Colorectal Cancer Literatures

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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 cancer and the colorectal.
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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

Abaza, M. S., A. Al-Saffar, et al. (2008). "c-myc antisense oligonucleotides sensitize human colorectal cancer cells to chemotherapeutic drugs." Tumour Biol **29**(5): 287-303.

BACKGROUND/AIMS: Overexpression of the c-myc oncogene frequently occurs in both colon tumors and colon carcinoma cell lines. We examined the sensitization of human colorectal cancer cells to chemotherapeutic drugs using c-myc antisense (AS) phosphorothioate oligonucleotides ([S]ODNs). METHODS: Cancer cells were treated with c-myc [S]ODNs, taxol, 5-fluorouracil (5-FU), doxorubicin and vinblastine individually and in combination. The antiproliferative effects, type of interaction between c-myc [S]ODNs and cytotoxic drugs, cell cycle, apoptosis and expression of cell-cycle- and apoptosis-regulatory genes were evaluated. RESULTS: After treatment with c-myc AS[S]ODNs, the growth of cancer cells was markedly inhibited in a dose- and time-dependent manner and the levels of c-myc mRNA and protein were greatly decreased ($p < 0.0001$). The combinations of c-myc AS[S]ODNs and cytotoxic drugs produced greater growth inhibition of human colorectal cancer cells compared to single treatment with either c-myc AS[S]ODNs ($p < 0.006$) or cytotoxic drugs ($p < 0.0001$). These combinations exhibited time- and dose-dependent additive and/or synergistic antiproliferative effects. Cancer cells treated with cytotoxic drugs were growth arrested in the S phase. In contrast, cells treated with either c-myc AS[S]ODNs or by the combination of c-myc AS[S]ODNs and cytotoxic drugs were growth arrested in the G(2)/M and S phases. The combination treatments also exhibited a marked apoptotic effect compared to single treatments. c-myc AS[S]ODN treatment reduced the mRNA levels of Bcl2, BclxL, cdk2, cyclin E1, cdk1 and cyclin B1, while increasing the mRNA levels of p21, p27, bax and caspase-3. CONCLUSION: This two-hit approach may be important in the quest to overcome drug resistance in cancer patients whose tumors carry an overexpressed c-myc gene.

Alhopuro, P., S. K. Ylisaukko-Oja, et al. (2005). "The MDM2 promoter polymorphism SNP309T-->G and the risk of uterine leiomyosarcoma, colorectal cancer, and squamous cell carcinoma of the head and neck." J Med Genet **42**(9): 694-8.

BACKGROUND: MDM2 acts as a principal regulator of the tumour suppressor p53 by targeting its destruction through the ubiquitin pathway. A polymorphism in the MDM2 promoter (SNP309) was recently identified. SNP309 was shown to result, via Sp1, in higher levels of MDM2 RNA and protein, and subsequent attenuation of the p53 pathway. Furthermore, SNP309 was proposed to be associated with accelerated soft tissue sarcoma formation in both hereditary (Li-Fraumeni) and sporadic cases in humans. METHODS: We evaluated the possible contribution of SNP309 to three tumour types known to be linked with the MDM2/p53 pathway, using genomic sequencing or restriction fragment length polymorphism as screening methods. Three separate Finnish tumour materials (population based sets of 68 patients with early onset uterine leiomyosarcomas and 1042 patients with colorectal cancer, and a series of

162 patients with squamous cell carcinoma of the head and neck) and a set of 185 healthy Finnish controls were analysed for SNP309. RESULTS: Frequencies of SNP309 were similar in all four cohorts. In the colorectal cancer series, SNP309 was somewhat more frequent in women and in patients with microsatellite stable tumours. Female SNP309 carriers were diagnosed with colorectal cancer approximately 2.7 years earlier than those carrying the wild type gene. However, no statistically significant association of SNP309 with patients' age at disease onset or to any other clinicopathological parameter was found in these three tumour materials. CONCLUSION: SNP309 had no significant contribution to tumour formation in our materials. Possible associations of SNP309 with microsatellite stable colorectal cancer and with earlier disease onset in female carriers need to be examined in subsequent studies.

Allen, W. L. and P. G. Johnston (2005). "Role of genomic markers in colorectal cancer treatment." J Clin Oncol **23**(20): 4545-52.

For the last four decades, fluorouracil (FU) has been the main treatment of choice in colorectal cancer (CRC) in both the advanced and adjuvant settings. In the advanced setting, FU monotherapy produces response rates of only 10% to 20%. Furthermore, in resected stage III CRC, FU monotherapy has increased overall survival by only 20%. The combination of FU with newer therapies such as oxaliplatin and irinotecan has significantly improved response rates to 40% to 50%. Despite these improvements, more than half of advanced CRC patients derive no benefit from treatment; this is due to either acquired or inherent drug resistance. This review aims to highlight the current prognostic and predictive markers that have been identified for CRC to date. The limited use of these predictive markers underscores the importance of and need for multiple marker testing in order to improve response rates and decrease toxicity. This review will also focus on high throughput methods to identify panels of predictive markers for CRC, which ultimately aim to tailor treatment according to an individual patient and tumor profile.

Alves, P. M., N. Levy, et al. (2008). "Identification of tumor-associated antigens by large-scale analysis of genes expressed in human colorectal cancer." Cancer Immun **8**: 11.

Despite the high prevalence of colon cancer in the world and the great interest in targeted anti-cancer therapy, only few tumor-specific gene products have been identified that could serve as targets for the immunological treatment of colorectal cancers. The aim of our study was therefore to identify frequently expressed colon cancer-specific antigens. We performed a large-scale analysis of genes expressed in normal colon and colon cancer tissues isolated from colorectal cancer patients using massively parallel signal sequencing (MPSS). Candidates were additionally subjected to experimental evaluation by semi-quantitative RT-PCR on a cohort of colorectal cancer patients. From a pool of more than 6000 genes identified unambiguously in the analysis, we found 2124 genes that were selectively expressed in colon cancer tissue and 147 genes that were differentially expressed to a significant degree between normal and cancer cells. Differential expression of many genes was confirmed by RT-PCR on a cohort of patients. Despite the fact that deregulated genes were involved in many different cellular pathways, we found that genes expressed in the extracellular space were significantly over-represented in colorectal cancer. Strikingly, we identified a transcript from a chromosome X-linked member of the human endogenous retrovirus (HERV) H family that was frequently and selectively expressed in colon cancer but not in normal tissues. Our data suggest that this sequence should be considered as a target of immunological interventions against colorectal cancer.

Andersen, C. L., T. Schepeler, et al. (2007). "Clusterin expression in normal mucosa and colorectal cancer." Mol Cell Proteomics **6**(6): 1039-48.

The gene Clusterin is a target for cancer therapy in clinical trials. The indication for intervention is up-regulated Clusterin expression. Clusterin has been reported to be deregulated in multiple cancer types, including colorectal cancer (CRC). However, for CRC the studies have disagreed on whether Clusterin is up- or down-regulated by neoplastic cells. In the present study we sought to clarify the expression and distribution of Clusterin mRNAs and proteins in normal and neoplastic colorectal tissue through laser microdissection, variant-specific real time RT-PCR, immunohistochemistry, immunofluorescence, Western blotting, and array-based transcriptional profiling. At the transcript level we demonstrated the expression of two novel Clusterin transcripts in addition to the known transcript, and at the protein level we demonstrated two Clusterin isoforms. Our analysis of normal epithelial cells revealed that among these, Clusterin was only expressed by rare neuroendocrine subtype. Furthermore our analysis showed that in the normal mucosa the majority of the observed Clusterin protein originated from the stromal compartment. In tumors we found that Clusterin was de novo synthesized by non-neuroendocrine cancer cells in approximately 25% of cases. Moreover we found that the overall Clusterin level in tumors often appeared to

be lower than in normal mucosa due to the stromal compartment often being suppressed in tumors. Although Clusterin in normal neuroendocrine cells showed a basal localization, the localization in cancer cells was often apical and in some cases associated with apical secretion. Collectively our results indicate that Clusterin expression is very complex. We conclude that Clusterin expression is associated with neuroendocrine differentiation in normal epithelia and that the Clusterin observed in neoplastic cells is de novo synthesized. The cases with de novo synthesized Clusterin define a distinct subgroup of CRC that may be of clinical importance as anti-Clusterin therapeutics are now in clinical trials.

Androulakis, X. M., S. J. Muga, et al. (2006). "Chemopreventive effects of Khaya senegalensis bark extract on human colorectal cancer." Anticancer Res **26**(3B): 2397-405.

An extract of the bark of Khaya senegalensis is commonly used in African traditional medicine for pain and inflammation. Khaya senegalensis bark extract (KSBE) was hypothesized to contain inhibitors of the cyclooxygenase-2 (COX-2) gene and to be useful in the prevention and treatment of colorectal cancer. The diphenyl-2-picrylhydrazyl (DPPH)- free radical activity and the total phenolic content of KSBE were measured, followed by an investigation of cell growth inhibition, COX and prostaglandin E 2 (PGE2) suppression, as well as apoptosis by Western blot analysis and ELISA. Our data clearly showed that KSBE displays anti-proliferative, antiinflammatory and pro-apoptotic effects on HT-29, HCT-15 and HCA-7 cells. Since all three cell lines, irrespective of COX-2 status (HCT-15 is COX-2-deficient), were affected by the treatment, it can be concluded that both COX-dependent and COX-independent pathways are activated by KSBE.

Arango, D., A. J. Wilson, et al. (2004). "Molecular mechanisms of action and prediction of response to oxaliplatin in colorectal cancer cells." Br J Cancer **91**(11): 1931-46.

The platinum compound oxaliplatin has been shown to be an effective chemotherapeutic agent for the treatment of colorectal cancer. In this study, we investigate the molecular mechanisms of action of oxaliplatin to identify means of predicting response to this agent. Exposure of colon cancer cells to oxaliplatin resulted in G2/M arrest and apoptosis. Immunofluorescent staining demonstrated that the apoptotic cascade initiated by oxaliplatin is characterised by translocation of Bax to the mitochondria and cytochrome c release into the cytosol. Oxaliplatin treatment resulted in caspase 3 activation and oxaliplatin-induced apoptosis was abrogated by inhibition of caspase activity with z-VAD-fmk, but was independent of Fas/FasL association. Targeted inactivation of Bax or p53 in HCT116 cells resulted in significantly increased resistance to oxaliplatin. However, the mutational status of p53 was unable to predict response to oxaliplatin in a panel of 30 different colorectal cancer cell lines. In contrast, the expression profile of these 30 cell lines, assessed using a 9216-sequence cDNA microarray, successfully predicted the apoptotic response to oxaliplatin. A leave-one-out cross-validation approach was used to demonstrate a significant correlation between experimentally observed and expression profile predicted apoptosis in response to clinically achievable doses of oxaliplatin (R=0.53; P=0.002). In addition, these microarray experiments identified several genes involved in control of apoptosis and DNA damage repair that were significantly correlated with response to oxaliplatin.

Aschele, C., D. Debernardis, et al. (2004). "Deleted in colon cancer protein expression in colorectal cancer metastases: a major predictor of survival in patients with unresectable metastatic disease receiving palliative fluorouracil-based chemotherapy." J Clin Oncol **22**(18): 3758-65.

PURPOSE: To determine whether deleted in colon cancer (DCC) protein expression in colorectal cancer (CRC) metastases could predict outcome to palliative fluorouracil (FU)-based chemotherapy and to assess whether it is similar to that observed in the corresponding primary tumors. PATIENTS AND METHODS: DCC protein expression was assessed immunohistochemically on archival specimens of CRC metastases from 42 patients homogeneously treated by methotrexate-modulated bolus FU alternated to 6-S-leucovorin-modulated infused FU and was retrospectively correlated with patient characteristics and clinical outcome. In a subset analysis, DCC immunoreactivity was compared between metastatic CRC and the corresponding primary tumors and regional lymph node metastases. RESULTS: Positive immunoreactivity for DCC was found in 45% of patients. Eighteen (78%) of 23 patients for whom multiple samples were available displayed a similar pattern of expression in distant metastases and primary tumors. The median survival time was 14.3 months in patients without DCC expression and 21.4 months in patients with DCC-positive tumors (log-rank test, P =.04); the 2-year survival rates were 8.5% and 42.5%, respectively. Response rates to chemotherapy were not significantly different between the two groups. By multivariate analysis, DCC protein expression maintained its prognostic value and showed to be the single best predictor of survival, with a relative risk of 2.16.

CONCLUSION: Our results indicate that expression of the DCC protein in CRC metastases is similar to that observed in the corresponding primary tumors and represents a dominant predictor of survival in patients with unresectable, advanced CRC who are undergoing palliative FU-based chemotherapy.

Ashida, R., K. Tominaga, et al. (2005). "AP-1 and colorectal cancer." Inflammopharmacology **13**(1-3): 113-25.

Activator protein-1 (AP-1) is a transcription factor that consists of either a Jun-Jun homodimer or a Jun-Fos heterodimer. AP-1 regulates the expression of multiple genes essential for cell proliferation, differentiation and apoptosis. Numerous reports suggest that AP-1 plays an important role in various human diseases. Among them, the roles relating to human cancers have been strongly suggested for a long time. In human cancers, colorectal cancer is still a leading cause of morbidity and mortality in the world. Since there are some reports about the role of AP-1 in colorectal cancer response to a number of stimuli, such as cytokines and growth factors, and oncogenictransformation, therapeutic inhibition of AP-1 activity has attracted considerable interest. Here, we demonstrate the biological properties of AP-1 and its role in colorectal cancer, and discuss a possibility of an AP-1 inhibitor, an adenovirus dominant-negative mutant of c-Jun, as a therapeutic agent for gene therapy.

Azuma, M., K. D. Danenberg, et al. (2006). "Epidermal growth factor receptor and epidermal growth factor receptor variant III gene expression in metastatic colorectal cancer." Clin Colorectal Cancer **6**(3): 214-8.

PURPOSE: The epidermal growth factor receptor (EGFR) variant type III (variously called EGFRvIII, de2-7 EGFR, or triangle upEGFR) has an in-frame deletion of the extracellular domain and is found in numerous types of human tumors. Because EGFRvIII has been reported to be tumor specific and has oncogenic potential, it is being investigated as a potential therapeutic target, but to our knowledge, there is only 1 previous report about EGFRvIII by immunohistochemistry in colorectal cancer. Our aim was to indicate the frequency of gene expressions of EGFRvIII and EGFR in metastatic colorectal cancer (mCRC). PATIENTS AND METHODS: Forty-five patients with mCRC who received the chemotherapy for metastatic disease were analyzed for the EGFRvIII variant. Paraffin-embedded tumor tissues were dissected using laser-captured microdissection and analyzed for the EGFR and EGFRvIII messenger RNA expression using a quantitative real-time reverse-transcriptase polymerase chain reaction method. Gene expression values (relative messenger RNA levels) are expressed as ratios (differences from the cycle threshold values) between the target gene and internal reference gene (beta-actin). Twenty-five women and 20 men with a median age of 55 years (range, 25-76 years) were included in this study. RESULTS: We did not find any expression of EGFRvIII in these 45 patients except for control cell lines as U87.EGFRvIII. However, EGFR gene expression was found in 43 of 45 (95.6%) with a range of 0.38-2.83. CONCLUSION: Our results demonstrate that the expression of EGFRvIII is rare, but most colon cancer demonstrates EGFR gene expression. We conclude that EGFRvIII does not play an important role in mCRC.

Becker, C., M. C. Fantini, et al. (2005). "IL-6 signaling promotes tumor growth in colorectal cancer." Cell Cycle **4**(2): 217-20.

Recent investigations support an important role for TGF-beta in the development of colorectal cancer. However, the molecular consequences of TGF-beta signaling in the colon remains incompletely understood. In a recent study in Immunity, we analyzed the role of TGF-beta in a murine model of colon cancer. Using transgenic mice overexpressing TGF-beta or a dominant negative TGF-beta receptor II under control of the CD2 minigene, we show that TGF-beta signaling in tumor infiltrating T lymphocytes regulates the growth of dysplastic colon epithelial cells, as determined by histology and a novel system for high resolution chromoendoscopy in vivo. At the molecular level, TGF-beta signaling in T cells regulated STAT-3 activation in tumor cells via IL-6. IL-6 signaling required tumor cell derived soluble IL-6R rather than membrane bound IL-6R and suppression of such TGF-beta-dependent IL-6 trans-signaling prevented tumor progression in vivo. Similar to these observations in mice, here we show that human colon cancer tissue expressed only low amounts of membrane bound IL-6R. In contrast, expression and activity of the matrix metalloproteinase TACE were increased. In summary, our data provide novel insights into the role of TGF-beta signaling in colorectal cancer and suggest novel therapeutic approaches for colorectal cancer based on an inhibition of TGF-beta-dependent IL-6 trans-signaling.

Behrend, L., A. Mohr, et al. (2005). "Manganese superoxide dismutase induces p53-dependent senescence in colorectal cancer cells." Mol Cell Biol **25**(17): 7758-69.

The mitochondrial enzyme manganese superoxide dismutase (MnSOD) is known to suppress cell growth in different tumor cell lines. However, the

molecular mechanism of this growth-retarding effect is not fully understood. Here we show that overexpression of MnSOD slows down growth of HCT116 human colorectal cancer cells by induction of cellular senescence. MnSOD overexpression causes up-regulation of p53 and its transcriptional target, the cyclin-dependent kinase inhibitor p21. Adenovirus-mediated knockdown of p53 by RNA interference rescues MnSOD-overexpressing clones from growth retardation. Accordingly, the overexpression of MnSOD in HCTp53(-/-) cells does not lead to senescence, whereas in HCTp21(-/-) cells we found induction of senescence by forced expression of MnSOD. These results indicate a pivotal role of p53, but not p21, in the observed effects. Analysis of the mitochondrial membrane potential revealed reduced polarization in MnSOD-overexpressing cells. In addition, depolarization of the mitochondrial membrane by mitochondrial inhibitors such as rotenone or antimycin A led colorectal cancer cells into p53-dependent senescence. Our data indicate that uncoupling of the electrochemical gradient by increased MnSOD activity gives rise to p53 up-regulation and induction of senescence. This novel mitochondrially mediated mechanism of tumor suppression might enable strategies that allow reactivation of cellular aging in tumor cells.

Belvedere, O., F. Puglisi, et al. (2004). "Lack of correlation between immunohistochemical expression of E2F-1, thymidylate synthase expression and clinical response to 5-fluorouracil in advanced colorectal cancer." Ann Oncol **15**(1): 55-8.

BACKGROUND: The level of the enzyme thymidylate synthase (TS) is known to inversely correlate with the clinical activity of 5-fluorouracil (FU) in advanced colorectal cancer patients. Since the correlation is not very strong, we have retrospectively analyzed the expression of E2F-1 in tumor samples or metastases from 25 patients with advanced colorectal cancer, homogeneously treated with an FU-based regimen. E2F-1 is a transcription factor regulating the expression of TS along with other crucial DNA synthesis related enzymes. MATERIALS AND METHODS: E2F-1 expression was analyzed by immunohistochemistry using the anti-E2F-1 monoclonal antibody KH95, scoring 2000 cells/case. Expression of TS was evaluated by immunohistochemistry using a rabbit anti-human polyclonal antibody. RESULTS: The level of E2F-1 expression did not correlate with TS expression, although a trend for correlation between E2F-1 level and maximal tumor shrinkage was observed (r = 0.42; P = 0.054). CONCLUSIONS: In spite of previous reports demonstrating that E2F-1 quantified by rt-PCR and western blot correlates with TS and could be used as a predictor to select colorectal cancer patients more likely to respond to FU treatment, our data suggest that, under these experimental conditions, immunohistochemistry cannot be used for such selection.

Bendardaf, R., H. Lamlum, et al. (2008). "Thymidylate synthase and microsatellite instability in colorectal cancer: implications for disease free survival, treatment response and survival with metastases." Acta Oncol **47**(6): 1046-53.

