expression predicts outcome of interleukin 2 therapy for renal cancer." Clin Cancer Res **11**(10): 3714-21.
7. Badeloe, S., A. J. van Geest, et al. (2008). "Absence of fumarate hydratase mutation in a family with cutaneous leiomyosarcoma and renal cancer." Int J Dermatol **47 Suppl 1**: 18-20.
8. Banks, R. E., P. Tirukonda, et al. (2006). "Genetic and epigenetic analysis of von Hippel-Lindau (VHL) gene alterations and relationship with clinical variables in sporadic renal cancer." Cancer Res **66**(4): 2000-11.
9. Barbero, G., F. Carta, et al. (2006). "Protein/RNA coextraction and small two-dimensional polyacrylamide gel electrophoresis for proteomic/gene expression analysis of renal cancer biopsies." Anal Biochem **349**(1): 62-71.
10. Bardi, E., A. V. Olah, et al. (2004). "Late effects on renal glomerular and tubular function in childhood cancer survivors." Pediatr Blood Cancer **43**(6): 668-73.
11. Beales, P. L., H. A. Reid, et al. (2000). "Renal cancer and malformations in relatives of patients with Bardet-Biedl syndrome." Nephrol Dial Transplant **15**(12): 1977-85.
12. Bilim, V., T. Kawasaki, et al. (2000). "Altered expression of beta-catenin in renal cell cancer and transitional cell cancer with the absence of beta-catenin gene mutations." Clin Cancer Res **6**(2): 460-6.

13. Blish, K. R., W. Wang, et al. (2008). "A human bone morphogenetic protein antagonist is down-regulated in renal cancer." Mol Biol Cell **19**(2): 457-64.
14. Board, R. E., F. C. Thistlethwaite, et al. (2007). "Anti-angiogenic therapy in the treatment of advanced renal cell cancer." Cancer Treat Rev **33**(1): 1-8.
15. Bodmer, D., M. Schepens, et al. (2003). "Disruption of a novel gene, DIRC3, and expression of DIRC3-HSPBAP1 fusion transcripts in a case of familial renal cell cancer and t(2;3)(q35;q21)." Genes Chromosomes Cancer **38**(2): 107-16.
16. Bodmer, D., W. van den Hurk, et al. (2002). "Understanding familial and non-familial renal cell cancer." Hum Mol Genet **11**(20): 2489-98.
17. Boni, J. P., C. Leister, et al. (2005). "Population pharmacokinetics of CCI-779: correlations to safety and pharmacogenomic responses in patients with advanced renal cancer." Clin Pharmacol Ther **77**(1): 76-89.
18. Bonne, A. C., D. Bodmer, et al. (2004). "Chromosome 3 translocations and familial renal cell cancer." Curr Mol Med **4**(8): 849-54.
19. Bonne, A., L. Vreede, et al. (2007). "Mapping of constitutional translocation breakpoints in renal cell cancer patients: identification of KCNIP4 as a candidate gene." Cancer Genet Cytogenet **179**(1): 11-8.
20. Bonsdorff, T. B., J. H. Jansen, et al. (2008). "Second hits in the FLCN gene in a hereditary renal cancer syndrome in dogs." Mamm Genome **19**(2): 121-6.
21. Brauch, H., G. Weirich, et al. (2004). "VHL mutations in renal cell cancer: does occupational exposure to trichloroethylene make a difference?" Toxicol Lett **151**(1): 301-10.
22. Bregni, M., A. Dodero, et al. (2002). "Nonmyeloablative conditioning followed by hematopoietic cell allografting and donor lymphocyte infusions for patients with metastatic renal and breast cancer." Blood **99**(11): 4234-6.
23. Brenner, W., F. Benzing, et al. (2004). "Regulation of beta1 integrin expression by PKCepsilon in renal cancer cells." Int J Oncol **25**(4): 1157-63.
24. Catherino, W. H., C. M. Mayers, et al. (2007). "Compensatory alterations in energy homeostasis characterized in uterine tumors from hereditary leiomyomatosis and renal cell cancer." Fertil Steril **88**(4 Suppl): 1039-48.
25. Chang, I. W., H. Y. Huang, et al. (2009). "Melanotic Xp11 translocation renal cancer: a case with PSF-TFE3 gene fusion and up-regulation of melanogenetic transcripts." Am J Surg Pathol **33**(12): 1894-901.
26. Cho, D., S. Signoretti, et al. (2007). "The role of mammalian target of rapamycin inhibitors in the treatment of advanced renal cancer." Clin Cancer Res **13**(2 Pt 2): 758s-763s.
27. Choyke, P. L. (2003). "Imaging of hereditary renal cancer." Radiol Clin North Am **41**(5): 1037-51.
28. Costa, V. L., R. Henrique, et al. (2007). "Quantitative promoter methylation analysis of multiple cancer-related genes in renal cell tumors." BMC Cancer **7**: 133.
29. Cozar, J. M., J. M. Romero, et al. (2007). "High incidence of CTLA-4 AA (CT60) polymorphism in renal cell cancer." Hum Immunol **68**(8): 698-704.
30. Crnkovic-Mertens, I., N. Wagener, et al. (2007). "Targeted inhibition of Livin resensitizes renal cancer cells towards apoptosis." Cell Mol Life Sci **64**(9): 1137-44.
31. da Silva, N. F., D. Gentle, et al. (2003). "Analysis of the Birt-Hogg-Dube (BHD) tumour suppressor gene in sporadic renal cell carcinoma and colorectal cancer." J Med Genet **40**(11): 820-4.
32. Datta, D., A. G. Contreras, et al. (2008). "Calcineurin inhibitors modulate CXCR3 splice variant expression and mediate renal cancer progression." J Am Soc Nephrol **19**(12): 2437-46.
33. Datta, D., A. G. Contreras, et al. (2009). "Calcineurin inhibitors activate the proto-oncogene Ras and promote protumorigenic signals in renal cancer cells." Cancer Res **69**(23): 8902-9.
34. Datta, K., J. Li, et al. (2004). "Protein kinase C zeta transactivates hypoxia-inducible factor alpha by promoting its association with p300 in renal cancer." Cancer Res **64**(2): 456-62.
35. Datta, K., R. Nambudripad, et al. (2000). "Inhibition of insulin-like growth factor-I-mediated cell signaling by the von Hippel-Lindau gene product in renal cancer." J Biol Chem **275**(27): 20700-6.
36. Del Monte, G., P. Ferroni, et al. (2008). "Interleukin-2 inhalation therapy in renal cell cancer: a case report and review of the literature." In Vivo **22**(4): 481-8.
37. Diaconu, I., L. Denby, et al. (2009). "Serotype chimeric and fiber-mutated adenovirus Ad5/19p-HIT for targeting renal cancer and untargeting the liver." Hum Gene Ther **20**(6): 611-20.
38. Donald, C. D., C. Q. Sun, et al. (2003). "Cancer-specific loss of beta-defensin 1 in renal and prostatic carcinomas." Lab Invest **83**(4): 501-5.
39. Dong, L. M., P. Brennan, et al. (2009). "An analysis of growth, differentiation and apoptosis genes with risk of renal cancer." PLoS One **4**(3): e4895.
40. Eleveld, M. J., D. Bodmer, et al. (2001). "Molecular analysis of a familial case of renal cell cancer and a t(3;6)(q12;q15)." Genes Chromosomes Cancer **31**(1): 23-32.
41. Farker, K., M. H. Lehmann, et al. (2000). "Analysis of point mutation in exon 2 of CYP2E1 gene in renal cell/urothelial cancer patients in comparison with control population." Int J Clin Pharmacol Ther **38**(1): 30-4.
42. Feijoo-Cuaresma, M., F. Mendez, et al. (2008). "Inadequate activation of the GTPase RhoA contributes to the lack of fibronectin matrix assembly in von Hippel-Lindau protein-defective renal cancer cells." J Biol Chem **283**(36): 24982-90.
43. Fergelot, P., N. Rioux-Leclercq, et al. (2005). "[Molecular pathways of tumour angiogenesis and new targeted therapeutic approaches in renal cancer]." Prog Urol **15**(6): 1021-9.
44. Fleming, S. (1999). "Renal cancer genetics: von Hippel Lindau and other syndromes." Int J Dev Biol **43**(5): 469-71.
45. Franco, O. E., T. Onishi, et al. (2003). "Phenylacetate inhibits growth and modulates cell cycle gene expression in renal cancer cell lines." Anticancer Res **23**(2B): 1637-42.
46. Franzini, A., S. C. Picozzi, et al. (2007). "A case of renal cancer with TFE3 gene fusion in an elderly man. Clinical, radiological and surgical findings." Urol Int **78**(2): 179-81.
47. Franzke, A., J. Buer, et al. (2001). "HLA phenotype and cytokine-induced tumor control in advanced renal cell cancer." Cancer Biother Radiopharm **16**(5): 401-9.
48. Fritzsche, F. R., K. Wassermann, et al. (2008). "ADAM9 is highly expressed in renal cell cancer and is associated with tumour progression." BMC Cancer **8**: 179.
49. Fujimoto, E., H. Sato, et al. (2005). "A Src family inhibitor (PP1) potentiates tumor-suppressive effect of connexin 32 gene in renal cancer cells." Life Sci **76**(23): 2711-20.
50. Fujimoto, E., T. Yano, et al. (2005). "Cytotoxic effect of the Her-2/Her-1 inhibitor PKI-166 on renal cancer cells expressing the connexin 32 gene." J Pharmacol Sci **97**(2): 294-8.
51. Gattinoni, L., M. Alu, et al. (2003). "Renal cancer treatment: a review of the literature." Tumori **89**(5): 476-84.
52. Godinot, C., E. de Laplanche, et al. (2007). "Actuality of Warburg's views in our understanding of renal cancer metabolism." J Bioenerg Biomembr **39**(3): 235-41.
53. Gordon, M. S. (2004). "Novel antiangiogenic therapies for renal cell cancer." Clin Cancer Res **10**(18 Pt 2): 6377S-81S.

54. Gordon, M. S., M. Hussey, et al. (2009). "Phase II study of erlotinib in patients with locally advanced or metastatic papillary histology renal cell cancer: SWOG S0317." J Clin Oncol **27**(34): 5788-93.
55. Gratama, J. W., A. H. Zea, et al. (1999). "Restoration of expression of signal-transduction molecules in lymphocytes from patients with metastatic renal cell cancer after combination immunotherapy." Cancer Immunol Immunother **48**(5): 263-9.