BACKGROUND: Colorectal cancer (CRC) cell lines displaying microsatellite instability (MSI) are resistant to 5-fluorouracil (5-FU) in vitro, which can be overcome by restoring DNA mismatch repair (MMR) competence. Thymidylate synthase (TS) is inhibited by 5-FU, being another potential mediator of therapeutic resistance to 5-FU. The clinical relevance of these observations remains unclear. OBJECTIVE: We examined the expression of TS and two MMR proteins (hMLH1 and hMSH2) in advanced CRC patients, to determine a) their mutual relationship, b) association to therapeutic response and c) impact on disease outcome. MATERIAL AND METHODS: Tumour samples from 73 patients CRC who were treated in advanced stage with either irinotecan alone or in combination with 5-FU/leucovorin, were analysed for expression of TS, hMLH1 and hMSH2 using immunohistochemistry (IHC). RESULTS: TS expression was closely correlated with hMLH1 expression (negative-weak/moderate-strong) (p=0.0001). TS-MMR expression was significantly (p=0.029 for whole series; p=0.004 for the 5-FU treated cases) related to response to treatment; tumours with low levels of both TS and MMR responded better (n=14/27, 51.8%) than those with high TS and MMR (n=3/18, 16.6%). Patients with high TS-MMR expression had a significantly longer DFS (47 months vs. 9 months, n=26) than those with low TS-MMR index (p=0.015), while the reverse was true concerning survival with metastases (WMS) (p=0.018) in all the patients (n=73). CONCLUSIONS: The present data suggest that MSI patients with low TS and deficient MMR demonstrate a significantly shorter DFS and longer WMS than patients with high expression of both markers, and they are also more likely to obtain the greatest benefit from 5-FU based chemotherapy.

Bialkowska, A. B., Y. Du, et al. (2009). "Identification of novel small-molecule compounds that inhibit the proproliferative Kruppel-like factor 5 in colorectal cancer cells by high-throughput screening." Mol Cancer Ther **8**(3): 563-70.

Colorectal cancer is one of the leading causes of cancer mortality and morbidity worldwide.

Previous studies indicate that the zinc finger-containing transcription factor Kruppel-like factor 5 (KLF5) positively regulates proliferation of intestinal epithelial cells and colorectal cancer cells. Importantly, inhibition of KLF5 expression in intestinal epithelial cells and colorectal cancer cells by pharmacologic or genetic means reduces their rate of proliferation. To identify additional and novel small molecules that inhibit KLF5 expression and thus colorectal cancer proliferation, we developed a reporter assay using colorectal cancer cell line (DLD-1) that stably expressed a luciferase reporter gene directed by 1,959 bp of the human KLF5 promoter upstream of the ATG start codon and performed a cell-based high-throughput screen with the Library of Pharmacologically Active Compounds that contains 1,280 biologically active compounds. The screen identified 8 potential inhibitors and 6 potential activators of the KLF5 promoter. Three potential inhibitors, wortmannin, AG17, and AG879, were further evaluated by secondary analyses. All three significantly reduced both KLF5 promoter-luciferase activity and protein level in DLD-1 cells in a dose- and time-dependent manner when compared with controls. They also significantly reduced the rate of proliferation of DLD-1 and two other colorectal cancer cell lines, HCT116 and HT29. Our results show the principle of using high-throughput screening to identify small-molecule compounds that modulate KLF5 activity and consequently inhibit colorectal cancer proliferation.

Bianchini, M., E. Levy, et al. (2006). "Comparative study of gene expression by cDNA microarray in human colorectal cancer tissues and normal mucosa." Int J Oncol **29**(1): 83-94.

The causative molecular pathways underlying the pathogenesis of colorectal cancer (CRC) need to be better characterized. The purpose of our study was to better understand the genetic mechanism of oncogenesis for human colorectal cancer and to identify new potential tumor markers of use in clinical practice. We used cDNA microarrays to compare gene expression profiles of colorectal biopsies from 25 CRC patients and 13 normal mucosa from adjacent non-cancerous tissues. Findings were validated by real-time PCR; in addition, western blotting and immunochemistry analysis were carried out as further confirmation of differential expression at a protein level. Comparing cancerous tissues with normal colonic mucosa we identified 584 known genes differentially expressed to a significant degree ($p<0.001$). Many of the transcripts that were more abundant in tumors than in non-neoplastic tissues appear to reflect important events for colon carcinogenesis. For example, a significant number of these genes serve as apoptotic inhibitors (e.g. BFAR, BIRC1, BIRC6). Furthermore, we observed the simultaneous up-regulation of HLA-E and the down-regulation of beta2-microglobulin; these genes strongly support a potential tumor escape strategy from immune surveillance in colon cancer tissues. Our study provides new gene candidates in the pathogenesis of human CRC disease. From our results we hypothesize that CRC cells escape immune surveillance through a specific gene expression alteration; moreover, over-expression of several survival genes seems to confer a more anti-apoptotic phenotype. These genes are involved in pathways not previously implicated in CRC pathogenesis and they may provide new targets for therapy.

Boldrini, L., P. Faviana, et al. (2004). "Regulation of telomerase and its hTERT messenger in colorectal cancer." Oncol Rep **11**(2): 395-400.

Telomeres are the distal ends of human chromosomes composed of tandem repeats of the sequence TTAGGG. In most human somatic cells, telomerase activity is undetectable, and the telomere length is progressively shortened during cell proliferation, leading to cellular senescence. In contrast, telomerase is activated in the vast majority of cancer cells, including colorectal cancer. The human telomerase complex is comprised of multiple components, but telomerase reverse transcriptase (hTERT) is the most important component for the control of telomerase activity. The p53 protein is a transcription factor with multiple biological activities, including cell cycle arrest and/or apoptosis upon DNA damage, hypoxia and oncogene activation; this requires transactivation or repression of specific target genes by wild-type p53. To better understand if a link between hTERT/telomerase regulation and p53 status exists in colorectal carcinogenesis, we analysed 43 cases of colorectal carcinoma for hTERT mRNA expression and telomerase activity. Moreover, a complete analysis of p53 status was performed. Alterations of p53 gene were found in 44.19% of cases and missense point mutations represented a high proportion of p53. Both telomerase activity ($p=0.014$) and hTERT expression ($p=0.03$) were significantly associated with p53 mutations, suggesting a role of p53 in the signaling pathway for telomerase control.

Bordonaro, M. (2009). "Modular Cre/lox system and genetic therapeutics for colorectal cancer." J Biomed Biotechnol **2009**: 358230.

The Cre/lox system is a powerful tool for targeting therapeutic effectors in a wide variety of human disorders. I review a Cre/lox Wnt-targeted system that has shown promise against Wnt-positive colorectal cancer cell lines. In addition to Wnt-specific

targeting of cell death inducers, the modular nature of this gene therapy model system can be exploited by designing positive and negative feedback loops to either amplify or inhibit Wnt activity for experimental or therapeutic benefit. I discuss the structural components and performance parameters of the system, the implication of these findings with respect to cancer stem cells, as well as the general applicability of this system to any disorder characterized by differential gene expression. I also consider the issue of gene delivery as well as in vivo testing requirements necessary for the further characterization and development of this system.

Bordonaro, M., D. L. Lazarova, et al. (2004). "Pharmacological and genetic modulation of Wnt-targeted Cre-Lox-mediated gene expression in colorectal cancer cells." Nucleic Acids Res **32**(8): 2660-74.

Wnt-targeted gene therapy has been proposed as a treatment for human colorectal cancer (CRC). The Cre-Lox system consists of methodology for enhancing targeted expression from tissue-specific or cancer-specific promoters. We analyzed the efficiency of Wnt-specific promoters as drivers of the Cre-mediated activity of a luciferase reporter gene or cell death effector gene in CRC cell lines in the presence and absence of two modulators of Wnt activity, sodium butyrate and lithium chloride. Butyrate is present in the colonic lumen after digestion of fiber-rich foods, whereas the colonic lumen is readily accessible to lithium chloride. In both SW620 and HCT-116 CRC cells, a physiologically relevant concentration of butyrate upregulated reporter and effector activity and altered the Wnt-specific expression pattern. Lithium chloride markedly enhanced Cre-Lox-mediated Wnt-specific reporter expression only in APC wild-type CRC cells. Possibilities for genetic modulation of the proposed CRC therapy included Wnt-specific expression of a floxed Lef1-VP16 fusion that enhanced Wnt-specific cell death and of a floxed dominant-negative Tcf4 that specifically downregulated endogenous Wnt activity. These findings demonstrated that the Cre-Lox system, in combination with pharmacological and genetic modulators, represents effective methodology for enhancing Wnt-targeted gene therapy.

Ceccarelli, C., G. Piazzi, et al. (2005). "Concurrent EGFr and Cox-2 expression in colorectal cancer: proliferation impact and tumour spreading." Ann Oncol **16 Suppl 4**: iv74-79.

BACKGROUND: Many reports were produced on single epidermal growth factor receptor (EGFr) and cyclo-oxygenase-2 (Cox-2) evaluation using immunohistochemical techniques (IHC), but very few works considered concurrent expression of these two proteins in the light of their impact on proliferation and tumour spreading. At least three molecular pathways (EGFr, Cox-2, and APC/beta-catenin molecular cascade) may interact in this malignancy giving rise to cross talking effects on proliferation and cancer spreading. PATIENTS AND METHODS: To better detail these two latter aggressive features, we studied 205 sporadic colorectal cancer patients, comparing concurrent expression of EGFr, Cox-2, Ki-67, Cyclins D1-A, and E, with tumour spreading (budding) (BUD) and pN status. RESULTS: Our results point to a different aggressive molecular profile due to Cox-2 expression. Cox-2 High expressing cases showed a clear EGFr proliferation-promoting role. On the contrary, EGFr seems directly involved in cancer cells spreading rather than in promoting cancer proliferation in Cox-2 Low/Negative cases. CONCLUSIONS: Immunohistochemical profiling of colorectal cancer seems to be a promising approach, not only to define prognostic impact, but also to detail proliferation-related molecular interplays between EGFr and Cox-2 pathways, with these two latter proteins, at present, being the hottest pharmacological targets for colorectal cancer (CRC) chemoprevention and therapy.

Chatel, G., C. Ganeff, et al. (2007). "Hedgehog signaling pathway is inactive in colorectal cancer cell lines." Int J Cancer **121**(12): 2622-7.

The Hedgehog (Hh) signaling pathway plays an important role in human development. Abnormal activation of this pathway has been observed in several types of human cancers, such as the upper gastro-intestinal tract cancers. However, activation of the Hh pathway in colorectal cancers is controversial. We analyzed the expression of the main key members of the Hh pathway in 7 colon cancer cell lines in order to discover whether the pathway is constitutively active in these cells. We estimated the expression of SHH, IHH, PTCH, SMO, GLI1, GLI2, GLI3, SUFU and HHIP genes by RT-PCR. Moreover, Hh ligand, Gli3 and Sufu protein levels were quantified by western blotting. None of the cell lines expressed the complete set of Hh pathway members. The ligands were absent from Colo320 and HCT116 cells, Smo from Colo205, HT29 and WiDr. GLI1 gene was not expressed in SW480 cells nor were GLI2/GLI3 in Colo205 or Caco-2 cells. Furthermore the repressive form of Gli3, characteristic of an inactive pathway, was detected in SW480 and Colo320 cells. Finally treatment of colon cancer cells with cyclopamine, a specific inhibitor of the Hh pathway, did not downregulate PTCH and GLI1 genes expression in the colorectal cells, whereas it did so in PANC1 control

cells. Taken together, these results indicate that the aberrant activation of the Hh signaling pathway is not common in colorectal cancer cell lines.

Chen, J., W. H. Ding, et al. (2009). "Impact of p27mt gene on transplantation model of human colorectal cancer in nude mice." World J Gastroenterol **15**(3): 369-72.

AIM: To investigate the inhibitory and anti-metastatic effect of mutant p27 gene (p27mt) on the growth of colorectal cancer xenografts in nude mice and its underlying mechanism. METHODS: Inhibitory effect of p27mt gene on the growth of colorectal cancer xenografts was determined by measurement of tumor size before and after direct intra-tumoral injection of Ad-p27mt in a pre-established transplantation model of human colorectal cancer in nude mice. Cell cycle and apoptosis were detected by flow cytometry performed on single-cell suspension from an isolated tumor. Expression of MMP-9 in tumor tissue was detected by immunohistochemistry. RESULTS: The average sizes of transplantation tumors were 1.94 +/- 0.67 cm(3), 2.75 +/- 0.83 cm(3) and 3.01 +/- 0.76 cm(3) in the Ad-p27mt, Ad-LacZ and control groups, respectively ($P < 0.05$). The average proliferation rates were 37.34% +/- 1.45%, 53.16% +/- 3.27% and 54.48% +/- 2.43%, in the Ad-p27mt, Ad-LacZ and control groups, respectively ($P < 0.05$). The average apoptosis rates were 19.79% +/- 3.32%, 6.38% +/- 4.91% and 7.25% +/- 5.20% in the Ad-p27mt, Ad-LacZ and control groups, respectively ($P < 0.01$). The average MMP-9 expression rates were 20%, 75% and 66.7% in the Ad-p27mt, Ad-LacZ and control groups, respectively ($P < 0.01$). CONCLUSION: p27mt inhibits the growth of transplanted tumor by blocking the proliferation of cancer xenografts and by promoting apoptosis of transplantated tumor cells, as well as decrease transplanted tumor metastasis.

Chen, L., J. Jiang, et al. (2007). "P53 dependent and independent apoptosis induced by lidamycin in human colorectal cancer cells." Cancer Biol Ther **6**(6): 965-73.

Enediyne compound is one class of antibiotics with very potent anti-cancer activity. However, the role of p53 in enediyne antibiotic-induced cell killing remains elusive. Here we reported the involvement of p53 signaling pathway in apoptosis induction by lidamycin (LDM), a member of the enediyne antibiotic family. We found that LDM at low drug concentration of 10 nmol/L induces apoptotic cell death much more effectively in human colorectal cancer cells with wild type p53 than those with mutant or deleted p53. p53 is functionally activated as an early event in response to low dose LDM that precedes the significant apoptosis induction. The primarily activation of mitochondria as well as the activation of p53 transcriptional targets such as Puma, Bad and Bax in HCT116 p53 wild type cells further demonstrates the key role of p53 in mediating the compound-induced apoptosis. This is further supported by the observation that the absence of Bax or Puma decreases apoptosis dramatically while Bcl-2 overexpression confers partially resistance after drug treatment. Activation of p53 signaling pathway leads to activation of caspases and caspases inhibitor VAD-fmk completely blocks low dose LDM induced apoptosis through the inhibition of mitochondria pathway. In contrast, LDM at higher concentration causes rapid apoptosis through more direct DNA damaging mechanism that is independent of activation of p53 and caspases and cannot be blocked by caspase inhibitor. Taken together, LDM induces apoptosis in a p53-dependent manner when given at low doses, but in a p53-independent manner when given at high doses. This dosage-dependent regimen can be applied to cancer clinic based upon the p53 status of cancer patients.

Chen, T. H., S. L. Pan, et al. (2008). "Denbinobin induces apoptosis by apoptosis-inducing factor releasing and DNA damage in human colorectal cancer HCT-116 cells." Naunyn Schmiedebergs Arch Pharmacol **378**(5): 447-57.

Denbinobin is a phenanthraquinone derivative present in the stems of Ephemerantha lonchophylla. We showed that denbinobin induces apoptosis in human colorectal cancer cells (HCT-116) in a concentration-dependent manner. The addition of a pan-caspase inhibitor (zVAD-fmk) did not suppress the denbinobin-induced apoptotic effect, and denbinobin-induced apoptosis was not accompanied by processing of procaspase-3, -6, -7, -9, and -8. However, denbinobin triggered the translocation of the apoptosis-inducing factor (AIF) from the mitochondria into the nucleus. Small interfering RNA targeting of AIF effectively protected HCT-116 cells against denbinobin-induced apoptosis. Denbinobin treatment also caused DNA damage, activation of the p53 tumor suppressor gene, and upregulation of numerous downstream effectors (p21WAF1/CIP1, Bax, PUMA, and NOXA). A HCT-116 xenograft model demonstrated the in vivo efficacy and low toxicity of denbinobin. Taken together, our findings suggest that denbinobin induces apoptosis of human colorectal cancer HCT-116 cells via DNA damage and an AIF-mediated pathway. These results indicate that denbinobin has potential as a novel anticancer agent.

Chen, W. C., Q. Liu, et al. (2004). "Expression of survivin and its significance in colorectal cancer." World J Gastroenterol **10**(19): 2886-9.

AIM: To study the expression of survivin, a novel member of inhibitors of apoptosis protein (IAP) and its significance in colorectal carcinoma. METHODS: Survivin mRNA expression was evaluated by semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR) in 52 colorectal carcinoma samples and 48 adjacent normal colorectal tissue samples. PCR product was sequenced to verify the desired result. Expressions of survivin protein, proliferating cell nuclear antigen labelling index (PI) and apoptotic index (AI) were detected immunohistochemically in 52 human colorectal carcinomas. RESULTS: The expression of survivin mRNA was detected in a significantly greater proportion of colorectal carcinoma samples than in adjacent normal colorectal tissues (67.3% vs 25%; P<0.01). There was no relationship between survivin mRNA expression in colorectal carcinomas and sex, tumor size, histological types, lymphnode metastasis, distant metastasis and Dukes' stage. The PCR product shared 99% of homology with human counterparts. Survivin expression was observed immunohistochemically in 27 of 52 cases of colorectal carcinoma (51.9%). The AI was significantly lower in survivin positive group than in survivin negative group (0.67+/-0.18% vs 1.14+/-0.42%; P<0.001), while the PI was greater in survivin positive group than in survivin negative group (51+/-22% vs 27+/-18%, P<0.001). CONCLUSION: Survivin is a special tumor marker independent of histopathological characteristics. It may play an important role during human colorectal tumorigenesis by inhibiting apoptosis and accelerating proliferative activity of colorectal tumor cells.

Chiacchiera, F. and C. Simone (2008). "Signal-dependent regulation of gene expression as a target for cancer treatment: inhibiting p38alpha in colorectal tumors." Cancer Lett **265**(1): 16-26.

In the last year, several evidences indicated that pharmacological manipulation of relevant signaling pathways could selectively affect gene expression to influence cell fate. These findings render of extreme importance the elucidation of how external stimuli are transduced to mediate chromatin modifications, resulting in a permissive or repressive environment for gene expression. These signaling cascades activate or repress the function of chromatin binding proteins that represent attractive pharmacological targets for human diseases. Actually, the closer the target is to chromatin, the more the transcriptional effect will be selective. Recent studies suggest that pharmacological manipulation of signaling pathways to modulate cell fate is indeed possible and that chromatin-associated kinases could represent an optimal target. The p38 MAPK is the prototype of this class of enzymes and its central role in the transcription process is evolutionary conserved. In this review we will focus on the possibility to inhibit p38alpha in colorectal cancer to arrest tumor progression and induce autophagic cell death.

Chien, C. C., S. H. Chen, et al. (2007). "Correlation of K-ras codon 12 mutations in human feces and ages of patients with colorectal cancer (CRC)." Transl Res **149**(2): 96-102.

Colorectal cancer (CRC) is the predominant gastrointestinal malignancy and constitutes a major medical and economic burden worldwide. A thorough understanding of the oncogenes or genes related to tumorigenesis is the key to developing successful therapeutic strategies. Molecular analysis of feces constitutes a potentially potent and noninvasive method for detection of CRC. Using nested reverse transcription-polymerase chain reaction (RT-PCR) and amplified restriction fragment length polymorphism analysis, sloughed cells from the entire length of the colon and rectum were analyzed for expression of activating K-ras codon 12 mutants, which are becoming attractive targets for antisense treatment. K-ras codon 12 mutant sequences were detected in feces of 5% (1/20) of healthy controls, in feces of 41% (12/29) of CRC patients, in 10% (3/29) of isolates of tissue complementary DNA (cDNA), and in 14% (4/29) of isolates of genomic DNA. Age of patient was significantly associated with K-ras codon 12 sequences in feces: Patients with wild-type K-ras codon 12 sequences were significantly younger than those with mutated forms of K-ras codon 12. Fecal ribonucleic acid (RNA) analysis was demonstrated to be a useful for diagnosis of CRC. This technique may be suitable for screening and determining the clinical significance of active mutations of the K-ras gene in feces and would possibly be useful for identifying patients that would benefit from antisense therapy.

Chiu, S. J., Y. J. Lee, et al. (2009). "Oxaliplatin-induced gamma-H2AX activation via both p53-dependent and -independent pathways but is not associated with cell cycle arrest in human colorectal cancer cells." Chem Biol Interact **182**(2-3): 173-82.