56. Guillen-Ahlers, H. (2008). "Wnt signaling in renal cancer." Curr Drug Targets **9**(7): 591-600.
57. Guse, K., T. Ranki, et al. (2007). "Treatment of metastatic renal cancer with capsid-modified oncolytic adenoviruses." Mol Cancer Ther **6**(10): 2728-36.
58. Gutwein, P., A. Schramme, et al. (2009). "Tumoural CXCL16 expression is a novel prognostic marker of longer survival times in renal cell cancer patients." Eur J Cancer **45**(3): 478-89.
59. Hansel, D. E. and B. I. Rini (2008). "Molecular genetics of hereditary renal cancer: new genes and diagnostic and therapeutic opportunities." Expert Rev Anticancer Ther **8**(6): 895-905.
60. Hao, D., S. D. Huan, et al. (2000). "A pilot study of low dose hydroxyurea as a novel resistance modulator in metastatic renal cell cancer." J Chemother **12**(4): 360-6.
61. Haviv, Y. S. and D. T. Curiel (2008). "Gene therapy for renal cancer." Contrib Nephrol **159**: 135-50.
62. Haviv, Y. S., J. L. Blackwell, et al. (2002). "Adenoviral gene therapy for renal cancer requires retargeting to alternative cellular receptors." Cancer Res **62**(15): 4273-81.
63. Haviv, Y. S., W. J. van Houdt, et al. (2004). "Transcriptional targeting in renal cancer cell lines via the human CXCR4 promoter." Mol Cancer Ther **3**(6): 687-91.
64. Hervouet, E., H. Simonnet, et al. (2007). "Mitochondria and reactive oxygen species in renal cancer." Biochimie **89**(9): 1080-8.
65. Hirata, H., Y. Hinoda, et al. (2009). "The bcl2 -938CC genotype has poor prognosis and lower survival in renal cancer." J Urol **182**(2): 721-7.
66. Hirata, H., Y. Hinoda, et al. (2009). "Wnt antagonist gene DKK2 is epigenetically silenced and inhibits renal cancer progression through apoptotic and cell cycle pathways." Clin Cancer Res **15**(18): 5678-87.
67. Hirata, H., Y. Hinoda, et al. (2009). "Wnt antagonist gene polymorphisms and renal cancer." Cancer **115**(19): 4488-503.
68. Honke, K., M. Tsuda, et al. (1998). "Cancer-associated expression of glycolipid sulfotransferase gene in human renal cell carcinoma cells." Cancer Res **58**(17): 3800-5.
69. Hoque, M. O., S. Begum, et al. (2004). "Quantitative detection of promoter hypermethylation of multiple genes in the tumor, urine, and serum DNA of patients with renal cancer." Cancer Res **64**(15): 5511-7.
70. Hueber, P. A., D. Iglesias, et al. (2008). "In vivo validation of PAX2 as a target for renal cancer therapy." Cancer Lett **265**(1): 148-55.
71. Ibanez de Caceres, I., E. Dulaimi, et al. (2006). "Identification of novel target genes by an epigenetic reactivation screen of renal cancer." Cancer Res **66**(10): 5021-8.
72. Iliopoulos, O. (2006). "Molecular biology of renal cell cancer and the identification of therapeutic targets." J Clin Oncol **24**(35): 5593-600.
73. Isaacs, J. S., Y. J. Jung, et al. (2005). "HIF overexpression correlates with biallelic loss of fumarate hydratase in renal cancer: novel role of fumarate in regulation of HIF stability." Cancer Cell **8**(2): 143-53.
74. Ishizawa, J., S. Yoshida, et al. (2004). "Inhibition of the ubiquitin-proteasome pathway activates stress kinases and induces apoptosis in renal cancer cells." Int J Oncol **25**(3): 697-702.
75. Joensuu, T. K., S. Nilsson, et al. (2004). "Phase I trial on sms-D70 somatostatin analogue in advanced prostate and renal cell cancer." Ann N Y Acad Sci **1028**: 361-74.
76. Johannesma, P. C., J. W. Lammers, et al. (2009). "[Spontaneous pneumothorax as the first manifestation of a hereditary condition with an increased renal cancer risk]." Ned Tijdschr Geneeskd **153**: A581.
77. Jonasdottir, T. J., C. S. Mellersh, et al. (2000). "Genetic mapping of a naturally occurring hereditary renal cancer syndrome in dogs." Proc Natl Acad Sci U S A **97**(8): 4132-7.
78. Jones, J. and T. A. Libermann (2007). "Genomics of renal cell cancer: the biology behind and the therapy ahead." Clin Cancer Res **13**(2 Pt 2): 685s-692s.
79. Jones, J., H. Otu, et al. (2005). "Gene signatures of progression and metastasis in renal cell cancer." Clin Cancer Res **11**(16): 5730-9.
80. Karami, S., P. Brennan, et al. (2009). "Analysis of SNPs and haplotypes in vitamin D pathway genes and renal cancer risk." PLoS One **4**(9): e7013.
81. Karumanchi, S. A., J. Merchan, et al. (2002). "Renal cancer: molecular mechanisms and newer therapeutic options." Curr Opin Nephrol Hypertens **11**(1): 37-42.
82. Kasahara, T., V. Bilim, et al. (2006). "Homozygous deletions of the INK4a/ARF locus in renal cell cancer." Anticancer Res **26**(6B): 4299-305.