Oxaliplatin, a chemotherapeutic drug, induces DNA double-strand breaks (DSBs) and apoptosis in colorectal cancer cells. It has been shown that gamma-H2AX acts as a marker of DSBs. However, the molecular events associated with oxaliplatin-mediated cell cycle arrest and cell death remain unclear. In this study, we investigated the roles

of p53 and gamma-H2AX following oxaliplatin treatment, as they are important effector proteins for apoptosis and DSB repair, respectively. Both phosphorylated-p53 (Ser-15) and gamma-H2AX were up-regulated and accumulated in the nuclei of p53-wild type human colorectal cancer HCT116 cells after exposure to oxaliplatin. Concomitantly, oxaliplatin-induced G2/M arrest was associated with a reduction in both cyclin B1 expression and phosphorylated-CDC2 (Thr-161). Release of G2/M arrest by caffeine was accompanied by a decrease in the levels of p53/p21; however, gamma-H2AX levels were unchanged. Furthermore, inhibition of p53 phosphorylation by pifithrin-alpha was sufficient to reduce the oxaliplatin-induced up-regulation of gamma-H2AX and apoptosis. Oxaliplatin-induced gamma-H2AX via a p53-independent pathway but did not cause caspase-3 activation in p53-null HCT116 cells. Interestingly, no changes were observed in the H2AX gene knockdown with regards to oxaliplatin-induced G2/M arrest in p53-wild type and S phase arrest in p53-null HCT116 cells. Taken together, these data indicate that a molecular pathway involving p53, gamma-H2AX and cell cycle arrest plays a pivotal role in the cellular response to oxaliplatin.

Coluccia, A. M., D. Benati, et al. (2006). "SKI-606 decreases growth and motility of colorectal cancer cells by preventing pp60(c-Src)-dependent tyrosine phosphorylation of beta-catenin and its nuclear signaling." Cancer Res **66**(4): 2279-86.

Inhibition of deregulated protein tyrosine kinases represents an attractive strategy for controlling cancer growth. However, target specificity is an essential aim of this strategy. In this report, pp60(c-Src) kinase and beta-catenin were found physically associated and constitutively activated on tyrosine residues in human colorectal cancer cells. The use of specific small-interfering RNAs (siRNA) validated pp60(c-Src) as the major kinase responsible for beta-catenin tyrosine phosphorylation in colorectal cancer. Src-dependent activation of beta-catenin was prevented by SKI-606, a novel Src family kinase inhibitor, which also abrogated beta-catenin nuclear function by impairing its binding to the TCF4 transcription factor and its trans-activating ability in colorectal cancer cells. These effects were seemingly specific, as cyclin D1, a crucial beta-catenin/TCF4 target gene, was also down-regulated by SKI-606 in a dose-dependent manner accounting, at least in part, for the reduced growth (IC50, 1.5-2.4 micromol/L) and clonogenic potential of colorectal cancer cells. Protein levels of beta-catenin remained substantially unchanged by SKI-606, which promoted instead a cytosolic/membranous retention of beta-catenin as judged by immunoblotting analysis of cytosolic/nuclear extracts and cell immunofluorescence staining. The SKI-606-mediated relocalization of beta-catenin increased its binding affinity to E-cadherin and adhesion of colorectal cancer cells, with ensuing reduced motility in a wound healing assay. Interestingly, the siRNA-driven knockdown of beta-catenin removed the effect of SKI-606 on cell-to-cell adhesion, which was associated with prolonged stability of E-cadherin protein in a pulse-chase experiment. Thus, our results show that SKI-606 operates a switch between the transcriptional and adhesive function of beta-catenin by inhibiting its pp60(c-Src)-dependent tyrosine phosphorylation; this could constitute a new therapeutic target in colorectal cancer.

Cortez, C., E. Tomaskovic-Crook, et al. (2007). "Influence of size, surface, cell line, and kinetic properties on the specific binding of A33 antigen-targeted multilayered particles and capsules to colorectal cancer cells." ACS Nano **1**(2): 93-102.

There has been increased interest in the use of polymer capsules formed by the layer-by-layer (LbL) technique as therapeutic carriers to cancer cells due to their versatility and ease of surface modification. We have investigated the influence of size, surface properties, cell line, and kinetic parameters such as dosage (particle concentration) and incubation time on the specific binding of humanized A33 monoclonal antibody (huA33 mAb)-coated LbL particles and capsules to colorectal cancer cells. HuA33 mAb binds to the A33 antigen present on almost all colorectal cancer cells and has demonstrated great promise in clinical trials as an immunotherapeutic agent for cancer therapy. Flow cytometry experiments showed the cell binding specificity of huA33 mAb-coated particles to be size-dependent, with the optimal size for enhanced selectivity at approximately 500 nm. The specific binding was improved by increasing the dosage of particles incubated with the cells. The level of specific versus nonspecific binding was compared for particles terminated with various polyelectrolytes to examine the surface dependency of antibody attachment and subsequent cell binding ability. The specific binding of huA33 mAb-coated particles is also reported for two colorectal cancer cell lines, with an enhanced binding ratio between 4 and 10 obtained for the huA33 mAb-functionalized particles. This investigation aims to improve the level of specific targeting of LbL particles, which is important in targeted drug and gene delivery applications.

Cortina, C., S. Palomo-Ponce, et al. (2007). "EphB-ephrin-B interactions suppress colorectal cancer

progression by compartmentalizing tumor cells." Nat Genet **39**(11): 1376-83.

The genes encoding tyrosine kinase receptors EphB2 and EphB3 are beta-catenin and Tcf4 target genes in colorectal cancer (CRC) and in normal intestinal cells. In the intestinal epithelium, EphB signaling controls the positioning of cell types along the crypt-villus axis. In CRC, EphB activity suppresses tumor progression beyond the earliest stages. Here we show that EphB receptors compartmentalize the expansion of CRC cells through a mechanism dependent on E-cadherin-mediated adhesion. We demonstrate that EphB-mediated compartmentalization restricts the spreading of EphB-expressing tumor cells into ephrin-B1-positive territories in vitro and in vivo. Our results indicate that CRC cells must silence EphB expression to avoid repulsive interactions imposed by normal ephrin-B1-expressing intestinal cells at the onset of tumorigenesis.

Coss, A., M. Tosetto, et al. (2009). "Increased topoisomerase IIalpha expression in colorectal cancer is associated with advanced disease and chemotherapeutic resistance via inhibition of apoptosis." Cancer Lett **276**(2): 228-38.

Topoisomerase IIalpha is a nuclear enzyme that regulates the tertiary structure of DNA. The influence of topoisomerase IIalpha gene (TOP2A) or protein alterations on disease progression and treatment response in colorectal cancer (CRC) is unknown. The study investigated the clinical relevance of topoisomerase IIalpha in CRC using in vivo and in vitro models. Differentially expressed genes in early and late-stage CRC were identified by array comparative genomic hybridization (CGH). Cellular location of gene amplifications was determined by fluorescence in situ hybridization (FISH). Topoisomerase IIalpha levels, proliferation index, and HER2 expression were examined in 228 colorectal tumors by immunohistochemistry. Overexpression of topoisomerase IIalpha in vitro was achieved by liposome-based transfection. Cell growth inhibition and apoptosis were quantified using the crystal violet assay and flow cytometry, respectively, in response to drug treatment. Amplification of TOP2A was identified in 3 (7.7%) tumors using array CGH and confirmed using FISH. At the protein level, topoisomerase IIalpha staining was observed in 157 (69%) tumors, and both staining and intensity levels were associated with an aggressive tumor phenotype (p values 0.04 and 0.005, respectively). Using logistic regression analysis, topoisomerase IIalpha remained significantly associated with advanced tumor stage when corrected for tumor proliferation (p=0.007) and differentiation (p=0.001). No association was identified between topoisomerase IIalpha and HER2. In vitro, overexpression of topoisomerase IIalpha was associated with resistance to irinotecan (p=0.001) and etoposide chemotherapy (p=0.03), an effect mediated by inhibition of apoptosis. Topoisomerase IIalpha overexpression is significantly associated with alterations in tumor behavior and response to drug treatment in CRC. Our results suggest that gene amplification may represent an important mechanism underlying these changes.

Cross, H. S., G. Bises, et al. (2005). "The Vitamin D endocrine system of the gut--its possible role in colorectal cancer prevention." J Steroid Biochem Mol Biol **97**(1-2): 121-8.

While Vitamin D insufficiency in the US and European population is rising, epidemiological studies suggest an inverse correlation between low serum levels of 25-hydroxyvitamin D(3) (25-OH-D(3)) and colorectal cancer incidence. The antimitotic, prodifferentiating and proapoptotic active metabolite 1alpha,25-dihydroxyvitamin D(3) (1,25-(OH)(2)-D(3)) is synthesized also by colonocytes, since these possess Vitamin D synthesizing (CYP27B1) and catabolic (CYP24) hydroxylases similar to the kidney. Early during colon tumor progression, expression of CYP27B1 and of the Vitamin D receptor increases, suggesting an autocrine/paracrine growth control in colon tissue as a physiological restriction against tumor progression. However, in human adenocarcinomas expression of the catabolic CYP24 is also enhanced when compared with adjacent normal mucosa. Therefore, to maintain colonic accumulation of 1,25-(OH)(2)-D(3) its catabolism needs to be restricted. Our studies in mice show that low nutritional calcium causes hyperproliferation of colon crypts and significant elevation of CYP24 expression, which can be completely abrogated by soy feeding. We suggest that phytoestrogens in soy, known to be estrogen receptor modulators, are responsible for decreased CYP24 expression. These results and our observation that 17beta-estradiol can elevate CYP27B1 expression in rectal tissue of postmenopausal women, may underlie the observed protective effect of estrogens against colorectal cancer in females.

Dahan, L., A. Sadok, et al. (2009). "Modulation of cellular redox state underlies antagonism between oxaliplatin and cetuximab in human colorectal cancer cell lines." Br J Pharmacol **158**(2): 610-20.

BACKGROUND AND PURPOSE: Oxaliplatin is the first platinum-based compound effective in the treatment of colorectal cancer. Oxaliplatin combined with cetuximab for metastatic colorectal cancer is under evaluation. The preliminary

results seem controversial, particularly for the use of cetuximab in K-Ras mutated patients. K-Ras mutation is known to affect redox homeostasis. Here we evaluated how the efficacy of oxaliplatin alone or combined with cetuximab varied according to the Ras mutation and redox status in a panel of colorectal tumour cell lines. EXPERIMENTAL APPROACH: Viability was evaluated by methylthiazoletetrazolium assay, reactive oxygen species production by DCFDA and lucigenin on HT29-D4, Caco-2, SW480 and SW620 cell lines. KEY RESULTS: Combination of oxaliplatin and cetuximab was less cytotoxic than oxaliplatin alone in colorectal cells harbouring wild-type Ras and membrane expression of receptors for epidermal growth factor receptor (EGFR), such as HT29-D4 and Caco-2 cells. In contrast, cetuximab did not affect oxaliplatin efficiency in cells harbouring K-Ras(V12) mutation, irrespective of membrane EGFR expression (SW620 and SW480 cells). Transfection of HT29-D4 with K-Ras(V12) decreased oxaliplatin IC(50) and impaired cetuximab sensitivity, without affecting expression of membrane EGFR compared with HT29-D4 control. Oxaliplatin efficacy relies on endogenous production of H(2)O(2). Cetuximab inhibits H(2)O(2) production inhibiting the EGFR/Nox1 NADPH oxidase pathway. Oxaliplatin efficacy was impaired by short hairpin RNA for Nox1 and by catalase (H(2)O(2) scavenger). CONCLUSIONS AND IMPLICATIONS: Cetuximab limited oxaliplatin efficiency by affecting the redox status of cancer cells through Nox1. Such combined therapy might be improved by controlling H(2)O(2) elimination.

Dakshinamurthy, A. G., R. Ramesar, et al. (2008). "Infrequent and low expression of cancer-testis antigens located on the X chromosome in colorectal cancer: implications for immunotherapy in South African populations." Biotechnol J **3**(11): 1417-23.

Cancer-testis (CT) antigens are a group of tumor antigens that are expressed in the testis and aberrantly in cancerous tissue but not in somatic tissues. The testis is an immune-privileged site because of the presence of a blood-testis barrier; as a result, CT antigens are considered to be essentially tumor specific and are attractive targets for immunotherapy. CT antigens are classified as the CT-X and the non-X CT antigens depending on the chromosomal location to which the genes are mapped. CT-X antigens are typically highly immunogenic and hence the first step towards tailored immunotherapy is to elucidate the expression profile of CT-X antigens in the respective tumors. In this study we investigated the expression profile of 16 CT-X antigen genes in 34 colorectal cancer (CRC) patients using reverse transcription-polymerase chain reaction. We observed that 12 of the 16 CT-X antigen genes studied did not show expression in any of the CRC samples analyzed. The other 4 CT-X antigen genes showed low frequency of expression and exhibited a highly variable expression profile when compared to other populations. Thus, our study forms the first report on the expression profile of CT-X antigen genes among CRC patients in the genetically diverse South African population. The results of our study suggest that genetic and ethnic variations in population might have a role in the expression of the CT-X antigen genes. Thus our results have significant implications for anti-CT antigen-based immunotherapy trials in this population.

de Angelis, P. M., B. Fjell, et al. (2004). "Molecular characterizations of derivatives of HCT116 colorectal cancer cells that are resistant to the chemotherapeutic agent 5-fluorouracil." Int J Oncol **24**(5): 1279-88.

5-Fluorouracil (5-FU) is the chemotherapeutic drug of choice for the treatment of metastatic colorectal cancer, but resistance to 5-FU remains a major obstacle to successful therapy. We generated 5-FU-resistant derivatives of the HCT116 human colon cancer cell line by serial passage of these cells in the presence of increasing 5-FU concentrations in an attempt to elucidate the biological mechanisms involved in resistance to 5-FU. Two resultant resistant derivatives, HCT116 ResB and ResD, were characterized for resistance phenotypes, genotypes, and gene expression using cells maintained long-term in 5-FU-free media. Compared to parental HCT116 cells that respond to 5-FU challenge by inducing high levels of apoptosis, ResB and ResD derivatives had significantly reduced apoptotic fractions when transiently challenged with 5-FU. ResB and ResD cells were respectively 27- and 121-fold more resistant to 5-FU, had increased doubling times, and significantly increased plating efficiencies compared to the parental cells. Both resistant derivatives retained the wild-type TP53 genotype, TP53 copy number and CGH profile characteristic of the parental line. Alterations in gene expression in the resistant derivatives compared to the parental line were assessed using oligonucleotide microarrays. Overall, the 5-FU-resistant derivatives were characterized by reduced apoptosis and a more aggressive growth phenotype, consistent with the observed up-regulation of apoptosis-inhibitory genes (e.g., IRAK1, MALT1, BIRC5), positive growth-regulatory genes (e.g., CCND3, CCNE2, CCNF, CYR61), and metastasis genes (e.g., LMNB1, F3, TMSNB), and down-regulation of apoptosis-promoting genes (e.g., BNIP3, BNIP3L, FOXO3A) and negative growth-regulatory genes (e.g., AREG, CCNG2, CDKN1A, CDKN1C, GADD45A). 5-FU

metabolism-associated genes (e.g., TYMS, DTYMK, UP) and DNA repair genes (e.g., FEN1, FANCG, RAD23B) were also up-regulated in one or both resistant derivatives, suggesting that the resistant derivatives might be able to overcome both 5-FU inhibition of thymidylate synthase and the DNA damage caused by 5-FU, respectively. Development of 5-FU resistance thus appears to encompass deregulation of apoptosis-, proliferation-, DNA repair-, and metastasis-associated regulatory pathways.

Di Nicolantonio, F., M. Martini, et al. (2008). "Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer." J Clin Oncol **26**(35): 5705-12.

PURPOSE Cetuximab or panitumumab are effective in 10% to 20% unselected metastatic colorectal cancer (CRC) patients. KRAS mutations account for approximately 30% to 40% patients who are not responsive. The serine-threonine kinase BRAF is the principal effector of KRAS. We hypothesized that, in KRAS wild-type patients, BRAF mutations could have a predictive/prognostic value. PATIENTS AND METHODS We retrospectively analyzed objective tumor responses, time to progression, overall survival (OS), and the mutational status of KRAS and BRAF in 113 tumors from cetuximab- or panitumumab-treated metastatic CRC patients. The effect of the BRAF V600E mutation on cetuximab or panitumumab response was also assessed using cellular models of CRC. Results KRAS mutations were present in 30% of the patients and were associated with resistance to cetuximab or panitumumab (P = .011). The BRAF V600E mutation was detected in 11 of 79 patients who had wild-type KRAS. None of the BRAF-mutated patients responded to treatment, whereas none of the responders carried BRAF mutations (P = .029). BRAF-mutated patients had significantly shorter progression-free survival (P = .011) and OS (P < .0001) than wild-type patients. In CRC cells, the introduction of BRAF V600E allele impaired the therapeutic effect of cetuximab or panitumumab. Treatment with the BRAF inhibitor sorafenib restored sensitivity to panitumumab or cetuximab of CRC cells carrying the V600E allele. CONCLUSION BRAF wild-type is required for response to panitumumab or cetuximab and could be used to select patients who are eligible for the treatment. Double-hit therapies aimed at simultaneous inhibition of epidermal growth factor receptor and BRAF warrant exploration in CRC patients carrying the V600E oncogenic mutation.

Din, F. V., L. A. Stark, et al. (2005). "Aspirin-induced nuclear translocation of NFkappaB and apoptosis in colorectal cancer is independent of p53 status and DNA mismatch repair proficiency." Br J Cancer **92**(6): 1137-43.

Substantial evidence indicates nonsteroidal anti-inflammatory drugs (NSAIDs) protect against colorectal cancer (CRC). However, the molecular basis for this anti-tumour activity has not been fully elucidated. We previously reported that aspirin induces signal-specific IkappaBalpha degradation followed by NFkappaB nuclear translocation in CRC cells, and that this mechanism contributes substantially to aspirin-induced apoptosis. We have also reported the relative specificity of this aspirin-induced NFkappaB-dependent apoptotic effect for CRC cells, in comparison to other cancer cell types. It is now important to establish whether there is heterogeneity within CRC, with respect to the effects of aspirin on the NFkappaB pathway and apoptosis. p53 signalling and DNA mismatch repair (MMR) are known to be deranged in CRC and have been reported as potential molecular targets for the anti-tumour activity of NSAIDs. Furthermore, both p53 and MMR dysfunction have been shown to confer resistance to chemotherapeutic agents. Here, we set out to determine the p53 and hMLH1 dependency of the effects of aspirin on NFkappaB signalling and apoptosis in CRC. We specifically compared the effects of aspirin treatment on cell viability, apoptosis and NFkappaB signalling in an HCT-116 CRC cell line with the p53 gene homozygously disrupted (HCT-116(p53-/-)) and an HCT-116 cell line rendered MMR proficient by chromosomal transfer (HCT-116(+ch3)), to the parental HCT-116 CRC cell line. We found that aspirin treatment induced apoptosis following IkappaBalpha degradation, NFkappaB nuclear translocation and repression of NFkappaB-driven transcription, irrespective of p53 and DNA MMR status. These findings are relevant for design of both novel chemopreventative agents and chemoprevention trials in CRC.

Douard, R., S. Moutereau, et al. (2006). "Sonic Hedgehog-dependent proliferation in a series of patients with colorectal cancer." Surgery **139**(5): 665-70.

BACKGROUND: The Hedgehog (Hh) gene family is known to regulate development of stem cells. In addition, activation is responsible for the induction of GLI1 proto-oncogene and subsequent cellular proliferation. Sonic Hedgehog (SHh), one of the Hh family members promotes carcinogenesis in airway and pancreatic epithelia, is expressed in colonic stem cells. As differentiated colonic cells arise from constant renewal of Hedgehog-expressing colonic stem cells, SHh could be involved in human colonic carcinogenesis. METHODS: Tissue samples of colorectal adenocarcinoma (T) and adjacent normal

colon tissue (NT) were sampled from each of 44 consecutive patients with colorectal cancer. Specific transcription of SHh, GLI1, and the GLI1 downstream target FOXM1 were evaluated using semiquantitative reverse transcriptase polymerase chain reaction. Similar in vitro measurements of mRNA of GLI1 and FOXM1 transcription levels after specific induction by SHh-Np were performed in the HT-29 colorectal tumor cell line to confirm the in vivo results. RESULTS: SHh mRNA was overexpressed in colorectal adenocarcinomas in 38 of 44 (86%) patients. Expression of transcription levels of GLI1 and FOXM1 correlated with SHh expression (SHh vs GLI1, r = 0.77, P < .0001; GLI1 vs FOXM1, r = 0.68, P < .0001; SHh vs FOXM1, r = 0.79, P < .0001). SHh overexpression did not appear to correlate with the patient characteristics evaluated. Similarly, when studied in the HT-29 colorectal cell line, exogenous SHh promoted cell proliferation, while inhibition of SHh expression decreased proliferation. Expression of GLI1 and FOXM1 mRNA increased with exogenous exposure to SHh. CONCLUSIONS: We demonstrated increased expression of SHh mRNA in human colonic adenocarcinomas and in a colorectal cell line with downstream increased expression of GLI1 and FOXM1 mRNA known to promote cell proliferation. This upregulation within human colorectal adenocarcinoma tissue confirms the potential role of the Hh pathway in colorectal carcinogenesis and suggests a potential therapeutic target of Hh blockade in colorectal cancer.

Dronamraju, S. S., J. M. Coxhead, et al. (2009). "Cell kinetics and gene expression changes in colorectal cancer patients given resistant starch: a randomised controlled trial." Gut **58**(3): 413-20.

OBJECTIVE: This study investigated the effects of oral supplementation of resistant starch (RS) on tumour cell and colonic mucosal cell kinetics and on gene expression in patients with colorectal cancer (CRC), and its potential role in colon cancer prevention. METHODS: 65 patients with CRC were randomised to treatment with RS or ordinary starch (OS) and were given starch treatment for up to 4 weeks. Pretreatment and post-treatment biopsies were obtained from the tumour and colonic mucosa, and the effects of the starch treatment on cell proliferation and expression of the cell cycle regulatory genes CDK4 (cyclin-dependent kinase 4) and GADD45A (growth arrest and DNA damage-inducible, alpha) were investigated. RESULTS: The proportion of mitotic cells in the top half of the colonic crypt was significantly lower following RS treatment (3.1 (1.5), mean (SEM)) as compared with OS treatment (13.7 (3.2)) (p = 0.028). However, there was no effect of RS treatment on crypt dimensions and tumour cell proliferation index. There was significant upregulation in expression of CDK4 (p<0.01) and downregulation in expression of GADD45A (p<0.001) in the tumour tissue when compared with macroscopically normal mucosa. Following RS treatment, CDK4 expression in tumours (0.88 (0.15)) was twofold higher than that in the OS group (0.37 (0.16)) (p = 0.02). The expression of GADD45A, which was downregulated in the presence of cancer, was significantly upregulated (p = 0.048) following RS treatment (1.41 (0.26)) as compared with OS treatment (0.56 (0.3)). However, there were no significant differences in the expression of these genes in the normal mucosa following starch treatment. CONCLUSIONS: Cell proliferation in the upper part of colonic crypts is a premalignant marker and its reduction by RS supplementation is consistent with an antineoplastic action of this food component. Differential expression of the key cell cycle regulatory genes may contribute to the molecular mechanisms underlying these antineoplastic effects of RS.

Dylla, S. J., L. Beviglia, et al. (2008). "Colorectal cancer stem cells are enriched in xenogeneic tumors following chemotherapy." PLoS One **3**(6): e2428.

BACKGROUND: Patients generally die of cancer after the failure of current therapies to eliminate residual disease. A subpopulation of tumor cells, termed cancer stem cells (CSC), appears uniquely able to fuel the growth of phenotypically and histologically diverse tumors. It has been proposed, therefore, that failure to effectively treat cancer may in part be due to preferential resistance of these CSC to chemotherapeutic agents. The subpopulation of human colorectal tumor cells with an ESA(+)CD44(+) phenotype are uniquely responsible for tumorigenesis and have the capacity to generate heterogeneous tumors in a xenograft setting (i.e. CoCSC). We hypothesized that if non-tumorigenic cells are more susceptible to chemotherapeutic agents, then residual tumors might be expected to contain a higher frequency of CoCSC. METHODS AND FINDINGS: Xenogeneic tumors initiated with CoCSC were allowed to reach approximately 400 mm(3), at which point mice were randomized and chemotherapeutic regimens involving cyclophosphamide or Irinotecan were initiated. Data from individual tumor phenotypic analysis and serial transplants performed in limiting dilution show that residual tumors are enriched for cells with the CoCSC phenotype and have increased tumorigenic cell frequency. Moreover, the inherent ability of residual CoCSC to generate tumors appears preserved. Aldehyde dehydrogenase 1 gene expression and enzymatic activity are elevated in CoCSC and using an in vitro culture system that maintains CoCSC as demonstrated by serial transplants and lentiviral marking of single cell-derived clones, we further show

that ALDH1 enzymatic activity is a major mediator of resistance to cyclophosphamide: a classical chemotherapeutic agent. CONCLUSIONS: CoCSC are enriched in colon tumors following chemotherapy and remain capable of rapidly regenerating tumors from which they originated. By focusing on the biology of CoCSC, major resistance mechanisms to specific chemotherapeutic agents can be attributed to specific genes, thereby suggesting avenues for improving cancer therapy.

Engesaeter, B. O., A. Bonsted, et al. (2006). "Photochemically mediated delivery of AdhCMV-TRAIL augments the TRAIL-induced apoptosis in colorectal cancer cell lines." Cancer Biol Ther **5**(11): 1511-20.

Tumor targeting is an important issue in cancer gene therapy. We have developed a light-specific transduction method, named photochemical internalization (PCI), to enhance gene expression from adenoviral vectors selectively in illuminated areas. Tumor necrosis factor related apoptosis inducing ligand (TRAIL) has been shown to induce apoptosis in cancer cells, and the aim of this study was to investigate the potential of PCI to enhance transgene expression from AdhCMV-TRAIL and evaluate its impact on apoptotic induction in the two human colorectal cancer cell lines HCT116 and WiDr. PCI-mediated delivery of AdhCMV-TRAIL enabled an increased expression of TRAIL, induced a synergistic reduction in cell viability compared to the individual action of AdhCMV-TRAIL and photochemical treatment, and enhanced the induction of apoptosis demonstrated by an increase in cytoplasmic histone-associated DNA fragments, caspase-8 and caspase-3 activation, PARP cleavage and a decrease in the mitochondrial membrane potential. The synergistic effect could be related to the enhanced TRAIL expression in PCI-treated samples and a modest sensitization of the cancer cells to TRAIL induced apoptosis due to the photochemical treatment. Furthermore, an increased cleavage of Bid and a cell line dependent reduction in the expression levels of anti-apoptotic Bcl-2 family members were observed and could possibly contribute to the enhanced apoptotic level in samples exposed to the combined treatment. The presented results indicate that photochemically mediated delivery of AdhCMV-TRAIL allows a selective enhancement in cell killing, and suggest that PCI may be relevant and advantageous for therapeutic gene delivery in vivo.

Ezumi, K., H. Yamamoto, et al. (2008). "Aberrant expression of connexin 26 is associated with lung metastasis of colorectal cancer." Clin Cancer Res **14**(3): 677-84.

PURPOSE: Connexin 26 (Cx26) is one of the gap junction-forming family members classically considered to be tumor suppressors. However, recent studies show association of elevated expression of Cx26 with poor prognosis in several human malignancies. Furthermore, Cx26 has been observed to be indispensable to spontaneous metastasis of melanoma cells. Here, we assessed Cx26 expression in primary colorectal cancer (CRC) and the metastatic lesions to elucidate its role in metastasis. EXPERIMENTAL DESIGN: Cx26 expression was assessed in 25 adenomas, 167 CRCs, and normal mucosa, together with the metastatic lesions. RESULTS: Normal mucosa and adenomatous tissue expressed Cx26 mainly in the plasma membrane, whereas cancer cells mostly contained Cx26 in the cytoplasm. The incidence of aberrant Cx26 expression varied widely in CRC (mean, 49.5 +/- 35.5%), and the expression levels were confirmed by Western blot and quantitative reverse transcription-PCR. Clinicopathologic survey revealed association of high expression with less differentiated histology and venous invasion ($P = 0.0053$ and $P = 0.0084$, respectively). Notably, high Cx26 expression was associated with shorter disease-free survival and shorter lung metastasis-free survival in 154 curatively resected CRC sets ($P = 0.041$ and $P = 0.028$, respectively). Survey of metastatic lesions revealed that lung metastasis, but not liver and lymph nodes metastases, expressed higher Cx26 than the CRC series or corresponding primary CRCs ($P < 0.0001$ and $P = 0.0001$, respectively). CONCLUSIONS: These findings suggest that aberrant expression of Cx26 plays an essential role in lung metastasis. Thus, Cx26 is a promising therapeutic target, particularly for CRC patients who develop lung metastasis.

Fan, F., M. J. Gray, et al. (2008). "Effect of chemotherapeutic stress on induction of vascular endothelial growth factor family members and receptors in human colorectal cancer cells." Mol Cancer Ther **7**(9): 3064-70.

Vascular endothelial growth factor (VEGF) is induced by stress. We determined whether chemotherapy (genotoxic stress) could induce expression of VEGF and VEGF receptors (VEGFR) in human colorectal cancer cells. The colorectal cancer cell lines HT29, RKO, and HCT116 were acutely exposed to increasing doses of oxaliplatin or 5-fluorouracil for 2, 6, and 24 h in vitro. Expression of VEGF ligand family members, VEGFRs, and signaling intermediates was determined by reverse transcription-PCR and Northern and Western blotting. The effect of oxaliplatin on VEGF-A transcriptional activity was determined by promoter assays. Acute exposure of human colorectal cancer cells to

oxaliplatin led to a marked induction of VEGF-A mRNA and protein, whereas 5-fluorouracil alone or when added to oxaliplatin did not cause a further increase in VEGF levels. VEGF-A promoter activity was induced by oxaliplatin exposure. Expression of VEGF-C, placental growth factor, VEGFR-1, and neuropilin-1 levels were also increased when cells were treated with oxaliplatin. Oxaliplatin led to an increase in Akt and Src activation in HT29 cells. In contrast, Akt activation did not change in RKO cells whereas phospho-Src and phospho-p44/42 mitogen-activated protein kinase was dramatic increased by oxaliplatin. Inhibition of Akt or Src activation with wortmannin or PP2 blocked induction of VEGF-A by oxaliplatin in HT29 or RKO cells, respectively. VEGFRs may reflect the adaptive stress responses by which tumor cells attempt to protect themselves from genotoxic stress. Neutralization of prosurvival responses with anti-VEGF therapy might explain, in part, some of the beneficial effects of anti-VEGF therapy when added to chemotherapy.

Fioravanti, A., B. Canu, et al. (2009). "Metronomic 5-fluorouracil, oxaliplatin and irinotecan in colorectal cancer." Eur J Pharmacol **619**(1-3): 8-14.

Metronomic chemotherapy (the frequent, long term, low dose administration of chemotherapeutic drugs) is a promising therapy because it enhances the anti-endothelial activity of conventional chemotherapeutics, but with lower or no toxic effects compared to maximum tolerated dose administration. The aims of the present study were to compare, in vitro and in vivo, the antiangiogenic and antitumor activities of metronomic irinotecan (CPT-11), oxaliplatin (L-OHP) and 5-fluorouracil (5-FU) in colorectal cancer and to investigate the metronomic combination of these drugs. In vitro cell proliferation, combination studies and vascular endothelial growth factor (VEGF) secretion analyses were performed on endothelial (HMVEC-d) and colorectal cancer (HT-29) cells exposed for 144 h to metronomic concentrations of SN-38, the active metabolite of CPT-11, L-OHP and 5-FU. HT-29 human colorectal cancer xenograft model was used and tumour growth, microvessel density and VEGF quantification were performed in tumours after the administration of metronomic CPT-11, L-OHP, 5-FU and their simultaneous combination. Low concentrations of SN-38, but not 5-FU and L-OHP, preferentially inhibited endothelial cell proliferation. Simultaneous and continuous exposure of HT-29 and HMVEC-d cells to low concentrations SN-38+L-OHP+5-FU for 144 h showed a strong antagonism and an unfavorable dose-reduction index. Moreover, the ternary combination resulted in a significant increase of VEGF secretion in HT-29 cancer cells. In a xenograft model metronomic CPT-11, but not 5-FU and L-OHP, significantly inhibits HT-29 tumor growth and microvessel density in the absence of toxicity. On the contrary, metronomic 5-FU+L-OHP+CPT-11 therapy did not affect the microvascular count. The metronomic concept might not universally apply to every cytotoxic drug in colorectal cancer and metronomic combination regimens should be used with caution.

Frattini, M., P. Saletti, et al. (2007). "PTEN loss of expression predicts cetuximab efficacy in metastatic colorectal cancer patients." Br J Cancer **97**(8): 1139-45.

To evaluate whether the epidermal growth factor receptor (EGFR), K-Ras and PTEN, all members of the EGFR signalling pathway, may affect the clinical response in cetuximab-treated metastatic colorectal cancer (mCRC) patients. Twenty-seven cetuximab-treated mCRC patients were evaluated for drug response and investigated for EGFR protein expression and gene status, K-Ras mutational status and PTEN protein expression. Ten patients achieved a partial response (PR) to cetuximab-based therapy. All 27 patients showed EGFR protein overexpression. Epidermal growth factor receptor gene amplification was observed in eight out of 27 (30%) and chromosome 7 marked polysomy in 16 (59%) patients. Partial response was observed in six out of eight patients with EGFR gene amplification, four out of 16 with marked polysomy and none out of three with eusomy ($P<0.05$). The K-Ras wild-type sequence was observed in 17 patients, and nine of them experienced a PR. Conversely, K-Ras was mutated in 10 cases, of which one patient experienced a PR ($P<0.05$). The PTEN protein was normally expressed in 16 patients, and 10 of them achieved a PR. In contrast, no benefit was documented in 11 patients with loss of PTEN activity ($P<0.001$). Patients with EGFR gene amplification or chromosome 7 marked polysomy respond to cetuximab. In addition to K-Ras mutations, we demonstrate for the first time that the loss of PTEN protein expression is associated with nonresponsiveness to cetuximab.

Fritzmann, J., M. Morkel, et al. (2009). "A colorectal cancer expression profile that includes transforming growth factor beta inhibitor BAMBI predicts metastatic potential." Gastroenterology **137**(1): 165-75.

BACKGROUND & AIMS: Much is known about the genes and mutations that cause colorectal cancer (CRC), yet only a few have been associated with CRC metastasis. We performed expression-profiling experiments to identify genetic markers of risk and to elucidate the molecular mechanisms of CRC metastasis. METHODS: We compared gene

expression patterns between metastatic and nonmetastatic stage-matched human colorectal carcinomas by microarray analysis. Correlations between BAMBI and metastasis-free survival were examined by quantitative real-time polymerase chain reaction (PCR) using an independent set of human colon carcinomas. Human colon cancer cell lines were analyzed for BAMBI regulation, cell motility, and experimental metastasis. RESULTS: We established a signature of 115 genes that differentiated metastatic from nonmetastatic primary tumors. Among these, the transforming growth factor (TGF) beta inhibitor BAMBI was highly expressed in approximately half of metastatic primary tumors and metastases but not in nonmetastatic tumors. BAMBI is a target of canonical Wnt signaling that involves the beta-catenin coactivator BCL9-2. We observed an inverse correlation between level of BAMBI expression and metastasis-free survival time of patients. BAMBI inhibits TGF-beta signaling and increases migration in colon cancer cells. In mice, overexpression of BAMBI caused colon cancer cells to form tumors that metastasized more frequently to liver and lymph nodes than control cancer cells. CONCLUSIONS: BAMBI regulates CRC metastasis by connecting the Wnt/beta-catenin and TGF-beta-signaling pathways. The metastatic expression signature we describe, along with BAMBI levels, can be used in prognosis. Developmental signaling pathways appear to act in hierarchies and cooperate in tumor cell migration, invasion, and metastasis.

Gal, R., E. Sadikov, et al. (2004). "Deleted in colorectal cancer protein expression as a possible predictor of response to adjuvant chemotherapy in colorectal cancer patients." Dis Colon Rectum **47**(7): 1216-24.

PURPOSE: The deleted in colorectal cancer (DCC) gene predicts a poor outcome for patients with colorectal carcinoma. This study was designed to investigate whether the expression of the DCC protein also can predict response to adjuvant chemotherapy. METHODS: The expression of DCC was evaluated immunohistochemically in 74 paraffin-embedded tumor samples from patients with Stage II (n = 41) and Stage III (n = 33) colorectal carcinomas. Follow-up time was at least 60 (median, 64) months. Follow-up was at least five years for all patients who are alive. End points of the study were recurrence of disease and death. Forty-eight patients received adjuvant therapy of 5-fluorouracil + levamisole; 28 were not treated. RESULTS: Fifty percent of tumors were deleted in colorectal cancer-positive (DCC+). Proportion of survival and disease-free survival were higher in the DCC+ patients (83 percent) than in deleted in colorectal cancer-negative (DCC-; 54 percent). In the DCC+ group, adjuvant treatment was a strong positive predictive factor for survival and disease-free survival. All DCC+ patients who received adjuvant chemotherapy (CHEMO+) are alive with no evidence of disease, whereas without chemotherapy (CHEMO-) only 54 percent are alive (P = 0.0001). When stratification was performed by stage, patients in Stage II who were DCC+/CHEMO+ had survival and disease-free survival of 100 percent, whereas in DCC+/CHEMO- survival rate was 75 percent and disease-free survival rate 62 percent (P = 0.042). Patients in Stage III who were DCC+/ CHEMO+ had survival and disease-free survival of 100 percent, whereas in DCC+/CHEMO- both dropped to zero (P = 0.0002). On the other hand, in the DCC- tumors, there was no statistical significant relationship between chemotherapy and survival or disease-free survival (DCC-/CHEMO- had 57 percent survival; DCC-/CHEMO+ had 52 percent survival). CONCLUSIONS: DCC is a prognostic factor for colorectal cancer. Positive expression of DCC identifies a subgroup of patients who respond favorably to adjuvant chemotherapy, which resulted in our cases, in 100 percent survival and disease-free survival rates. Without treatment, the survival rate of DCC+ patients dropped significantly. We suggest that DCC immunostaining should be performed routinely. All DCC+ patients should receive adjuvant chemotherapy. For DCC- tumors, a larger cohort of patients should be studied before definitive conclusions can be drawn; however, clinical trials of new drug combinations should focus on DCC- patients.

Gali-Muhtasib, H., M. Diab-Assaf, et al. (2004). "Thymoquinone extracted from black seed triggers apoptotic cell death in human colorectal cancer cells via a p53-dependent mechanism." Int J Oncol **25**(4): 857-66.

For centuries, the black seed (Nigella sativa) herb and oil have been used in Asia, Middle East and Africa to promote health and fight disease. Thymoquinone (TQ), the most abundant constituent present in black seed, is a promising dietary chemopreventive agent. We investigated the effects of thymoquinone (TQ) against HCT-116 human colon cancer cells and attempted to identify its potential molecular mechanisms of action. We report that TQ inhibits the growth of colon cancer cells which was correlated with G1 phase arrest of the cell cycle. Furthermore, TUNEL staining and flow cytometry analysis indicate that TQ triggers apoptosis in a dose- and time-dependent manner. Apoptosis induction by TQ was associated with a 2.5-4.5-fold increase in mRNA expression of p53 and the downstream p53 target gene, p21WAF1. Simultaneously, we found a

marked increase in p53 and p21WAF1 protein levels but a significant inhibition of anti-apoptotic Bcl-2 protein. Co-incubation with pifithrin-alpha (PFT-alpha), a specific inhibitor of p53, restored Bcl-2, p53 and p21WAF1 levels to the untreated control and suppressed TQ-induced cell cycle arrest and apoptosis. p53-null HCT-116 cells were less sensitive to TQ-induced growth arrest and apoptosis. These results indicate that TQ is antineoplastic and pro-apoptotic against colon cancer cell line HCT116. The apoptotic effects of TQ are modulated by Bcl-2 protein and are linked to and dependent on p53. Our data support the potential for using the agent TQ for the treatment of colon cancer.

Gan, Y., J. Gu, et al. (2008). "Adenovirus-mediated HCCS1 overexpression elicits a potent antitumor efficacy on human colorectal cancer and hepatoma cells both in vitro and in vivo." Cancer Gene Ther **15**(12): 808-16.

We had characterized earlier the novel tumor suppressor gene hepatocellular carcinoma suppressor 1 (HCCS1), and demonstrated that expression of exogenous HCCS1 gene in human hepatocarcinoma cells could remarkably suppress their abilities to develop tumors in nude mice and to form colonies in soft agar. In this study, we provide further experimental evidence to confirm the role of HCCS1 as a tumor suppressor gene and investigate its potential in therapeutic applications by using adenovirus vectors. We show that HCCS1 overexpression, mediated by replication-deficient adenovirus, significantly suppressed the growth of human colorectal cancer cells, as well as hepatocellular carcinoma cells in vitro and in vivo. To further improve its antitumor efficacy, we inserted the HCCS1 gene into an oncolytic adenovirus. This HCCS1-armed oncolytic adenovirus exhibited a dramatic inhibitory effect on cancer cells in vitro and in vivo, and led to a complete regression of 50% of established tumor xenografts in nude mice. Taken together, our data suggest that HCCS1 is a promising therapeutic gene for the treatment of human cancers.

Goi, T., M. Fujioka, et al. (2004). "Angiogenesis and tumor proliferation/metastasis of human colorectal cancer cell line SW620 transfected with endocrine glands-derived-vascular endothelial growth factor, as a new angiogenic factor." Cancer Res **64**(6): 1906-10.