83. Kawakami, T., K. Okamoto, et al. (2003). "Multipoint methylation and expression analysis of tumor suppressor genes in human renal cancer cells." Urology **61**(1): 226-30.
84. Kempkensteffen, C., F. R. Fritzsche, et al. (2009). "Down-regulation of the pro-apoptotic XIAP associated factor-1 (XAF1) during progression of clear-cell renal cancer." BMC Cancer **9**: 276.
85. Kiuru, M. and V. Launonen (2004). "Hereditary leiomyomatosis and renal cell cancer (HLRCC)." Curr Mol Med **4**(8): 869-75.
86. Kiuru, M., R. Lehtonen, et al. (2002). "Few FH mutations in sporadic counterparts of tumor types observed in hereditary leiomyomatosis and renal cell cancer families." Cancer Res **62**(16): 4554-7.
87. Kiuru, M., V. Launonen, et al. (2001). "Familial cutaneous leiomyomatosis is a two-hit condition associated with renal cell cancer of characteristic histopathology." Am J Pathol **159**(3): 825-9.
88. Kluijt, I., D. de Jong, et al. (2009). "Early onset of renal cancer in a family with Birt-Hogg-Dube syndrome." Clin Genet **75**(6): 537-43.
89. Kobayashi, M., T. Okada, et al. (2007). "Tissue-targeted in vivo gene transfer coupled with histone deacetylase inhibitor depsipeptide (FK228) enhances adenoviral infection in rat renal cancer allograft model systems." Urology **70**(6): 1230-6.
90. Kondo, Y., J. Hamada, et al. (2005). "Over expression of hypoxia-inducible factor-1alpha in renal and bladder cancer cells increases tumorigenic potency." J Urol **173**(5): 1762-6.
91. Kopper, L. and J. Timar (2006). "Genomics of renal cell cancer-- does it provide breakthrough?" Pathol Oncol Res **12**(1): 5-11.
92. Koski, T. A., H. J. Lehtonen, et al. (2009). "Array comparative genomic hybridization identifies a distinct DNA copy number profile in renal cell cancer associated with hereditary leiomyomatosis and renal cell cancer." Genes Chromosomes Cancer **48**(7): 544-51.
93. Kuefer, R., M. Autenrieth, et al. (2006). "[Translational research in renal cell cancer. Illustrated by the example of the vascular endothelial growth factor pathway]." Urologe A **45**(3): 328, 330-5.
94. Kuiper, R. P., L. Vreede, et al. (2009). "The tumor suppressor gene FBXW7 is disrupted by a constitutional t(3;4)(q21;q31)

in a patient with renal cell cancer." Cancer Genet Cytogenet **195**(2): 105-11.
95. Laing, M. E., E. Kay, et al. (2007). "Genetic factors associated with skin cancer in renal transplant patients." Photodermatol Photoimmunol Photomed **23**(2-3): 62-7.
96. Lamers, C. H., J. W. Gratama, et al. (2005). "Parallel detection of transduced T lymphocytes after immunogene therapy of renal cell cancer by flow cytometry and real-time polymerase chain reaction: implications for loss of transgene expression." Hum Gene Ther **16**(12): 1452-62.
97. Lamers, C. H., S. C. Langeveld, et al. (2007). "Gene-modified T cells for adoptive immunotherapy of renal cell cancer maintain transgene-specific immune functions in vivo." Cancer Immunol Immunother **56**(12): 1875-83.
98. Langbein, S., W. M. Frederiks, et al. (2008). "Metastasis is promoted by a bioenergetic switch: new targets for progressive renal cell cancer." Int J Cancer **122**(11): 2422-8.
99. Lau, K. W., Y. M. Tian, et al. (2007). "Target gene selectivity of hypoxia-inducible factor-alpha in renal cancer cells is conveyed by post-DNA-binding mechanisms." Br J Cancer **96**(8): 1284-92.
100. Launonen, V., O. Vierimaa, et al. (2001). "Inherited susceptibility to uterine leiomyomas and renal cell cancer." Proc Natl Acad Sci U S A **98**(6): 3387-92.
101. Leach, F. S. (2004). "Linking human genetics with molecular medicine: will hereditary renal cancer play a major role?" Cancer Biol Ther **3**(5): 441-6.
102. Lee, J. K., N. Seki, et al. (2005). "Constitutive expression of functional CD40 on mouse renal cancer cells: induction of Fas and Fas-mediated killing by CD40L." Cell Immunol **235**(2): 145-52.
103. Lee, T. J., J. T. Lee, et al. (2008). "Overexpression of Par-4 enhances thapsigargin-induced apoptosis via down-regulation of XIAP and inactivation of Akt in human renal cancer cells." J Cell Biochem **103**(2): 358-68.
104. Lehtonen, H. J., I. Blanco, et al. (2007). "Conventional renal cancer in a patient with fumarate hydratase mutation." Hum Pathol **38**(5): 793-6.
105. Li, G., K. Passebosc-Faure, et al. (2001). "The expression of G250/mn/CA9 antigen by flow cytometry: its possible implication for detection of micrometastatic renal cancer cells." Clin Cancer Res **7**(1): 89-92.
106. Lionello, I., P. Mangia, et al. (2007). "CD8(+) T lymphocytes isolated from renal cancer patients recognize tumour cells through an HLA- and TCR/CD3-independent pathway." Cancer Immunol Immunother **56**(7): 1065-76.