Endocrine glands-derived-vascular endothelial growth factor (EG-VEGF) was recently cloned as a new angiogenic factor that selectively acts on the endothelium of endocrine gland cells. We evaluated the involvement of EG-VEGF in colorectal cancer. The expression of EG-VEGF was confirmed in all of the colorectal cancer cell lines. (On the other hand, the expression of EG-VEGF mRNA was not detected in colorectal normal mucosae.) Stable EG-VEGF infectors of colorectal cancer cell line SW620 were produced, EG-VEGF transfectants were implanted into cecum and s.c., and cell proliferation was evaluated. Angiogenesis was evaluated by dorsal air sac method. Liver metastasis was evaluated after the implantation of EG-VEGF transfectants into the mouse spleen. Tumor proliferation (cecum, s.c.) was significantly higher in the EG-VEGF transfectants than in the control cells. The small vessels were significantly increased in EG-VEGF transfectants as compared with those in control cells. Also, liver metastatic ratio was higher in the EG-VEGF transfectants than in the control cells. In this study, EG-VEGF, a new angiogenic factor, may lead to angiogenesis, promoting cell proliferation and liver metastasis in colorectal cancers. When the EG-VEGF gene-overexpressing colorectal cancer cell line that had been treated with phosphorothioate antisense EG-VEGF oligonucleotides was injected s.c. into mice, angiogenesis and tumor growth were inhibited. Although the novel angiogenesis factor EG-VEGF was not expressed in the normal colorectal mucosa, it was expressed in colorectal cancer cells, which indicates that it is a cancer-specific and possibly tissue-specific angiogenesis factor in the large intestine, and which suggests that it can be targeted by a novel antiangiogenesis therapy.

Grabsch, H., M. Dattani, et al. (2006). "Expression of DNA double-strand break repair proteins ATM and BRCA1 predicts survival in colorectal cancer." Clin Cancer Res **12**(5): 1494-500.

PURPOSE: The double-strand break (DSB) is the major DNA lesion leading to chromosomal aberrations and faithful repair is crucial for maintaining genomic instability. Very little is known about the expression of DNA DSB repair proteins in colorectal cancer. To address this issue, we examined the expression pattern of DSB repair key proteins ATM, BRCA1, BRCA2, Ku70, and Ku80 and their putative role in patients survival in a large series of colorectal cancer. EXPERIMENTAL DESIGN: 342 sporadic colorectal cancer were subjected to immunohistochemistry by using specific antibodies for the various proteins investigated. Staining results were compared with clinicopathologic data, patient survival, as well as expression of mismatch repair proteins MLH1 and MSH2. RESULTS: The expression pattern of both ATM and BRCA1 predicted survival in all colorectal cancer patients as well as in the small subgroup of patients that received adjuvant therapy. Low expression of ATM and BRCA1 was associated with loss of MLH1 or MSH2 expression. CONCLUSIONS: This is the first study to

show a relationship between the expression of DNA DSB repair proteins ATM and BRCA1 and survival in colorectal cancer patients. Studies in tumors from large randomized trials are now necessary to validate our pilot data and establish the clinical usefulness of the immunohistochemical assay in predicting response to a particular adjuvant therapy regimen. Furthermore, our results indicate a possible link between expression of DNA mismatch repair and DNA DSB repair proteins in sporadic colorectal cancer, which warrants further investigation.

Greenhough, A., H. A. Patsos, et al. (2007). "The cannabinoid delta(9)-tetrahydrocannabinol inhibits RAS-MAPK and PI3K-AKT survival signalling and induces BAD-mediated apoptosis in colorectal cancer cells." Int J Cancer **121**(10): 2172-80.

Deregulation of cell survival pathways and resistance to apoptosis are widely accepted to be fundamental aspects of tumorigenesis. As in many tumours, the aberrant growth and survival of colorectal tumour cells is dependent upon a small number of highly activated signalling pathways, the inhibition of which elicits potent growth inhibitory or apoptotic responses in tumour cells. Accordingly, there is considerable interest in therapeutics that can modulate survival signalling pathways and target cancer cells for death. There is emerging evidence that cannabinoids, especially Delta(9)-tetrahydrocannabinol (THC), may represent novel anticancer agents, due to their ability to regulate signalling pathways critical for cell growth and survival. Here, we report that CB1 and CB2 cannabinoid receptors are expressed in human colorectal adenoma and carcinoma cells, and show for the first time that THC induces apoptosis in colorectal cancer cells. THC-induced apoptosis was rescued by pharmacological blockade of the CB1, but not CB2, cannabinoid receptor. Importantly, THC treatment resulted in CB1-mediated inhibition of both RAS-MAPK/ERK and PI3K-AKT survival signalling cascades; two key cell survival pathways frequently deregulated in colorectal tumours. The inhibition of ERK and AKT activity by THC was accompanied by activation of the proapoptotic BCL-2 family member BAD. Reduction of BAD protein expression by RNA interference rescued colorectal cancer cells from THC-induced apoptosis. These data suggest an important role for CB1 receptors and BAD in the regulation of apoptosis in colorectal cancer cells. The use of THC, or selective targeting of the CB1 receptor, may represent a novel strategy for colorectal cancer therapy.

Grone, J., O. Doebler, et al. (2006). "Robo1/Robo4: differential expression of angiogenic markers in colorectal cancer." Oncol Rep **15**(6): 1437-43.

The family of roundabout (Robo) proteins is related to the transmembrane receptors and plays a major role in the process of axonal guidance in neurogenesis. It has recently been shown that Robo proteins are also associated with tumor angiogenesis with Slit2 acting as the corresponding ligand. The aim of this study was to validate the differential expression by means of microarray analysis and real-time PCR and to analyze the in situ expression of Robo1 and Robo4 in colorectal cancer. Quantitative analyses of Robo1, Robo4 and Slit2 mRNA expression measured by large scale gene expression studies (Affymetrix U133A) showed a significant up-regulation of Robo1 in tumor vs. normal tissue, whereas Robo4 and Slit2 showed no significant deregulation. For subsequent real-time PCR experiments, paired colorectal tissue samples from cancerous and corresponding non-cancerous tissues were obtained from 50 colorectal cancer patients who underwent surgical resection. Robo1 mRNA overexpression in cancerous tissue compared with normal counterparts was observed in 80% of the patients with a 4-fold expression in 45% and a 12-fold expression in 15%. For Robo4, an up-regulation was detected in >70% (36/50). For Slit2, no differential expression was observed. The overexpression of Robo1 and Robo4 in tumor vs. normal tissue was verified using real-time PCR. The histological analysis revealed an expression of Robo1 mainly in tumor cells, whereas Robo4 is located primarily in endothelial cells of tumor vessels. Therefore, the Robo proteins provide potential target structures for the anti-tumorigenic and anti-angiogenic therapy of colorectal carcinoma.

Grone, J., B. Weber, et al. (2007). "Differential expression of genes encoding tight junction proteins in colorectal cancer: frequent dysregulation of claudin-1, -8 and -12." Int J Colorectal Dis **22**(6): 651-9.

BACKGROUND AND AIMS: As integral membrane proteins, claudins form tight junctions together with occludin. Several claudins were shown to be up-regulated in various cancer types. We performed an expression analysis of genes encoding tight junction proteins to display differential gene expression on RNA and protein level and to identify and validate potential targets for colorectal cancer (CRC) therapy. PATIENTS AND METHODS: Amplified and biotinylated cRNA from 30 microdissected CRC specimen and corresponding normal tissues was hybridized to Affymetrix U133set GeneChips. Quantification of differential protein expression of claudin-1, -8 and -12 between normal and corresponding tumour tissues was performed by

Western blot analyses. Paraffin-embedded CRC tissue samples, colon cancer cell lines and normal tissue microarray were analysed for protein expression of claudin-1 by immunohistochemistry (IHC). RESULTS: Claudin-1 (CLDN1) and -12 (CLDN12) are frequently overexpressed in CRC, whereas claudin-8 (CLDN8) shows down-regulation in tumour tissue on RNA level. Quantification of proteins confirmed the overexpression of claudin-1 in tumour tissues, whereas changes of claudin-8 and -12 were not significantly detectable on protein level. IHC confirmed the markedly elevated expression level of claudin-1 in the majority of CRC, showing membranous and intracellular vesicular staining. CONCLUSIONS: Differential expression of genes encoding claudins in CRC suggests that these tight junction proteins may be associated to and involved in tumorigenesis. CLDN1 is frequently up-regulated in large proportion of CRC and may represent potential target molecule for blocking studies in CRC.

Gupta, N., P. M. Martin, et al. (2006). "Down-regulation of BCRP/ABCG2 in colorectal and cervical cancer." Biochem Biophys Res Commun **343**(2): 571-7.

Expression of Breast Cancer Resistance Protein (BCRP/ABCG2) in tumor cells is associated with resistance to multiple chemotherapeutic agents. BCRP also protects against phototoxicity by mediating the efflux of protoporphyrins from cells. However, chemotherapy and photodynamic therapy are effective treatment options for cancer. Furthermore, protoporphyrins are essential, in the form of heme, for the synthesis of nitric oxide, over-production of which is associated with cancer. This raises the question as to whether the expression of this transporter is altered in cancer. To address this question, we investigated the expression of BCRP in colorectal cancer and cervical cancer. Paired normal and cancer tissues from colectomy specimens were used for the analysis of BCRP mRNA by RT-PCR and Northern blot. BCRP was analyzed by immunohistochemistry/immunofluorescence. Similar studies were also done with specimens of normal cervix and cancer cervix. A commercial dot blot was probed to quantify the expression of BCRP in paired normal and cancer cDNA samples from 154 patients with tumors in 19 different tissues. BCRP mRNA was present in normal colorectal tissue and showed a 6-fold decrease in cancer. BCRP was abundant in the normal colon and showed a decrease in colon cancer. The down-regulation of BCRP mRNA and protein was also evident in cervical cancer. There was also a decrease in BCRP mRNA in cancer in 12 of the 19 different tissues collected from 154 patients. These data show that cancer-associated down-regulation of BCRP is likely to be a common phenomenon in several tissues. Decreased expression of BCRP may have a role in tumorigenesis by allowing accumulation of genotoxins and over-production of nitric oxide.

Hata, T., H. Yamamoto, et al. (2005). "Role of p21waf1/cip1 in effects of oxaliplatin in colorectal cancer cells." Mol Cancer Ther **4**(10): 1585-94.

Clinical studies have shown that oxaliplatin, a novel platinum derivative, is a potent chemotherapeutic agent for colorectal cancer when combined with 5-fluorouracil and leucovorin. Although the toxic activity is based on covalent adducts between platinum and DNA, its actual biological behavior is mostly unknown. In an effort to explore the mechanism of tumor susceptibility to oxaliplatin, we examined the cytotoxic effects of oxaliplatin in colorectal cancer cell lines in reference to p53 gene status. Although p53 gene status did not clearly predict sensitivity to oxaliplatin, p53 wild-type cells including HCT116 were sensitive but HCT116 p53-/- were found to be resistant to oxaliplatin. Oxaliplatin caused strong p21waf1/cip1 induction and G0-G1 arrest in p53 wild-type cells, whereas cisplatin did not induce G0-G1 arrest. Assays using p53 wild but p21waf1/cip1 null HCT116 cells revealed that oxaliplatin did not show G0-G1 arrest and reduced growth-inhibitory effects, suggesting that p21waf1/cip1 may be a key element in oxaliplatin-treated p53 wild-type cells. Although HCT116 is DNA mismatch repair-deficient, a mismatch repair-proficient HCT116+ch3 cell line displayed similar responses with regard to p21waf1/cip1-mediated growth inhibition and G0-G1 arrest. In p53 mutant cells, on the other hand, oxaliplatin caused an abrupt transition from G1 to S phase and eventually resulted in G2-M arrest. This abrupt entry into S phase was associated with loss of the p21waf1/cip1 protein via proteasome-mediated degradation. These findings suggest that p21waf1/cip1 plays a role in oxaliplatin-mediated cell cycle and growth control in p53-dependent and -independent pathways.

Hawkins, N. J., J. H. Lee, et al. (2009). "MGMT methylation is associated primarily with the germline C>T SNP (rs16906252) in colorectal cancer and normal colonic mucosa." Mod Pathol **22**(12): 1588-99.

O(6)-methylguanine DNA methyltransferase (MGMT) is a DNA repair protein that restores mutagenic O(6)-methylguanine to guanine. MGMT methylation is frequently observed in sporadic colorectal cancer and was recently correlated with the C>T allele at SNP rs16906252, within the transcriptional enhancer element of the promoter. MGMT methylation has also been associated with

KRAS mutations, particularly G>A transitions. We studied 1123 colorectal carcinoma to define the molecular and clinicopathological profiles associated with MGMT methylation. Furthermore, we assessed factors contributing to MGMT methylation in the development of colorectal cancer by studying the allelic pattern of MGMT methylation using SNP rs16906252, and the methylation status of neighbouring genes within 10q26 in selected tumours and matched normal colonic mucosa. MGMT methylation was detected by combined bisulphite restriction analysis in 28% of tumours and was associated with a number of characteristics, including CDKN2A methylation, absent lymphovascular space invasion and KRAS mutations (but not specifically with KRAS G>A transitions). In a multivariate analysis adjusted for age and sex, MGMT methylation was associated with the T allele of SNP rs16906252 (P<0.0001, OR 5.5, 95% CI 3.8-7.9). Low-level methylation was detected by quantitative methylation-specific PCR in the normal colonic mucosa of cases, particularly those with a correspondingly methylated tumour, as well as controls without neoplasia, and this was also associated with the C>T SNP. We show that the T allele at SNP rs16906252 is a key determinant in the onset of MGMT methylation in colorectal cancer, whereas the association of methylation at MGMT and CDKN2A suggests that these loci may be targets of a common mechanism of epigenetic dysregulation.

Hershko, D. D. and M. Shapira (2006). "Prognostic role of p27Kip1 deregulation in colorectal cancer." Cancer **107**(4): 668-75.

p27Kip1, an inhibitor of cyclin-dependent kinases, is a negative cell cycle regulator that plays an important role in tumor suppression. Deregulation of p27 is commonly observed in many human cancers secondary to enhanced ubiquitin-mediated degradation, mediated and rate-limited by its specific ubiquitin ligase subunits Skp2 and Cks1. In the present study the prognostic implications of p27 and the mechanisms that down-regulate its expression in colorectal cancer (CRC) are reviewed. A review and analysis of the English literature was conducted. Loss of p27 was strongly associated with aggressive tumor behavior and poor clinical outcome in CRC. Overexpression of Skp2 and Cks1 was observed in aggressive CRC and is responsible for down-regulation of p27 levels. Both Skp2 and Cks1 were found to be independent prognostic markers for survival and provide predictive information additional to that provided by p27 alone. Deregulation of p27 has a profound effect on tumor progression in CRC and was found to be an accurate and independent prognostic marker. Thus, determination of levels of p27 and of its ubiquitin ligase subunits by readily available immunohistochemical studies may be a useful tool in the assessment of prognosis, especially in patients with intermediate disease, and may potentially assist in the planning of adjuvant therapy and development of novel interventional therapy.

Hibi, K., H. Mizukami, et al. (2009). "Aberrant methylation of the netrin-1 receptor genes UNC5C and DCC detected in advanced colorectal cancer." World J Surg **33**(5): 1053-7.

BACKGROUND: UNC5C and DCC, the netrin-1 receptors, belong to the functional dependence receptors family, which shares the ability to induce apoptosis in the absence of their ligands. Recently, two reports indicated that UNC5C and DCC methylation was closely associated with loss of gene expression in colorectal cancer. These results prompted us to examine the methylation status of the UNC5C and DCC genes in the colorectal carcinomas we surgically removed. METHODS: The methylation status of the UNC5C and DCC genes were examined in primary carcinomas and the corresponding normal tissues derived from 50 patients with colorectal cancer using quantitative methylation-specific polymerase chain reaction (qMSP). The correlation between the methylation status and the clinicopathologic findings was then evaluated. RESULTS: Aberrant methylation of the netrin-1 receptor genes were detected in 41 of the 50 (82%) primary colon cancers, suggesting that the aberrant methylation of netrin-1 receptors was frequently observed in colorectal cancer. The clinicopathologic data were then correlated with this result. CONCLUSIONS: A significant difference was observed in the Dukes stage (p = 0.0438). Netrin-1 receptors might act as a tumor suppressor in colorectal cancers, and thus methylation might present a malignant potential in colorectal cancer.

Hoffmann, D. and O. Wildner (2006). "Restriction of adenoviral replication to the transcriptional intersection of two different promoters for colorectal and pancreatic cancer treatment." Mol Cancer Ther **5**(2): 374-81.

In our current study, we developed oncolytic adenoviruses which preferentially lyse pancreatic and colon cancer cells by replacing viral E1 and/or E4 promoter with the tumor/tissue-specific promoters, cyclooxygenase-2 (COX-2), midkine (MK), or the cell cycle-dependent promoter, E2F1. We generated three sets of recombinant adenoviral vectors. In the first set, only the native E1A promoter was replaced by the COX-2, MK, or E2F1 promoter, respectively. In the second set, the viral E4 promoter was substituted by these heterologous promoters and the viral E1A promoter was substituted by the ubiquitously active cytomegalovirus-IE promoter. In the third set, we

substituted the viral E1A and E4 promoters with the COX-2, MK, or E2F1 promoter, respectively. In our system, transcriptional targeting of solitary viral E1A resulted in 50% enhanced restricted vector replication when compared with an unrestricted replication-competent adenovirus. Furthermore, a targeted expression of the viral E1A gene products had a greater effect on restricted adenoviral replication than that of the E4 region. With our vectors, Ad.COX.MK and Ad.MK.COX, using two different heterologous promoters to control E1A and E4 expression, we showed enhanced viral replication specificity when compared with Ad.COX.COX or Ad.MK.MK, respectively. In a s.c. xenograft tumor model, there was no significant difference in the antineoplastic efficacy of the double heterologous promoter-controlled vectors when compared with our unrestricted replication-competent control adenovirus or vectors with only E1A transcriptionally driven by a heterologous promoter.

Hong, S. K., Y. A. Gul, et al. (2004). "Expression of beta-catenin, COX-2 and iNOS in colorectal cancer: relevance of COX-2 adn iNOS inhibitors for treatment in Malaysia." Asian J Surg **27**(1): 10-7.

BACKGROUND: Promising new pharmacological agents and gene therapy targeting cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) could modulate treatment of colorectal cancer in the future. The aim of this study was to elucidate the expression fo beta-catenin and teh presence of COX-2 and iNOS in colorectal cancer specimens in Malaysia. This is a useful prelude to future studies investigating interventions directed towards COX-2 adn iNOS. METHODS: A cross-section study using retrospective data over a 2-year period (1999-2000) involved 101 archival, formalin-fixed, paraffin-embedded tissue samples of colorectal cancers that were surgically resected in a tertiary referral. RESULTS: COX-2 production was detected in adjacent normal tissue in 34 sample (33.7%) and in tumour tissue in 60 samples (59.4%). More tumours expressed iNOS (82/101, 81.2%) than COX-2. No iNOS expression was detected in adjacent normal tissue. Intense beta-catenin immunoreactivity at the cell-to-cell border. Poorly differentiated tumours had significantly lower total beta-catenin ($p = 0.009$) and COX-2 scores ($p = 0.031$). No significant relationships were established between pathological stage and beta-catenin, COX-2 and iNOS scores. CONCLUSIONS: the accumulation of beta-catenin does not seem to be sufficient to activate pathways that lead to increased COX-2 and iNOS expression. A high proportion of colorectal cancers were found to express COX-2 and a significant number produced iNOS, suggesting that their inhibitors may be potentially useful as chemotherapeutic agents in the management of colorectal cancer.

Hopfner, M., A. P. Sutter, et al. (2006). "Tyrosine kinase of insulin-like growth factor receptor as target for novel treatment and prevention strategies of colorectal cancer." World J Gastroenterol **12**(35): 5635-43.

AIM: To investigate the antineoplastic potency of the novel insulin-like growth factor 1 receptor (IGF-1R) tyrosine kinase inhibitor (TKI) NVP-AEW541 in cell lines and primary cell cultures of human colorectal cancer (CRC). METHODS: Cells of primary colorectal carcinomas were from 8 patients. Immunostaining and crystal violet staining were used for analysis of growth factor receptor protein expression and detection of cell number changes, respectively. Cytotoxicity was determined by measuring the release of the cytoplasmic enzyme lactate dehydrogenase (LDH). The proportion of apoptotic cells was determined by quantifying the percentage of sub-G1 (hypodiploid) cells. Cell cycle status reflected by the DNA content of the nuclei was detected by flow cytometry. RESULTS: NVP-AEW541 dose-dependently inhibited the proliferation of colorectal carcinoma cell lines and primary cell cultures by inducing apoptosis and cell cycle arrest. Apoptosis was characterized by caspase-3 activation and nuclear degradation. Cell cycle was arrested at the G1/S checkpoint. The NVP-AEW541-mediated cell cycle-related signaling involved the inactivation of Akt and extracellular signal-regulated kinase (ERK) 1/2, the upregulation of the cyclin-dependent kinase inhibitors p21(Waf1/CIP1) and p27(Kip1), and the downregulation of the cell cycle promoter cyclin D1. Moreover, BAX was upregulated during NVP-AEW541-induced apoptosis, whereas Bcl-2 was downregulated. Measurement of LDH release showed that the antineoplastic effect of NVP-AEW541 was not due to general cytotoxicity of the compound. However, augmented antineoplastic effects were observed in combination treatments of NVP-AEW541 with either 5-FU, or the EGFR-antibody cetuximab, or the HMG-CoA-reductase inhibitor fluvastatin. CONCLUSION: IGF-1R-TK inhibition is a promising novel approach for either mono- or combination treatment strategies of colorectal carcinoma and even for CRC chemoprevention.

Hou, L., D. Mori, et al. (2009). "Fumagillin inhibits colorectal cancer growth and metastasis in mice: in vivo and in vitro study of anti-angiogenesis." Pathol Int **59**(7): 448-61.

Fumagillin is an inhibitor of type 2 methionine aminopeptidase that can block blood vessel formation, but its molecular mechanism and

therapeutic value in colon cancer still remain to be elucidated. In this study, male severe combined immunodeficiency (SCID) mice were injected with colon cancer cells in the subcutis and then treated with Fumagillin and Cyclo (Arg-Gly-Asp-D-Phe-Val), an integrin alphavbeta(3) antagonist. The tumor weight, microvessel density (MVD), and number of pulmonary metastatic foci were examined. Gene expression profiles were examined by microarray analysis of human umbilical endothelial cells (HUVEC). The Fumagillin-treated mice had smaller tumor mass, fewer pulmonary metastases, and lower MVD-CD105 levels than control animals. In vitro proliferation and tube formation of HUVEC was also significantly decreased by Fumagillin. Microarray analysis of Fumagillin-treated HUVEC showed upregulation of 71 genes and downregulation of 143 genes. Expression changes were involved in cell proliferation, migration, adhesion, and gene transcription. Quantitative real-time-polymerase chain reaction and western blotting showed decreased expression of cyclin E2, activated leukocyte cell adhesion molecule (ALCAM), and intercellular adhesion molecule-1 (ICAM-1) genes in the presence of Fumagillin. This downregulation by Fumagillin may be involved in the anti-angiogenesis by Fumagillin. In conclusion, Fumagillin was found to suppress colorectal cancer growth and metastasis by suppressing angiogenesis.

Hsi, L. C., X. Xi, et al. (2004). "The histone deacetylase inhibitor suberoylanilide hydroxamic acid induces apoptosis via induction of 15-lipoxygenase-1 in colorectal cancer cells." Cancer Res **64**(23): 8778-81.

Histone deacetylases (HDACs) mediate changes in nucleosome conformation and are important in the regulation of gene expression. HDACs are involved in cell cycle progression and differentiation, and their deregulation is associated with several cancers. HDAC inhibitors have emerged recently as promising chemotherapeutic agents. One such agent, suberoylanilide hydroxamic acid, is a potent inhibitor of HDACs that causes growth arrest, differentiation, and/or apoptosis of many tumor types in vitro and in vivo. Because of its low toxicity, suberoylanilide hydroxamic acid is currently in clinical trials for the treatment of cancer. HDAC inhibitors induce the expression of <2% of genes in cultured cells. In this study, we show that low micromolar concentrations of suberoylanilide hydroxamic acid induce the expression of 15-lipoxygenase-1 in human colorectal cancer cells. The expression of 15-lipoxygenase-1 correlates with suberoylanilide hydroxamic acid-induced increase in 13-S-hydroxyoctadecadienoic acid levels, growth inhibition, differentiation, and apoptosis observed with these cells. Furthermore, specific inhibition of 15-lipoxygenase-1 significantly reduced the suberoylanilide hydroxamic acid-induced effects. These novel findings are the first demonstration of a mechanistic link between the induction of 15-lipoxygenase-1 by a HDAC inhibitor and apoptosis in cancer cells. This result has important implications for the study of suberoylanilide hydroxamic acid and other HDAC inhibitors in the prevention and therapy of colorectal cancer and supports future investigations of the mechanisms by which HDAC inhibitors up-regulate 15-lipoxygenase-1.

Hsi, L. C., X. Xi, et al. (2005). "The methyltransferase inhibitor 5-aza-2-deoxycytidine induces apoptosis via induction of 15-lipoxygenase-1 in colorectal cancer cells." Mol Cancer Ther **4**(11): 1740-6.

DNA methylation by DNA methyltransferases in CpG-rich promoter regions of genes is a well-described component of epigenetic silencing in human cells. Dysregulation of this process in cancer cells may lead to hypermethylation of promoter CpG islands, thus disabling transcription initiation of certain genes, such as tumor suppressor genes. Reversing epigenetic silencing and up-regulating genes involved in preventing or reversing the malignant phenotype has become a new, important targeted approach for cancer prevention and treatment. Therefore, methyltransferase inhibitors (MTI) have emerged recently as promising chemotherapeutic or preventive agents. The potent MTI 5-aza-2-deoxycytidine (5-Azadc) causes growth arrest, differentiation, and/or apoptosis of many tumor types in vitro and in vivo. The present study shows that low micromolar concentrations of 5-Azadc induce the expression of 15-lipoxygenase-1 (15-LOX-1) in human colorectal cancer cells. The expression of 15-LOX-1 correlates with 5-Azadc-induced increases in 13-S-hydroxyoctadecadienoic acid levels, growth inhibition, and apoptosis in these cells. Furthermore, specific inhibition of 15-LOX-1 by pharmacologic means or small interfering RNA significantly reduced the 5-Azadc-induced effects. These novel findings are the first demonstration of a mechanistic link between the induction of 15-LOX-1 by a MTI and apoptosis in cancer cells. This result has important implications for the study of 5-Azadc and other MTIs in the prevention and therapy of colorectal cancer and supports future investigations of the mechanisms by which MTIs up-regulate 15-LOX-1.

Hu, X. T., W. Chen, et al. (2009). "Depletion of the proteasome subunit PSMA7 inhibits colorectal cancer cell tumorigenicity and migration." Oncol Rep **22**(5): 1247-52.

Colorectal cancer is one of the most common causes of cancer-related deaths throughout the world. Recently, we reported that proteasome subunit PSMA7 located on 20q13 amplicon was overexpressed and associated with liver metastasis of colorectal cancer. The results indicate that PSMA7 may play an important role in the colorectal cancer progression and provide a unique target site for the development of therapeutic drugs. However, it is unknown how aberrant PSMA7 activation critically regulates the metastatic behavior of colorectal cancer cells. To investigate the role of PSMA7 in the progression of colorectal cancer, we employed the RNA interference technology to knock down the PSMA7 gene in human colon cancer cell line RKO and analyzed its effect and explored the involved mechanisms. Depletion of PSMA7 by shRNA in RKO cells inhibited their anchorage-independent growth and cell invasion and migration. Moreover, PSMA7 depletion was able to strongly suppress the in vivo tumorigenic ability of RKO cells. These effects may be induced by inhibiting CD44 expression directly or indirectly. Genetic or pharmacological inhibition of PSMA7 may therefore be a beneficial strategy in the treatment of colorectal cancer patients.

Huo, Q., T. Kinugasa, et al. (2009). "Claudin-1 protein is a major factor involved in the tumorigenesis of colorectal cancer." Anticancer Res **29**(3): 851-7.

BACKGROUND: The molecular and morphological alterations of the tight junctions in colorectal cancer (CRC) are still poorly understood. The possible involvement of claudin-1 (CL-1), one of the major tight junctional proteins (TJPs), was investigated in the tumorigenesis of CRC. PATIENTS AND METHODS: Adenocarcinoma tissue and paired normal mucosa specimens were resected from surgical specimens of CRC patients and analyzed to determine whether the expression of CL-1 correlated with the clinicopathological factors and to determine the role of CL-1 in the alteration of tight junctions during tumorigenesis. RESULTS: The expression of CL-1 at the mRNA and protein levels was analyzed in 41 cases and was found to increase in the CRC tissue in comparison to that in the normal tissue specimens. The mRNA levels of CL-1 were correlated with tumor depth, but not with the preoperative carcinoembryonic antigen (CEA) serum level. When T84 cells, a human colon cancer cell line, were transfected with the CL-1 gene, the CL-1 overexpressing cells grew as aggregates in contrast to the monolayer formation of the parental cells. In addition, trypsin-treated CL-1 overexpressing cells aggregated more easily than did the parental cells. CONCLUSION: CL-1 plays a pivotal role in cell morphology and behavior in the colonic epithelium. CL-1 protein may therefore be one of the major factors involved in the tumorigenesis of CRC.

Huynh, D., E. Vincan, et al. (2007). "Oncogenic properties of HIV-Tat in colorectal cancer cells." Curr HIV Res **5**(4): 403-9.

With the advent of Highly-Active-Anti-Retroviral-Therapy (HAART), HIV patients can expect to live beyond 10-15 years following diagnosis. An unexpected result of increased survival is the emergence of opportunistic, oncogenic virus-associated cancers such as Burkitt's lymphoma (Epstein-Barr Virus), cervical cancer (Human Papilloma Virus) and Kaposi's sarcoma (Kaposi's sarcoma-associated herpesvirus) in this immuno-compromised population. Furthermore, there are reports of colorectal cancers (CRC) in long-term HIV-AIDS survivors. Compared to the general, non-immuno-compromised population, long-term AIDS patients have 4 and 3.3-fold increased risk of developing colorectal and anorectal cancer respectively. Unlike oncogenic virus-associated cancers, CRC is not known to have a viral etiology. Our study aimed to investigate one aspect of HIV infection and colorectal carcinogenesis. We proposed that the HIV transactivator protein Tat; a protein with known oncogenic properties that is secreted and can re-enter non-infected cells may have a role in CRC. Using two CRC cell lines, LIM1215 and LIM2537 we found that Tat inhibits epithelial cyto-differentiation, blocks apoptosis in vitro and accelerates tumour formation in vivo. In addition, Tat significantly increases in vitro migration in the absence of foetal calf serum. These properties underpin CRC, and as HIV infection is initiated in the gut lymphoid system, these data provide a basis for the increased incidence of CRC in long term AIDS patients.

Ide, M., K. Saito, et al. (2007). "Over-expression of 14-3-3sigma in budding colorectal cancer cells modulates cell migration in the presence of tenascin-C." Oncol Rep **18**(6): 1451-6.

Epigenetic silencing of the 14-3-3sigma gene by CpG hypermethylation has been reported in many kinds of cancers, but has been considered inapplicable in the colorectal variety. The expression of 14-3-3sigma in colorectal cancer is located primarily in the invasive area. The interaction between tumor cells and the extracellular matrix (ECM) is involved in tumor invasion. In the current study, we investigated the correlation between 14-3-3sigma expression and the ECM, focusing especially on the presence of tenascin-C (TNC) at the invasive area of colorectal cancers. Correlations between the immunohistochemical expression of 14-3-3sigma and TNC, as well as other clinicopathological factors, were evaluated in 123

colorectal carcinoma tissues. 14-3-3sigma expression was frequently observed in budding tumor cells in the invasive area and expression was significantly correlated with budding formation (p=0.001), pTNM classification (p=0.001) and stromal TNC expression (p=0.004). Using colorectal cancer cell lines and ECMs, the up-regulation of 14-3-3sigma mRNA levels was investigated by semi-quantitative RT-PCR. TNC surrounding the tumor cells increased 14-3-3sigma mRNA expression 1.8- to 2.2-fold in HCT116 cells. The effect of 14-3-3sigma over-expression on tumor cell migration was investigated using an agarose-cell droplet migration assay. Over-expression of 14-3-3sigma up-regulated HCT116 cell migration on TNC (p<0.001). We concluded that the expression of 14-3-3sigma in the invasive area modulates tumor cell migration in certain types of colorectal cancer and thus facilitates tumor progression.

Ide, T., Y. Kitajima, et al. (2008). "Expression of the hMLH1 gene is a possible predictor for the clinical response to 5-fluorouracil after a surgical resection in colorectal cancer." Oncol Rep **19**(6): 1571-6.

The loss of a DNA mismatch repair occurs in approximately 15% of sporadic colorectal cancer (CRC) and is usually caused by the lack of expression of the hMLH1 gene due to promoter methylation. Despite undergoing adjuvant 5-fluorouracil (5-FU) therapy after a curative surgical resection, some patients with advanced-stage CRC develop recurrence. In the present study, we investigated whether the hMLH1 mRNA expression or promoter methylation is a prognostic factor in CRC patients treated with adjuvant 5-FU. The hMLH1 mRNA expression levels were measured by quantitative reverse transcription PCR in cancer and normal epithelial cells that were obtained from 94 CRC patients using a laser capture microdissection. Then, the methylation status of the hMLH1 promoter in the CRC tissues was examined by methylation-specific PCR. The hMLH1 mRNA expression levels were significantly lower in the cancer cells than in the normal mucosa (p<0.01) and the hMLH1 mRNA expression levels in the cancer cells were significantly lower in the CRC tissues with methylated versus unmethylated hMLH1 (p<0.01) in the 94 patients. Among the 35 patients receiving adjuvant 5-FU, the disease-free survival rate was significantly better in the patients demonstrating a low hMLH1 mRNA expression in the cancer cells in comparison to that of the patients with a high hMLH1 mRNA expression (p<0.01). Moreover, a multivariate analysis revealed that hMLH1 mRNA expression was a significant independent prognostic factor for tumor recurrence in CRC patients treated with adjuvant 5-FU. However, hMLH1 methylation was not correlated with the survival in these 35 patients. These data suggest that the hMLH1 mRNA quantitation in colorectal cancer cells may be helpful for evaluating the prognosis of CRC patients receiving 5-FU-based adjuvant chemotherapy after a surgical resection.

Italiano, A., P. Follana, et al. (2008). "Cetuximab shows activity in colorectal cancer patients with tumors for which FISH analysis does not detect an increase in EGFR gene copy number." Ann Surg Oncol **15**(2): 649-54.

BACKGROUND: EGFR (epidermal growth factor receptor) gene gain assessed by FISH (fluorescence in situ hybridization) has been shown to be predictive of response to EGFR-targeted therapies in patients with non-small cell lung cancer. The aim or our study was to relate the EGFR gene copy number to therapeutic results in patients with metastatic colorectal cancer (CRC) treated with a cetuximab-containing regimen. METHODS: Forty-seven patients with metastatic CRC treated with a cetuximab-containing regimen between August 2004 and September 2006 were included in our study. EGFR status was assessed by immunohistochemistry (IHC) and by FISH on fixed paraffin-embedded sections of tumor specimens. RESULTS: By IHC (n = 47), 39 patients (83%) had EGFR-positive tumors. EGFR gene copy gain was detected in 8 (19.5%) of 41 tumors. Neither EGFR expression assessed by IHC nor EGFR gene copy gain assessed by FISH were statistically significantly correlated with objective response rate, disease control rate, progression-free survival, and overall survival. Of the 33 patients whose tumors were FISH negative, 8 patients (24.2%) had a partial response, and 10 (30.3%) had stable disease. CONCLUSIONS: EGFR FISH analysis does not seem to be a sufficiently robust test for selecting candidate CRC patients for cetuximab therapy.

Jardim, M. J., Q. Wang, et al. (2009). "Reduced ATR or Chk1 expression leads to chromosome instability and chemosensitization of mismatch repair-deficient colorectal cancer cells." Mol Biol Cell **20**(17): 3801-9.

Genomic instability in colorectal cancer is categorized into two distinct classes: chromosome instability (CIN) and microsatellite instability (MSI). MSI is the result of mutations in the mismatch repair (MMR) machinery, whereas CIN is often thought to be associated with a disruption in the APC gene. Clinical data has recently shown the presence of heterozygous mutations in ATR and Chk1 in human cancers that exhibit MSI, suggesting that those mutations may contribute to tumorigenesis. To determine whether reduced activity in the DNA damage checkpoint pathway would cooperate with MMR deficiency to induce CIN, we used siRNA strategies to partially decrease the expression of ATR

or Chk1 in MMR-deficient colorectal cancer cells. The resultant cancer cells display a typical CIN phenotype, as characterized by an increase in the number of chromosomal abnormalities. Importantly, restoration of MMR proficiency completely inhibited induction of the CIN phenotype, indicating that the combination of partial checkpoint blockage and MMR deficiency is necessary to trigger CIN. Moreover, disruption of ATR and Chk1 in MMR-deficient cells enhanced the sensitivity to treatment with the commonly used colorectal chemotherapeutic compound, 5-fluorouracil. These results provide a basis for the development of a combination therapy for those cancer patients.

Jensen, S. A., B. Vainer, et al. (2008). "Prognostic significance of numeric aberrations of genes for thymidylate synthase, thymidine phosphorylase and dihydrofolate reductase in colorectal cancer." Acta Oncol **47**(6): 1054-61.

BACKGROUND: Most human cancer cells have structural aberrations of chromosomal regions leading to loss or gain of gene specific alleles. This study aimed to assess the range of gene copies per nucleus of thymidylate synthase (TYMS), thymidine phosphorylase (TP) and dihydrofolate reductase (DHFR) in colorectal cancer, and to evaluate its prognostic significance following adjuvant chemotherapy, since these enzymes are closely related to efficacy of 5-fluorouracil (5FU). PATIENTS AND METHODS: Consecutive patients (n = 314), who were completely resected for colorectal cancer stages II-IV and adjuvantly treated with 5-FU were retrospectively evaluated. Paraffin embedded tumor specimens were assessed for gene copies per nucleus of TYMS, TP and DHFR by fluorescence in situ hybridisation (FISH) using specific peptide nucleic acid probes. Outcome according to gene copies per nucleus above and below the median were compared. Also TYMS expression, assessed by immunohistochemistry, was associated with TYMS copies per nucleus. RESULTS: The number of gene copies per nucleus were 1.7 (0.7-2.8), 1.8 (0.9-3.1) and 1.8 (1.1-2.7) median (range) for TYMS, TP and DHFR, respectively. TYMS expression was directly associated with TYMS genes per nucleus ($p = 0.05$). Cox multivariate analysis, adjusted for the prognostic impact of disease stage, vascular tumor invasion, and bowel obstruction at resection, revealed that high TYMS gene copy number was associated with significantly higher risk of recurrence (HR = 1.6; 95%CI 1.1-2.2; $p = 0.02$) and death (HR = 1.6; 95%CI 1.1-2.3; $p = 0.01$). No significant differences in outcome appeared according to TP and DHFR gene ratios. CONCLUSION: Aberration of TYMS gene is of significance to expression of TYMS, which may influence the biology and 5-FU sensitivity of colorectal cancer. This may be utilized in the allocation of patients for treatment approaches and for decision on follow-up programs.

Jiang, X., J. Tan, et al. (2008). "DACT3 is an epigenetic regulator of Wnt/beta-catenin signaling in colorectal cancer and is a therapeutic target of histone modifications." Cancer Cell **13**(6): 529-41.

Genetic and epigenetic defects in Wnt/beta-catenin signaling play important roles in colorectal cancer progression. Here we identify DACT3, a member of the DACT (Dpr/Frodo) gene family, as a negative regulator of Wnt/beta-catenin signaling that is transcriptionally repressed in colorectal cancer. Unlike other Wnt signaling inhibitors that are silenced by DNA methylation, DACT3 repression is associated with bivalent histone modifications. Remarkably, DACT3 expression can be robustly derepressed by a pharmacological combination that simultaneously targets both histone methylation and deacetylation, leading to strong inhibition of Dishevelled (Dvl)-mediated Wnt/beta-catenin signaling and massive apoptosis of colorectal cancer cells. Our study identifies DACT3 as an important regulator of Wnt/beta-catenin signaling in colorectal cancer and suggests a potential strategy for therapeutic control of Wnt/beta-catenin signaling in colorectal cancer.

Jin, G., V. Ramanathan, et al. (2009). "Inactivating cholecystokinin-2 receptor inhibits progastrin-dependent colonic crypt fission, proliferation, and colorectal cancer in mice." J Clin Invest **119**(9): 2691-701.