107. Lipworth, L., R. E. Tarone, et al. (2009). "Epidemiologic characteristics and risk factors for renal cell cancer." Clin Epidemiol **1**: 33-43.
108. Liu, Y. H., C. Y. Lin, et al. (2008). "Up-regulation of vascular endothelial growth factor-D expression in clear cell renal cell carcinoma by CD74: a critical role in cancer cell tumorigenesis." J Immunol **181**(9): 6584-94.
109. Los, M., O. A. Kerckhaert, et al. (2000). "Mutational analysis of endothelial cells derived from von Hippel-Lindau-related renal cancer." J Natl Cancer Inst **92**(20): 1688-9.
110. Luan, F. L., R. Ding, et al. (2003). "Rapamycin is an effective inhibitor of human renal cancer metastasis." Kidney Int **63**(3): 917-26.
111. Macher-Goeppinger, S., S. Aulmann, et al. (2009). "Prognostic value of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and TRAIL receptors in renal cell cancer." Clin Cancer Res **15**(2): 650-9.
112. Madej, A., M. Puzianowska-Kuznicka, et al. (2003). "Vitamin D receptor binding to DNA is altered without the change in its expression in human renal clear cell cancer." Nephron Exp Nephrol **93**(4): e150-7.
113. Maestro, M. L., V. del Barco, et al. (2000). "Loss of heterozygosity on the short arm of chromosome 3 in renal cancer." Oncology **59**(2): 126-30.
114. Mahajan, S., V. Dammai, et al. (2008). "Hypoxia-inducible factor-2alpha regulates the expression of TRAIL receptor DR5 in renal cancer cells." Carcinogenesis **29**(9): 1734-41.
115. Majid, S., A. A. Dar, et al. (2009). "BTG3 tumor suppressor gene promoter demethylation, histone modification and cell cycle arrest by genistein in renal cancer." Carcinogenesis **30**(4): 662-70.
116. Mancini, V., M. Battaglia, et al. (2008). "Current insights in renal cell cancer pathology." Urol Oncol **26**(3): 225-38.
117. Margolin, K., T. W. Synold, et al. (2007). "Oblimersen and alpha-interferon in metastatic renal cancer: a phase II study of the California Cancer Consortium." J Cancer Res Clin Oncol **133**(10): 705-11.
118. Marshall, F. F. (2005). "Quantitative detection of promoter hypermethylation of multiple genes in the tumor, urine, and serum DNA of patients with renal cancer." J Urol **173**(6): 1918.
119. Marshall, S. E., C. Bordea, et al. (2000). "Glutathione S-transferase polymorphisms and skin cancer after renal transplantation." Kidney Int **58**(5): 2186-93.
120. Marshall, S. E., C. Bordea, et al. (2000). "p53 codon 72 polymorphism and susceptibility to skin cancer after renal transplantation." Transplantation **69**(5): 994-6.
121. Masuda, K., M. Ono, et al. (2003). "Downregulation of Cap43 gene by von Hippel-Lindau tumor suppressor protein in human renal cancer cells." Int J Cancer **105**(6): 803-10.
122. Mertz, K. D., F. Demichelis, et al. (2008). "Association of cytokeratin 7 and 19 expression with genomic stability and favorable prognosis in clear cell renal cell cancer." Int J Cancer **123**(3): 569-76.
123. Michelsen, J., H. Thiesson, et al. (2006). "Tissue expression and plasma levels of adrenomedullin in renal cancer patients." Clin Sci (Lond) **111**(1): 61-70.
124. Mikhailenko, D. S., R. B. Kurynin, et al. (2008). "[Inactivation of the VHL gene in sporadic clear cell renal cancer]." Mol Biol (Mosk) **42**(1): 71-7.
125. Moch, H. (2008). "[Molecular basis of targeted therapy in metastatic renal cancer]." Pathologe **29 Suppl 2**: 184-6.
126. Moch, H. (2008). "[Von-Hippel-Lindau (VHL) protein function by initiation and progression of renal cancer]." Pathologe **29 Suppl 2**: 149-52.
127. Mongiat-Artus, P., C. Miquel, et al. (2006). "Spectrum of molecular alterations in colorectal, upper urinary tract, endocervical, and renal carcinomas arising in a patient with hereditary non-polyposis colorectal cancer." Virchows Arch **449**(2): 238-43.
128. Moore, L. E., R. Hung, et al. (2008). "Folate metabolism genes, vegetable intake and renal cancer risk in central Europe." Int J Cancer **122**(8): 1710-5.
129. Moore, L. E., R. T. Wilson, et al. (2005). "Lifestyle factors, exposures, genetic susceptibility, and renal cell cancer risk: a review." Cancer Invest **23**(3): 240-55.
130. Moschella, F., R. P. Catanzaro, et al. (2003). "Shifting gene expression profiles during ex vivo culture of renal tumor cells: implications for cancer immunotherapy." Oncol Res **14**(3): 133-45.
131. Nanus, D. M., Y. Geng, et al. (2000). "Interaction of retinoic acid and interferon in renal cancer cell lines." J Interferon Cytokine Res **20**(9): 787-94.
132. Noguchi, S., T. Shuin, et al. (1998). "High alkaline phosphatase activity in 3p-induced human renal cancer cells." Cancer Lett **131**(2): 223-7.
133. Nyhan, M. J., G. C. O'Sullivan, et al. (2008). "Role of the VHL (von Hippel-Lindau) gene in renal cancer: a multifunctional tumour suppressor." Biochem Soc Trans **36**(Pt 3): 472-8.
134. O'Connor, D. P., E. W. Kay, et al. (2001). "Altered p53 expression in benign and malignant skin lesions from renal transplant recipients and immunocompetent patients with

skin cancer: correlation with human papillomaviruses?" Diagn Mol Pathol **10**(3): 190-9.
135. Ohba, K., Y. Miyata, et al. (2005). "Expression of nm23-H1 gene product in sarcomatous cancer cells of renal cell carcinoma: correlation with tumor stage and expression of matrix metalloproteinase-2, matrix metalloproteinase-9, sialyl Lewis X, and c-erbB-2." Urology **65**(5): 1029-34.
136. Okamura, K., H. Koike, et al. (2009). "Survivin and its spliced isoform gene expression is associated with proliferation of renal cancer cells and clinical stage of renal cancer." Cancer Epidemiol **33**(2): 137-41.
137. O'Kane, H. F., C. J. Watson, et al. (2006). "Targeting death receptors in bladder, prostate and renal cancer." J Urol **175**(2): 432-8.
138. Okegawa, T., J. R. Sayne, et al. (2007). "A histone deacetylase inhibitor enhances adenoviral infection of renal cancer cells." J Urol **177**(3): 1148-56.
139. Okimoto, K., J. Sakurai, et al. (2004). "A germ-line insertion in the Birt-Hogg-Dube (BHD) gene gives rise to the Nihon rat model of inherited renal cancer." Proc Natl Acad Sci U S A **101**(7): 2023-7.
140. Ornstein, D. K., I. A. Lubensky, et al. (2000). "Prevalence of microscopic tumors in normal appearing renal parenchyma of patients with hereditary papillary renal cancer." J Urol **163**(2): 431-3.
141. Osawa, A., Y. Sumiyama, et al. (2006). "Single case of renal cell carcinoma and endocrine pancreatic head cancer occurring with von Hippel-Lindau disease." J Hepatobiliary Pancreat Surg **13**(2): 174-80.
142. Peter, C., J. T. Kielstein, et al. (2007). "A novel bioluminescent tumor model of human renal cancer cell lines: an in vitro and in vivo characterization." J Urol **177**(6): 2342-6.
143. Piekielko-Witkowska, A., A. Master, et al. (2009). "Disturbed expression of type 1 iodothyronine deiodinase splice variants in human renal cancer." Thyroid **19**(10): 1105-13.
144. Pollard, P., N. Wortham, et al. (2005). "Evidence of increased microvessel density and activation of the hypoxia pathway in tumours from the hereditary leiomyomatosis and renal cell cancer syndrome." J Pathol **205**(1): 41-9.
145. Queille, S., L. Luron, et al. (2007). "Analysis of skin cancer risk factors in immunosuppressed renal transplant patients shows high levels of UV-specific tandem CC to TT mutations of the p53 gene." Carcinogenesis **28**(3): 724-31.
146. Radulovic, S. and S. K. Bjelogrlic (2007). "Sunitinib, sorafenib and mTOR inhibitors in renal cancer." J Buon **12 Suppl 1**: S151-62.
147. Ramsay, H. M., P. N. Harden, et al. (2001). "Polymorphisms in glutathione S-transferases are associated with altered risk of nonmelanoma skin cancer in renal transplant recipients: a preliminary analysis." J Invest Dermatol **117**(2): 251-5.
148. Refae, M. A., N. Wong, et al. (2007). "Hereditary leiomyomatosis and renal cell cancer: an unusual and aggressive form of hereditary renal carcinoma." Nat Clin Pract Oncol **4**(4): 256-61.
149. Rennel, E., S. Mellberg, et al. (2007). "Endocan is a VEGF-A and PI3K regulated gene with increased expression in human renal cancer." Exp Cell Res **313**(7): 1285-94.
150. Richard, S., C. Beroud, et al. (1998). "[Von Hippel-Lindau disease and renal cancer: 10 years of genetic progress. GEFVHL (French-Speaking Study Group on von Hippel-Lindau disease)]." Prog Urol **8**(3): 330-9.
151. Rini, B. I., S. Halabi, et al. (2004). "Cancer and Leukemia Group B 90206: A randomized phase III trial of interferon-alpha or interferon-alpha plus anti-vascular endothelial growth factor antibody (bevacizumab) in metastatic renal cell carcinoma." Clin Cancer Res **10**(8): 2584-6.
152. Saenz Lopez, P., F. Vazquez Alonso, et al. (2009). "[Polymorphisms in inflammatory response genes in metastatic renal cancer]." Actas Urol Esp **33**(5): 474-81.
153. Sanz-Ortega, J., C. Olivier, et al. (2009). "[Hereditary renal cancer]." Actas Urol Esp **33**(2): 127-33.
154. Sasaki, M., Y. Tanaka, et al. (2004). "Polymorphisms of the CYP1B1 gene as risk factors for human renal cell cancer." Clin Cancer Res **10**(6): 2015-9.