Hyperproliferation of the colonic epithelium, leading to expansion of colonic crypt progenitors, is a recognized risk factor for colorectal cancer. Overexpression of progastrin, a nonamidated and incompletely processed product of the gastrin gene, has been shown to induce colonic hyperproliferation and promote colorectal cancer in mice, but the mechanism of pathogenesis has not been defined. Cholecystokinin-2 receptor (CCK2R) is the primary receptor for cholecystokinin (CCK) and amidated gastrin. Here, we show that Cck2r was expressed in murine colonic crypts and upregulated in the transgenic mice that overexpress human progastrin. Murine deletion of Cck2r abrogated progastrin-dependent increases in colonic proliferation, mucosal thickness, and beta-catenin and CD44 expression in the colon tumor. In addition, either deletion or antagonism of Cck2r resulted in the inhibition of progastrin-dependent increases in progenitors expressing doublecortin and CaM kinase-like-1 (DCAMKL1), stem cells expressing leucine rich repeat-containing G protein-coupled receptor 5

(LgR5), and colonic crypt fission. Furthermore, in the azoxymethane mouse model of colorectal carcinogenesis, Cck2r deletion in human progastrin-overexpressing mice resulted in markedly decreased aberrant crypt foci formation and substantially reduced tumor size and multiplicity. Taken together, these observations indicate that progastrin induces proliferative effects, primarily in colonic progenitor cells, through a CCK2R-dependent pathway. Moreover, our data suggest that CCK2R may be a potential target in the treatment or prevention of colorectal cancer.

Kaulfuss, S., P. Burfeind, et al. (2009). "Dual silencing of insulin-like growth factor-I receptor and epidermal growth factor receptor in colorectal cancer cells is associated with decreased proliferation and enhanced apoptosis." Mol Cancer Ther **8**(4): 821-33.

Overexpression and activation of tyrosine kinase receptors are common features of colorectal cancer. Using the human colorectal cancer cell lines DLD-1 and Caco-2, we evaluated the role of the insulin-like growth factor-I (IGF-I) receptor (IGF-IR) and epidermal growth factor receptor (EGFR) in cellular functions of these cells. We used the small interfering RNA (siRNA) technology to specifically down-regulate IGF-IR and EGFR expression. Knockdown of IGF-IR and EGFR resulted in inhibition of cell proliferation of DLD-1 and Caco-2 cells. An increased rate of apoptosis was associated with siRNA-mediated silencing of IGF-IR and EGFR as assessed by activation of caspase-3/caspase-7. The combined knockdown of both EGFR and IGF-IR decreased cell proliferation and induced cell apoptosis more effectively than did silencing of either receptor alone. Comparable effects on cell proliferation and apoptosis were observed after single and combinational treatment of cells by the IGF-IR tyrosine kinase inhibitor NVP-AEW541 and/or the EGFR tyrosine kinase inhibitor erlotinib. Combined IGF-IR and EGFR silencing by either siRNAs or tyrosine kinase inhibitors diminished the phosphorylation of downstream signaling pathways AKT and extracellular signal-regulated kinase (ERK)-1/2 more effectively than did the single receptor knockdown. Single IGF-IR knockdown inhibited IGF-I-dependent phosphorylation of AKT but had no effect on IGF-I- or EGF-dependent phosphorylation of ERK1/2, indicating a role of EGFR in ligand-dependent ERK1/2 phosphorylation. The present data show that inhibition of the IGF-IR transduction cascade augments the antipoliferative and proapoptotic effects of EGFR inhibition in colorectal cancer cells. A clinical application of combination therapy targeting both EGFR and IGF-IR could be a promising therapeutic strategy.

Kim-Schulze, S., H. S. Kim, et al. (2008). "Intrarectal vaccination with recombinant vaccinia virus expressing carcinoembronic antigen induces mucosal and systemic immunity and prevents progression of colorectal cancer." J Immunol **181**(11): 8112-9.

The gastrointestinal mucosa contains an intact immune system that protects the host from pathogens and communicates with the systemic immune system. Absorptive epithelial cells in the mucosa give rise to malignant tumors although the interaction between tumor cells and the mucosal immune system is not well defined. The pathophysiology of colorectal cancer has been elucidated through studies of hereditary syndromes, such as familial adenomatous polyposis, a cancer predisposition syndrome caused by germline mutations in the adenomatous polyposis coli tumor suppressor gene. Patients with FAP develop adenomas and inevitably progress to invasive carcinomas by the age of 40. To better delineate the role of mucosal immunity in colorectal cancer, we evaluated the efficacy of intrarectal recombinant vaccinia virus expressing the human carcinoembryonic Ag (CEA) in a murine FAP model in which mice are predisposed to colorectal cancer and also express human CEA in the gut. Mucosal vaccination reduced the incidence of spontaneous adenomas and completely prevented progression to invasive carcinoma. The therapeutic effects were associated with induction of mucosal CEA-specific IgA Ab titers and CD8(+) CTLs. Mucosal vaccination was also associated with an increase in systemic CEA-specific IgG Ab titers, CD4(+) and CD8(+) T cell responses and resulted in growth inhibition of s.c. implanted CEA-expressing tumors suggesting communication between mucosal and systemic immune compartments. Thus, intrarectal vaccination induces mucosal and systemic antitumor immunity and prevents progression of spontaneous colorectal cancer. These results have implications for the prevention of colorectal cancer in high-risk individuals.

Koda, M., M. Sulkowska, et al. (2007). "Expression of the obesity hormone leptin and its receptor correlates with hypoxia-inducible factor-1 alpha in human colorectal cancer." Ann Oncol **18 Suppl 6**: vi116-9.

BACKGROUND: The obesity hormone, leptin, has been found to play a role in development and proliferation of normal and malignant tissues. Leptin activity is mediated through the leptin receptor (ObR) that is often expressed in different human cancer cells. Previously, we found that the expression of leptin and ObR can be stimulated by hypoxia-mimetic agents. The aim of this study was to analyze the abundance of and relationships among leptin, ObR

and hypoxia-inducible factor-1alpha (HIF-1alpha, transcriptional regulator) in human colorectal cancer. MATERIALS AND METHODS: We investigated the expression of leptin, ObR and HIF-1alpha in colorectal cancer specimens from 135 patients who underwent curative resection. RESULTS: Immunoreactivity for leptin, ObR and HIF-1alpha protein was observed in 69 of 135 (51.1%), 129 of 135 (95.5%) and 88 of 135 (65.2%) of colorectal cancers, respectively. Statistically significant positive correlations were noted between leptin and HIF-1alpha ($P = 0.005$, $r = 0.243$), ObR and HIF-1alpha ($P < 0.001$, $r = 0.325$) as well as leptin and ObR ($P < 0.001$, $r = 0.426$) in the group of all patients as well as in various subgroups depending on clinicopathological features. CONCLUSIONS: The results indicate that the leptin system is overexpressed in human colorectal cancer and this overexpression appears to be associated with the abundance of HIF-1alpha.

Kodach, L. L., S. A. Bleuming, et al. (2007). "The effect of statins in colorectal cancer is mediated through the bone morphogenetic protein pathway." Gastroenterology **133**(4): 1272-81.

BACKGROUND & AIMS: Epidemiological evidence suggests that statins prevent colorectal cancer (CRC), but the biological mechanism remains obscure. Statins induce bone morphogenetic protein (BMP) expression in bone cells. We have previously shown that BMPs act as tumor suppressors in CRC. We hypothesized that the action of statins in CRC involves the induction of BMPs. METHODS: We investigated the effects of statins on CRC cell lines using immunoblotting, measurements of apoptosis and cell proliferation, and luciferase reporter assays. The effect of statins was confirmed in a xenograft mouse model. RESULTS: CRC cell lines show widely differing sensitivities to statin treatment. Sensitive cell lines show induction of BMP2 protein levels and a BMP2 reporter construct, activation of the BMP pathway, and induction of the BMP target gene ID-2, whereas resistant cell lines do not. The addition of the specific inhibitor of BMPs, noggin, completely prevents lovastatin-induced apoptosis in sensitive cells. Sensitive cell lines express the central BMP pathway element SMAD4, whereas the resistant cell lines do not. Targeted knockout of SMAD4 leads to the loss of statin sensitivity and reconstitution with SMAD4, to the restoration of statin sensitivity. In a xenograft mouse model, tumors from sensitive and insensitive cell lines were treated with oral simvastatin. Significant inhibition of tumor growth using sensitive cells but increased tumor growth when using insensitive cells was observed. CONCLUSIONS: Statins induce apoptosis in CRC cells through induction of BMP2. Statin therapy may only be effective in SMAD4-expressing CRCs and may have adverse effects in SMAD4-negative tumors.

Koga, Y., M. Yasunaga, et al. (2008). "Detection of colorectal cancer cells from feces using quantitative real-time RT-PCR for colorectal cancer diagnosis." Cancer Sci **99**(10): 1977-83.

Early detection of colorectal cancer (CRC) is desired for reducing its mortality rate. Recently, the feasibility of a new method for isolating colonocytes from feces was demonstrated, followed by direct sequencing analysis for detecting colorectal cancer. In the present study, gene expression analysis was conducted using quantitative real-time reverse transcriptase-polymerase chain reaction (RT-PCR). One hundred and sixty-six patients with CRC and 134 healthy volunteers were enrolled. Messenger RNA expressions of CEA, MMP7, MYBL2, PTGS2 and TP53 in the colonocytes isolated from feces were analyzed by quantitative real-time RT-PCR. Beta-2-microglobulin, used for internal control, could not be detected in approximately 25% each of the CRC patients (39/166) and healthy volunteers (33/134). CEA expression did not differ significantly between CRC patients and healthy volunteers ($P = 0.21$). MMP7, MYBL2, PTGS2 and TP53 gene expressions were significantly higher in CRC patients than in healthy volunteers ($P < 0.001$). The overall sensitivity and specificity using these gene expressions were 58.3% (74/127, 95% CI; 49.2-67.0) and 88.1% (89/101, 95% CI; 80.2-93.7), respectively. The sensitivity was dependent on the tumor location ($P = 0.01$) and tumor size ($P = 0.02$), but not the tumor depth ($P = 0.06$) or cancer stage ($P = 0.37$). Gene expression analysis of colonocytes isolated from feces may be a useful method for CRC screening, if the number of isolated colonocytes is sufficiently high for analysis by quantitative real-time PCR. Therefore, improvement of the colonocyte retrieval system from feces may be necessary for the technique to be developed for clinical use.

Konishi, T., S. Sasaki, et al. (2005). "Exogenous expression of hRFI induces multidrug resistance through escape from apoptosis in colorectal cancer cells." Anticancer Res **25**(4): 2737-41.

BACKGROUND: hRFI is a newly discovered gene encoding a Ring Finger domain highly homologous to that of the X-chromosome-linked inhibitor of apoptosis protein, which is among the most potent inhibitors of apoptosis. The hRFI protein is preferentially expressed in colorectal cancers, although its actual function is unknown. The aim of this study was to determine whether hRFI possesses an anti-apoptotic function in colorectal cancer cells against chemotherapeutic agents.

MATERIALS AND METHODS: HCT116 colorectal cancer cells were exogenously transfected with hRFI or LacZ as a control. After exposure to either cisplatin, irinotecan or 5-fluorouracil, apoptosis and caspase-3 activity were evaluated by flow cytometry analysis. RESULTS: The hRFI transfectant exhibited significant resistance to apoptosis induced by each of the three agents, along with inactivation of caspase-3. Growth in the normal medium was not altered. CONCLUSION: hRFI plays an important role in the resistance to chemotherapeutic agent-induced apoptosis in colorectal cancer cells.

Konishi, T., S. Sasaki, et al. (2006). "Overexpression of hRFI inhibits 5-fluorouracil-induced apoptosis in colorectal cancer cells via activation of NF-kappaB and upregulation of BCL-2 and BCL-XL." Oncogene **25**(22): 3160-9.

Resistance to apoptosis is one of the important determinants of resistance to 5-fluorouracil (5-FU) in colorectal cancer cells. Human Ring-Finger homologous to Inhibitor of apoptosis protein type (hRFI) is a newly discovered gene that has been shown to inhibit death receptor-mediated apoptosis in colorectal cancer cells. However, the molecular mechanism of the inhibition of apoptosis is presently unknown. In order to investigate the molecular function of hRFI in the regulation of 5-FU-induced apoptosis in colorectal cancer cells, HCT116 cells were stably transfected with hRFI or LacZ as a control. hRFI overexpression resulted in cellular resistance to 5-FU through an inhibition of the mitochondrial apoptotic pathway and specific upregulation of Bcl-2 and Bcl-XL. Futhermore, hRFI overexpression resulted in the activation of nuclear factor-kappaB (NF-kappaB). Inhibition of NF-kappaB effectively reversed the resistance to apoptosis as well as the upregulation of Bcl-2 and Bcl-XL in the hRFI transfectant, indicating that the activation of NF-kappaB is the key mechanism for all these findings. Overexpression of hRFI in SW480 and COLO320 colorectal cancer cells similarly resulted in resistance to 5-FU with the activation of NF-kappaB and upregulation of Bcl-2 and Bcl-XL. hRFI might be a novel therapeutic target for gene therapy in colorectal cancer.

Koopman, M., S. Venderbosch, et al. (2009). "Predictive and prognostic markers for the outcome of chemotherapy in advanced colorectal cancer, a retrospective analysis of the phase III randomised CAIRO study." Eur J Cancer **45**(11): 1999-2006.

We have tested several biomarkers [dihydropyrimidine dehydrogenase (DPD), orotate phosphoribosyl transferase (OPRT), thymidine phosphorylase (TP), thymidylate synthase (TS) and excision cross-complementing gene (ERCC1)] for their prognostic and predictive value in relation to the outcome of chemotherapy in tumour tissues of 556 advanced colorectal cancer (ACC) patients who were randomised between sequential treatment and combination treatment in the CApecitabine, IRinotecan, Oxaliplatin (CAIRO) study. DPD expression showed a statistically significant predictive value for combination treatment with capecitabine plus irinotecan with low versus high values resulting in an improved median progression-free survival (PFS) and median overall survival (OS) of 8.9 (95% confidence interval (CI) 8.3-9.9) versus 7.2 months (95% CI 6.5-8.1, p=0.006), and 21.5 months (95% CI 17.9-26.5) versus 16.9 months (95% CI 13.0-19.1, p=0.04), respectively. In the overall patient population a high OPRT expression in stromal cells was a favourable prognostic parameter for OS, with 21.5 months (95% CI 17.9-27.3) versus 17.2 months (95% CI 15.1-18.6, p=0.036), respectively. A similar effect was observed for PFS. In a multivariate analysis that included known prognostic factors these results remained significant and also showed that a high OPRT expression in tumour cells was an unfavourable prognostic parameter for PFS and OS. In conclusion, in this largest study on capecitabine with or without irinotecan to date we found a predictive value of DPD expression. Our results on the prognostic value of OPRT expression warrant further studies on the role of stromal cells in the outcome of treatments. The divergent results of ours and previous studies underscore the complexity of these biomarkers and currently prevent the routine use of these markers in daily clinical practice.

Koukourakis, M. I., A. Giatromanolaki, et al. (2006). "Lactate dehydrogenase 5 expression in operable colorectal cancer: strong association with survival and activated vascular endothelial growth factor pathway--a report of the Tumour Angiogenesis Research Group." J Clin Oncol **24**(26): 4301-8.

PURPOSE: Lactate dehydrogenase 5 (LDH-5) regulates, under hypoxic conditions, the anaerobic transformation of pyruvate to lactate for energy acquisition. Several studies have shown that serum LDH may be an ominous prognostic marker in malignant tumors. The clinical significance of tissue LDH-5, however, remains largely unexplored. PATIENTS AND METHODS: We investigated the immunohistochemical expression of LDH-5 in a series of 128 stage II/III colorectal adenocarcinomas treated with surgery alone. In addition, markers of tumor hypoxia (hypoxia-inducible factor 1 alpha [HIF1alpha]), angiogenesis (vascular endothelial growth factor [VEGF] and phosporylated kinase domain receptor [pKDR]/flk-1 receptor) and the tumor

vascular density (CD31 positive standard vascular density [sVD] and pKDR positive activated vascular density [aVD]) were assessed. RESULTS: The expression of LDH-5, together with that of HIF1alpha and pKDR, was both nuclear and cytoplasmic. Assessment, with minimal interobserver variability, was achieved using a previously described scoring system. LDH-5 was significantly associated with HIF1alpha (P = .01), aVD (P = .001) and, particularly, with pKDR expression in cancer cells (P = .0001). Tissue LDH-5 expression was linked with elevated serum LDH levels, but serum levels failed to reflect tissue expression in 71% of LDH-5 positive cases. In univariate analysis tissue LDH-5 was associated with poor survival (P = .0003, HR 15.1), whereas in multivariate analysis this isoenzyme was the strongest independent prognostic factor (P = .0009). VEGF, pKDR, aVD, sVD and vascular invasion were all significantly related to unfavorable prognosis. CONCLUSION: The immunohistochemical assessment of tissue LDH-5 and pKDR provides important prognostic information in operable colorectal cancer. The strong association between LDH-5 and pKDR expression would justify their use as surrogate markers to screen patients for tyrosine kinase inhibitor therapy.

Koyanagi, K., A. J. Bilchik, et al. (2008). "Prognostic relevance of occult nodal micrometastases and circulating tumor cells in colorectal cancer in a prospective multicenter trial." Clin Cancer Res **14**(22): 7391-6.

PURPOSE: Nodal micrometastasis and circulating tumor cells detected by multimarker quantitative real-time reverse transcription-PCR (qRT-PCR) may have prognostic importance in patients with colorectal cancer. EXPERIMENTAL DESIGN: Paraffin-embedded sentinel lymph nodes from 67 patients and blood from 34 of these patients were evaluated in a prospective multicenter trial of sentinel lymph node mapping in colorectal cancer. Sentinel lymph nodes were examined by H&E staining and cytokeratin immunohistochemistry. Sentinel lymph nodes and blood were examined by a four-marker qRT-PCR assay (c-MET, melanoma antigen gene-A3 family, beta1-->4-N-acetylgalactosaminyltransferase, and cytokeratin-20); qRT-PCR results were correlated with disease stage and outcome. RESULTS: In H&E-negative sentinel lymph node patients that recurred, cytokeratin immunohistochemistry and qRT-PCR detected metastasis in 30% and 60% of patients, respectively. Disease-free survival differed significantly by multimarker qRT-PCR upstaged sentinel lymph node (P = 0.014). qRT-PCR analysis of blood for circulating tumor cells correlated with overall survival (P = 0.040). CONCLUSION: Molecular assessment for micrometastasis in sentinel lymph node and blood specimens may help identify patients at high risk for recurrent colorectal cancer, who could benefit from adjuvant therapy.

Kunnumakkara, A. B., P. Diagaradjane, et al. (2009). "Curcumin sensitizes human colorectal cancer to capecitabine by modulation of cyclin D1, COX-2, MMP-9, VEGF and CXCR4 expression in an orthotopic mouse model." Int J Cancer **125**(9): 2187-97.

Because of the poor prognosis and the development of resistance against chemotherapeutic drugs, the current treatment for advanced metastatic colorectal cancer (CRC) is ineffective. Whether curcumin (a component of turmeric) can potentiate the effect of capecitabine against growth and metastasis of CRC was investigated. The effect of curcumin on proliferation of CRC cell lines was examined by mitochondrial dye-uptake assay, apoptosis by esterase staining, nuclear factor-kappaB (NF-kappaB) by electrophoretic mobility shift assay and gene expression by Western blot analysis. The effect of curcumin on the growth and metastasis of CRC was also examined in orthotopically implanted tumors in nude mice. In vitro, curcumin inhibited the proliferation of human CRC cell lines, potentiated capecitabine-induced apoptosis, inhibited NF-kappaB activation and suppressed NF-kappaB-regulated gene products. In nude mice, the combination of curcumin and capecitabine was found to be more effective than either agent alone in reducing tumor volume (p = 0.001 vs. control; p = 0.031 vs. capecitabine alone), Ki-67 proliferation index (p = 0.001 vs. control) and microvessel density marker CD31. The combination treatment was also highly effective in suppressing ascites and distant metastasis to the liver, intestines, lungs, rectum and spleen. This effect was accompanied by suppressed expression of activated NF-kappaB and NF-kappaB-regulated gene products (cyclin D1,c-myc, bcl-2, bcl-xL, cIAP-1, COX-2, ICAM-1, MMP-9, CXCR4 and VEGF). Overall, our results suggest that curcumin sensitizes CRC to the antitumor and antimetastatic effects of capecitabine by suppressing NF-kappaB cell signaling pathway.

Kunnumakkara, A. B., P. Diagaradjane, et al. (2008). "Curcumin sensitizes human colorectal cancer xenografts in nude mice to gamma-radiation by targeting nuclear factor-kappaB-regulated gene products." Clin Cancer Res **14**(7): 2128-36.