155. Sato, A., M. Oya, et al. (2006). "Survivin associates with cell proliferation in renal cancer cells: regulation of survivin expression by insulin-like growth factor-1, interferon-gamma and a novel NF-kappaB inhibitor." Int J Oncol **28**(4): 841-6.
156. Schmidt-Wolf, I. G., S. Finke, et al. (1999). "Phase I clinical study applying autologous immunological effector cells transfected with the interleukin-2 gene in patients with metastatic renal cancer, colorectal cancer and lymphoma." Br J Cancer **81**(6): 1009-16.
157. Seki, N., A. D. Brooks, et al. (2002). "Tumor-specific CTL kill murine renal cancer cells using both perforin and Fas ligand-mediated lysis in vitro, but cause tumor regression in vivo in the absence of perforin." J Immunol **168**(7): 3484-92.
158. Shears, L., L. Plowright, et al. (2008). "Disrupting the interaction between HOX and PBX causes necrotic and apoptotic cell death in the renal cancer lines CaKi-2 and 769-P." J Urol **180**(5): 2196-201.
159. Shinojima, T., M. Oya, et al. (2007). "Renal cancer cells lacking hypoxia inducible factor (HIF)-1alpha expression maintain vascular endothelial growth factor expression through HIF-2alpha." Carcinogenesis **28**(3): 529-36.
160. Shioi, K., A. Komiya, et al. (2006). "Vascular cell adhesion molecule 1 predicts cancer-free survival in clear cell renal carcinoma patients." Clin Cancer Res **12**(24): 7339-46.
161. Skubitz, K. M. and A. P. Skubitz (2002). "Differential gene expression in renal-cell cancer." J Lab Clin Med **140**(1): 52-64.
162. Smith, K., L. Gunaratnam, et al. (2005). "Silencing of epidermal growth factor receptor suppresses hypoxia-inducible factor-2-driven VHL-/- renal cancer." Cancer Res **65**(12): 5221-30.
163. Smith, W. M., X. P. Zhou, et al. (2001). "Opposite association of two PPARG variants with cancer: overrepresentation of H449H in endometrial carcinoma cases and underrepresentation of P12A in renal cell carcinoma cases." Hum Genet **109**(2): 146-51.
164. Smits, K. M., L. J. Schouten, et al. (2008). "Genetic and epigenetic alterations in the von hippel-lindau gene: the influence on renal cancer prognosis." Clin Cancer Res **14**(3): 782-7.
165. Smits, K. M., L. J. Schouten, et al. (2008). "Polymorphisms in genes related to activation or detoxification of carcinogens might interact with smoking to increase renal cancer risk: results from The Netherlands Cohort Study on diet and cancer." World J Urol **26**(1): 103-10.
166. Smyth, A., H. M. Reid, et al. (2007). "Modifications of the radiosensitivity of a renal cancer cell line as a consequence of stable TIMP-1 overexpression." Int J Radiat Biol **83**(1): 13-25.
167. Sommerer, C., W. Hartschuh, et al. (2008). "Pharmacodynamic immune monitoring of NFAT-regulated genes predicts skin cancer in elderly long-term renal transplant recipients." Clin Transplant **22**(5): 549-54.
168. Sosman, J. A., I. Puzanov, et al. (2007). "Opportunities and obstacles to combination targeted therapy in renal cell cancer." Clin Cancer Res **13**(2 Pt 2): 764s-769s.
169. Stewart, L., G. M. Glenn, et al. (2008). "Association of germline mutations in the fumarate hydratase gene and uterine fibroids in women with hereditary leiomyomatosis and renal cell cancer." Arch Dermatol **144**(12): 1584-92.
170. Sudarshan, S., C. Sourbier, et al. (2009). "Fumarate hydratase deficiency in renal cancer induces glycolytic addiction and

hypoxia-inducible transcription factor 1alpha stabilization by glucose-dependent generation of reactive oxygen species." Mol Cell Biol **29**(15): 4080-90.
171. Sudarshan, S., P. A. Pinto, et al. (2007). "Mechanisms of disease: hereditary leiomyomatosis and renal cell cancer--a distinct form of hereditary kidney cancer." Nat Clin Pract Urol **4**(2): 104-10.
172. Sudarshan, S., W. M. Linehan, et al. (2007). "HIF and fumarate hydratase in renal cancer." Br J Cancer **96**(3): 403-7.
173. Tamura, T., T. Nishi, et al. (2001). "Intratumoral delivery of interleukin 12 expression plasmids with in vivo electroporation is effective for colon and renal cancer." Hum Gene Ther **12**(10): 1265-76.
174. Tanaka, Y., H. Hirata, et al. (2007). "Polymorphisms of catechol-O-methyltransferase in men with renal cell cancer." Cancer Epidemiol Biomarkers Prev **16**(1): 92-7.
175. Tani, K., M. Azuma, et al. (2004). "Phase I study of autologous tumor vaccines transduced with the GM-CSF gene in four patients with stage IV renal cell cancer in Japan: clinical and immunological findings." Mol Ther **10**(4): 799-816.
176. Tani, K., Y. Nakazaki, et al. (2000). "Progress reports on immune gene therapy for stage IV renal cell cancer using lethally irradiated granulocyte-macrophage colony-stimulating factor-transduced autologous renal cancer cells." Cancer Chemother Pharmacol **46 Suppl**: S73-6.
177. Thrash-Bingham, C. A. and K. D. Tartof (1999). "aHIF: a natural antisense transcript overexpressed in human renal cancer and during hypoxia." J Natl Cancer Inst **91**(2): 143-51.
178. Tolle, A., M. Jung, et al. (2009). "Brain-type and liver-type fatty acid-binding proteins: new tumor markers for renal cancer?" BMC Cancer **9**: 248.
179. Toloczko-Grabarek, A., A. Sikorski, et al. (2005). "Nuclear Pedigree Criteria for the Identification of Individuals Suspected to be at Risk of an Inherited Predisposition to Renal Cancer." Hered Cancer Clin Pract **3**(3): 129-34.
180. Tomlinson, I. P., N. A. Alam, et al. (2002). "Germline mutations in FH predispose to dominantly inherited uterine fibroids, skin leiomyomata and papillary renal cell cancer." Nat Genet **30**(4): 406-10.
181. Toro, J. R., M. L. Nickerson, et al. (2003). "Mutations in the fumarate hydratase gene cause hereditary leiomyomatosis and renal cell cancer in families in North America." Am J Hum Genet **73**(1): 95-106.
182. Toschi, A., J. Edelstein, et al. (2008). "HIF alpha expression in VHL-deficient renal cancer cells is dependent on phospholipase D." Oncogene **27**(19): 2746-53.
183. Trigo, J. M. and J. Bellmunt (2008). "[Current strategies in the treatment of renal-cell cancer: targeted therapies]." Med Clin (Barc) **130**(10): 380-92.
184. Trinder, P., U. Seitzer, et al. (1999). "Constitutive and IFN-gamma regulated expression of IL-7 and IL-15 in human renal cell cancer." Int J Oncol **14**(1): 23-31.
185. Tsao, C. C., B. T. Teh, et al. (2008). "Inhibition of Mxi1 suppresses HIF-2alpha-dependent renal cancer tumorigenesis." Cancer Biol Ther **7**(10): 1619-27.
186. Turner, K. J., J. W. Moore, et al. (2002). "Expression of hypoxia-inducible factors in human renal cancer: relationship to angiogenesis and to the von Hippel-Lindau gene mutation." Cancer Res **62**(10): 2957-61.
187. Ueki, T., T. Takeuchi, et al. (2001). "Silencing of the caspase-1 gene occurs in murine and human renal cancer cells and causes solid tumor growth in vivo." Int J Cancer **91**(5): 673-9.
188. Uemura, H. (1999). "[Molecular detection of circulating cancer cells in patients with renal cell carcinoma]." Hinyokika Kiyo **45**(8): 571-5.
189. Unwin, R. D., R. A. Craven, et al. (2003). "Proteomic changes in renal cancer and co-ordinate demonstration of both the glycolytic and mitochondrial aspects of the Warburg effect." Proteomics **3**(8): 1620-32.
190. Vaziri, S. A., D. R. Grabowski, et al. (2009). "Inhibition of proteasome activity by bortezomib in renal cancer cells is p53 dependent and VHL independent." Anticancer Res **29**(8): 2961-9.
191. Velickovic, M., B. Delahunt, et al. (2001). "VHL and FHIT locus loss of heterozygosity is common in all renal cancer morphotypes but differs in pattern and prognostic significance." Cancer Res **61**(12): 4815-9.
192. Wang, E., R. Lichtenfels, et al. (2004). "Ontogeny and oncogenesis balance the transcriptional profile of renal cell cancer." Cancer Res **64**(20): 7279-87.
193. sWeber, A., I. Kristiansen, et al. (2008). "The FUSE binding proteins FBP1 and FBP3 are potential c-myc regulators in renal, but not in prostate and bladder cancer." BMC Cancer **8**: 369.
194. Wei, M. H., O. Toure, et al. (2006). "Novel mutations in FH and expansion of the spectrum of phenotypes expressed in families with hereditary leiomyomatosis and renal cell cancer." J Med Genet **43**(1): 18-27.
195. Wiesenhutter, B., S. Selinski, et al. (2007). "Re-assessment of the influence of polymorphisms of phase-II metabolic enzymes on renal cell cancer risk of trichloroethylene-exposed workers." Int Arch Occup Environ Health **81**(2): 247-51.
196. Winquist, E., J. Knox, et al. (2006). "Phase II trial of DNA methyltransferase 1 inhibition with the antisense oligonucleotide MG98 in patients with metastatic renal carcinoma: a National Cancer Institute of Canada Clinical Trials Group investigational new drug study." Invest New Drugs **24**(2): 159-67.
197. Wysocki, P. J., J. Zolnierek, et al. (2008). "Targeted therapy of renal cell cancer." Curr Opin Investig Drugs **9**(6): 570-5.
198. Zellweger, T., H. Miyake, et al. (2001). "Chemosensitization of human renal cell cancer using antisense oligonucleotides targeting the antiapoptotic gene clusterin." Neoplasia **3**(4): 360-7.
199. Zivkovic, S., M. Kostov, et al. (2008). "[Histopathological characteristics and coexpression of p53 and p16(INK4a) proteins in renal cancer]." Vojnosanit Pregl **65**(11): 820-4.
200. PubMed (2013). http://www.ncbi.nlm.nih.gov/pubmed.
201. Cancer. Wikipedia. (2013) http://en.wikipedia.org/wiki/Cancer.

12/11/2012