PURPOSE: How colorectal cancer develops resistance to gamma-radiation is not fully understood, but the transcription factor nuclear factor-kappaB (NF-kappaB) and NF-kappaB-regulated gene products have been proposed as mediators. Because curcumin,

a component of turmeric (Curcuma longa), has been shown to suppress NF-kappaB activation, whether it can sensitize the colorectal cancer to gamma-radiation was investigated in colorectal cancer xenografts in nude mice. EXPERIMENTAL DESIGN: We established HCT 116 xenograft in nude mice, randomized into four groups, and treated with vehicle (corn oil), curcumin, gamma-radiation, and curcumin in combination with gamma-radiation. NF-kappaB modulation was ascertained using electrophoretic mobility shift assay and immunohistochemistry. Markers of proliferation, angiogenesis, and invasion were monitored by immunohistochemistry and Western blot analysis. RESULTS: Curcumin significantly enhanced the efficacy of fractionated radiation therapy by prolonging the time to tumor regrowth (P=0.02) and by reducing the Ki-67 proliferation index (P<0. 001). Moreover, curcumin suppressed NF-kappaB activity and the expression of NF-kappaB-regulated gene products (cyclin D1, c-myc, Bcl-2, Bcl-xL, cellular inhibitor of apoptosis protein-1, cyclooxygenase-2, matrix metalloproteinase-9, and vascular endothelial growth factor), many of which were induced by radiation therapy and mediate radioresistance. The combination of curcumin and radiation therapy also suppressed angiogenesis, as indicated by a decrease in vascular endothelial growth factor and microvessel density (P=0.002 versus radiation alone). CONCLUSION: Collectively, our results suggest that curcumin potentiates the antitumor effects of radiation therapy in colorectal cancer by suppressing NF-kappaB and NF-kappaB-regulated gene products, leading to inhibition of proliferation and angiogenesis.

Kurer, M. A. (2007). "Protein and mRNA expression of tissue factor pathway inhibitor-1 (TFPI-1) in breast, pancreatic and colorectal cancer cells." Mol Biol Rep **34**(4): 221-4.

BACKGROUND: Patients with solo tumour malignancy are at higher risk of developing venous thromboembolism. When prophylactic anticoagulation (and in particular heparin) is used during cancer therapy however, patients appear to have a prolonged survival. Tumours express large quantities of procoagulant molecules, which predispose patients to these conditions. Tissue Factor (TF) is an important example, which may have a role in the biology of malignant disease. Intra-tumour vessel coagulation however is not a common phenomenon. Our hypothesis is that cancer cells produce anticoagulant molecules, which may prevent intra-tumour vessel auto-coagulation. Our results show that one such factor--Tissue Factor Pathway Inhibitor (TFPI-1) is expressed by a number of different cancer cells. METHODS: Seven human cancer cell lines were studied: three breast, two colorectal and two pancreatic. Cells were maintained in cell culture, and at 90% confluence protein and RNA were extracted. RNA integrity was confirmed using an RNA integrity gel and RNA purity determined by spectrophotometry. Reverse transcription polymerase chain reaction (RT-PCR) was used for TFPI-1 mRNA detection and immunoblotting used for TFPI-1 protein detection. RESULTS: Six cell lines (two breast, two colorectal, and two pancreatic) expressed the TFPI-1 gene. Gene function was confirmed by detection of TFPI-1 protein expression in these cell lines. CONCLUSION: TFPI-1 is expressed by breast cancer and other cancer cell lines maintained in cell culture. This has not been previously reported. Functional expression of TFPI-1 by cancer cells suggests that it has an important role in cancer biology. Further experiments are required to establish its function.

Lee, J. H., J. S. Lee, et al. (2006). "Tautomycetin inhibits growth of colorectal cancer cells through p21cip/WAF1 induction via the extracellular signal-regulated kinase pathway." Mol Cancer Ther **5**(12): 3222-31.

Tautomycetin is an antifungal antibiotic retaining potent immunosuppressive function. We have identified the roles of tautomycetin on cellular proliferation and transformation of colorectal cancer cells. The proliferation and anchorage-independent growth of HCT-15, HT-29, and DLD-1 colorectal cancer cells were efficiently inhibited without induction of apoptosis by 150 nmol tautomycetin. These growth inhibitory effects were dependent on p21Cip/WAF induction via the extracellular signal-regulated kinase pathway, and the tautomycetin effects were abolished in HCT-116 colon cells and eight other types of cells that did not induce p21Cip/WAF by 150 nmol tautomycetin. The crucial role of p21Cip/WAF1 in the extracellular signal-regulated kinase pathway-dependent antiproliferative responses by tautomycetin was confirmed by using p21Cip/WAF1 gene-deleted HCT-116 cells. The growth inhibitory effect of tautomycetin was acquired by regulation of Raf-1 activity through inhibition of protein phosphatase type 1 and protein phosphatase type 2A with high preference toward protein phosphatase type 1. Tautomycetin could be a potential drug for colorectal cancer.

Lee, S. H., M. Cekanova, et al. (2008). "Multiple mechanisms are involved in 6-gingerol-induced cell growth arrest and apoptosis in human colorectal cancer cells." Mol Carcinog **47**(3): 197-208.

6-Gingerol, a natural product of ginger, has been known to possess anti-tumorigenic and pro-apoptotic activities. However, the mechanisms by

which it prevents cancer are not well understood in human colorectal cancer. Cyclin D1 is a proto-oncogene that is overexpressed in many cancers and plays a role in cell proliferation through activation by beta-catenin signaling. Nonsteroidal anti-inflammatory drug (NSAID)-activated gene-1 (NAG-1) is a cytokine associated with pro-apoptotic and anti-tumorigenic properties. In the present study, we examined whether 6-gingerol influences cyclin D1 and NAG-1 expression and determined the mechanisms by which 6-gingerol affects the growth of human colorectal cancer cells in vitro. 6-Gingerol treatment suppressed cell proliferation and induced apoptosis and G(1) cell cycle arrest. Subsequently, 6-gingerol suppressed cyclin D1 expression and induced NAG-1 expression. Cyclin D1 suppression was related to inhibition of beta-catenin translocation and cyclin D1 proteolysis. Furthermore, experiments using inhibitors and siRNA transfection confirm the involvement of the PKCepsilon and glycogen synthase kinase (GSK)-3beta pathways in 6-gingerol-induced NAG-1 expression. The results suggest that 6-gingerol stimulates apoptosis through upregulation of NAG-1 and G(1) cell cycle arrest through downregulation of cyclin D1. Multiple mechanisms appear to be involved in 6-gingerol action, including protein degradation as well as beta-catenin, PKCepsilon, and GSK-3beta pathways.

Levy, E. M., M. Bianchini, et al. (2008). "Human leukocyte antigen-E protein is overexpressed in primary human colorectal cancer." Int J Oncol **32**(3): 633-41.

HLA-E is a non-classical MHC molecule whose expression by tumour cells has been recently reported in several human cancer types. We studied HLA-E expression in colorectal cancer patients, its clinical significance and prognostic value, as well as characterized its expression in colorectal cancer cell lines. We analysed HLA-E expression at the transcript level by qRT-PCR in micro-dissected samples and at the protein level by semiquantitative immunohistochemistry on paraffin-embedded tissue sections from 42 biopsies of colorectal cancer patients. We observed that HLA-E transcript and protein are spontaneously overexpressed in a significant proportion of colorectal tumour biopsies, as compared to normal mucosae. We also found a negative correlation between HLA-E expression and the CD57+ cells infiltrate. Moreover, we analysed HLA-E expression in several colorectal cancer cell lines and demonstrated that IFN-gamma upregulates the expression of membrane HLA-E in vitro. Interestingly, we demonstrated that colorectal cancer cell lines overexpressing HLA-E at the cell surface inhibited NK-mediated cell lysis. Although IFN-gamma regulatory role needs further investigation, we provide evidence suggesting that this cytokine, within the tumour microenvironment, could promote HLA-E translocation to the surface of tumour epithelial cells. Furthermore, we showed that upregulation of HLA-E could be a marker of shorter disease-free survival in Dukes' C patients and we suggest that this molecule renders tumours less susceptible to immune attack.

Li, H. J., M. Everts, et al. (2009). "Combined transductional untargeting/retargeting and transcriptional restriction enhances adenovirus gene targeting and therapy for hepatic colorectal cancer tumors." Cancer Res **69**(2): 554-64.

Unresectable hepatic colorectal cancer (CRC) metastases are a leading cause of cancer mortality. These tumors and other epithelial tumors often express both cyclooxygenase-2 (COX-2) and carcinoembryonic antigen (CEA). Because adenovirus (Ad) vectors infect the liver and lack tumor tropism, they cannot be used for systemic therapy of hepatic metastases. We used COX-2 transcriptional restriction, in combination with transductional Ad hepatic untargeting and tumor retargeting by a bispecific adapter, sCARhMFE, composed of sCAR [the coxsackie/Ad receptor (CAR) ectodomain] and MFE-23 (a single-chain anti-CEA antibody), to untarget liver after i.v. administration of Ad vectors expressing firefly luciferase and to retarget virus to hepatic colorectal tumor xenografts and non-small cell lung tumor xenografts. To improve both liver untargeting and tumor retargeting, we developed sCARfMFE, a trimerized sCARhMFE adapter. Trimerization greatly improves both untargeting of CAR-dependent Ad infection and CEA-dependent virus retargeting in culture and in vivo. Combining sCARfMFE bispecific adapter transductional liver untargeting and transductional tumor retargeting with COX-2 transcriptional tumor-restricted transgene expression increases systemically administered Ad therapeutic efficacy for hepatic CRC tumors, using herpes virus type 1 thymidine kinase (HSV1-tk) as a therapeutic gene in conjunction with the prodrug ganciclovir (GCV). Both transductional untargeting and COX-2 transcriptional restriction also reduce HSV1-tk/GCV hepatic toxicity. In addition, transductional sCARfMFE untargeting reduces the innate immune response to systemic Ad administration. Combined transductional liver Ad untargeting, transductional tumor retargeting, and transcriptional transgene restriction suggests a means to engineer practical, effective therapeutic agents for hepatic CRC metastases in particular, as well as hepatic metastases of other epithelial cancers.

Li, P., J. E. Lin, et al. (2009). "GCC signaling in colorectal cancer: Is colorectal cancer a paracrine deficiency syndrome?" Drug News Perspect **22**(6): 313-8.

Guanylyl cyclase C (GCC) is the receptor expressed by intestinal cells for the paracrine hormones guanylin and uroguanylin that coordinate mucosal homeostasis and its silencing contributes to intestinal transformation. It orchestrates proliferative and metabolic circuits by limiting the cell cycle and programming metabolic transitions central to regeneration along the crypt-villus axis. Mice deficient in GCC are more susceptible to colon cancer induced by germline mutations or carcinogens. Moreover, guanylin and uroguanylin are the most commonly lost gene products in colon cancer. The role of GCC as a tumor suppressor and the universal loss of its hormones in transformation suggest a paradigm in which colorectal cancer is a disease of paracrine hormone insufficiency. Indeed, GCC signaling reverses the tumorigenic phenotype of human colon cancer cells by regulating proliferation and metabolism. These data suggest a pathophysiological hypothesis in which GCC is a tumor suppressor coordinating proliferative homeostasis whose silencing through hormone loss initiates transformation. The correlative therapeutic hypothesis suggests that colorectal cancer is a disease of hormone insufficiency that can be prevented or treated by oral hormone replacement therapy employing GCC ligands.

Li, P., J. E. Lin, et al. (2008). "Colorectal cancer is a paracrine deficiency syndrome amenable to oral hormone replacement therapy." Clin Transl Sci **1**(2): 163-7.

The most commonly lost gene products in colorectal carcinogenesis include the paracrine hormones guanylin and uroguanylin, the endogenous ligands for guanylyl cyclase C (GCC), the intestinal receptor for diarrheagenic bacterial enterotoxins. Recently, GCC-cGMP signaling has emerged as a principal regulator of proliferation, genetic integrity and metabolic programming in normal human enterocytes and colon cancer cells. Elimination of GCC in mice produced hyperplasia of the proliferating compartment associated with increases in rapidly cycling progenitor cells, and reprogrammed enterocyte metabolism, with a shift from oxidative phosphorylation to glycolysis. In addition, in colons of mice carrying mutations in Apc (Apc(Min) (/+)) or exposed to the carcinogen azoxymethane, elimination of GCC increased tumor initiation and promotion by disrupting genomic integrity and releasing cell cycle restriction. These previously unrecognized roles for GCC as a fundamental regulator of intestinal homeostasis and as an intestinal tumor suppressor suggest that receptor dysregulation reflecting paracrine hormone insufficiency is a key event during the initial stages of colorectal tumorigenesis. Together with the uniform over-expression of GCC in human tumors, these novel roles for GCC underscore the potential of oral replacement with GCC ligands for targeted prevention and therapy of colorectal cancer.

Lin, F., R. Wang, et al. (2008). "Knockdown of RCK/p54 expression by RNAi inhibits proliferation of human colorectal cancer cells in vitro and in vivo." Cancer Biol Ther **7**(10): 1669-76.

Colorectal cancer is the third most common cancer in both men and women around the world. Although much progress of the mechanism of colorectal carcinogenesis has been made, the studies centering on the mechanisms of tumorigenesis are much needed to be further exploited. The overexpression of RCK/p54 gene, a member of the DEAD box protein/RNA helicase family, has been found in this malignancy. Roles of RCK in the development of colon cancer, however, are unknown. In this report, we explored whether RCK/p54 plays a role in maintaining the malignant phenotype and functions in the canonical Wnt signaling pathway of colorectal cancer cells harboring an APC mutation. The ectopic overexpression of RCK/p54 gene in colorectal cancer cells by transfection with RCK/p54 cDNA could lead to a significant increase of Tcf transcriptional activity and expression levels of Wnt target genes. By RNAi assay, we also observed that the Tcf transcriptional activity in LoVo-shRNA cells was significantly decreased by approximately 61.3%, while the mRNA and protein expression levels of Wnt target genes were also obviously decreased. Furthermore, the anti-tumour effects and its possible mechanisms of actions in LoVo cells elicited by a decrease in the level of RCK/p54 by RNAi were examined. Results showed that RCK/p54 downregulation could significantly reduce the viability of LoVo cells, increased cell number of S phase, led to cell apoptosis induction, and inhibited tumor growth in nude mice. Taken together, RCK/p54 might be a determinant of colorectal cancer proliferation by activating the canonical Wnt pathway and RCK/p54-shRNA might be a potential strategy for colorectal cancer gene therapy.

Linder, N., E. Martelin, et al. (2009). "Xanthine oxidoreductase - clinical significance in colorectal cancer and in vitro expression of the protein in human colon cancer cells." Eur J Cancer **45**(4): 648-55.

Xanthine oxidoreductase (XOR) is a key enzyme in degradation of DNA and RNA, and has previously been shown to be decreased in aggressive

breast and gastric cancer. In this study, XOR expression was assessed in tissue microarray specimens of 478 patients with colorectal cancer and related to clinical parameters. In addition, we performed in vitro studies of XOR activity, protein and mRNA in colon cancer cells (Caco-2). Results from the tissue expression analyses show that XOR was decreased in 62% and undetectable in 22% of the tumours as compared to normal tissue. Loss of XOR was associated with poor grade of differentiation (p=0.006) and advanced Dukes stage (p=0.03). In multivariate survival analysis, XOR was a prognostic factor (p=0.008), independent of Dukes stage, histological grade, age and tumour location. The in vitro analyses show that XOR is not measurable in undifferentiated Caco-2 cells, but appears and increases with differentiation. We conclude that XOR expression is associated with histological grade of differentiation and extent of disease in colorectal cancer, and it provides significant prognostic information independently of established factors.

Lledo, S., R. Alfonso, et al. (2005). "Antisense gene therapy using anti-k-ras and antitelomerase oligonucleotides in colorectal cancer." Rev Esp Enferm Dig **97**(7): 472-80.

AIM: To test the efficacy of anti-k-ras and antitelomerase oligonucleotides for disabling colorectal cancer cell growth. MATERIAL AND METHODS: An established human colorectal cancer cell line (SW 480, ATTC) was used. Oligodeoxiribonucleotides (ODNs) have a phosphorotioate modification to ensure intracellular intake. We used an antitelomerase ODN (Telp5) and two anti-k-ras ODNs (AS-KRAS and ISIS). AS-KRAS is designed to join the k-ras oncogene s exon 1. ISIS links to the terminal transcription unit 5 of k-ras. Telp5 joins the template region of the hTR telomerase subunit. ODNs have been tested in different concentrations (1, 5, 10, 20 micromolar). Cell viability has been tested at 48 and 72 hours. Statistical analysis and graphic design were made with the statistical package "Analyzing Data with GraphPad Prism-1999", GraphPad Sofware Inc., San Diego CA. We used the Student's t test for statistical analysis. RESULTS: The lowest dose (1 microM) was not effective. Using the highest dose (20 microM for 48 hours) of combined AS-KRAS and Telp5 cell viability decreased to 99.67%. The rest of results varied depending on ODN type, dose, and exposure time. CONCLUSIONS: Tested antisense ODNs stop colorectal cancer cell growth, and a combination of anti-telomerase and anti-k-ras is the most useful treatment. Efficacy is best with a higher dose and longer treatment period.

Lubbe, W. J., Z. Y. Zhou, et al. (2006). "Tumor epithelial cell matrix metalloproteinase 9 is a target for antimetastatic therapy in colorectal cancer." Clin Cancer Res **12**(6): 1876-82.

BACKGROUND: The current paradigm suggests that matrix metalloproteinase 9 (MMP-9) expressed by stromal cells is a therapeutic target in human colorectal tumors which presumably regulates metastatic disease progression. Conversely, whereas cancer cells within those tumors may induce stromal cells to produce MMP-9 and may be targets for MMP-9 activity, they are not the source of MMP-9 underlying metastasis. METHODS: MMP-9 expression in matched colorectal tumors and normal adjacent mucosa from patients and human colon cancer cell lines was examined by real-time reverse transcription-PCR, laser capture microdissection, immunoelectron microscopy, and immunoblot analysis. The role of colon cancer cell MMP-9 in processes underlying metastasis was explored in vitro by examining degradation of extracellular matrix components by gelatin zymography and formation of locomotory organelles by cell spreading analysis and in vivo by quantifying hematogenous tumor cell seeding of mouse lungs. RESULTS: Primary colorectal tumors overexpress MMP-9 compared with matched normal adjacent mucosa. In contrast to the current paradigm, MMP-9 is expressed equally by cancer and stromal cells within human colon tumors. Cancer cell MMP-9 regulates metastatic behavior in vitro, including degradation of extracellular matrix components and formation of locomotory organelles. Moreover, this MMP-9 critically regulates hematogenous seeding of mouse lungs by human colon cancer cells in vivo. CONCLUSIONS: These observations reveal that MMP-9 produced by human colon cancer, rather than stromal, cells is central to processes underlying metastasis. They underscore the previously unrecognized potential of specifically targeting tumor cell MMP-9 in interventional strategies to reduce mortality from metastatic colorectal cancer.

Luo, X., C. Z. Wang, et al. (2008). "Characterization of gene expression regulated by American ginseng and ginsenoside Rg3 in human colorectal cancer cells." Int J Oncol **32**(5): 975-83.

American ginseng (Panax quinquefolius L., Araliaceae) possesses anti-cancer potential and is one of the most commonly used herbal medicines in the United States. Ginsenoside Rg3, one of the saponins in American ginseng, has been shown to inhibit tumor growth. In this study, we sought to characterize the downstream genes targeted by American ginseng extracts in HCT-116 human colorectal cancer cells. We first demonstrated that the content of Rg3 in

American ginseng steamed at 120 degrees C for 2 h (referred to as S2h) was significantly increased when compared with that of the unsteamed ginseng. Both S2h and Rg3 exhibited antiproliferative effects on HCT-116 cells. Using the Affymetrix high density genechips containing more than 40,000 genes and ESTs, the gene expression profiling of HCT-116 cells were assayed. Microarray data indicated that the expression levels of 76 genes were changed significantly after treatment with S2h or Rg3, whereby it was found that 52 of the 76 genes were up-regulated while the remaining 24 were down-regulated. Ingenuity pathways analysis of top functions affected by both S2h and Rg3 were carried out. The most effected pathway is the Ephrin receptor pathway. To validate the microarray data, quantitative real-time PCR of six candidate target genes was conducted, whereby it was found that three genes were up-regulated (AKAPA8L, PMPCB and PDE5A) and three were down-regulated (PITPNA, DUS2L and RIC8A). Although further studies are needed to elucidate the mechanisms of action, our findings should expand the understanding of the molecular framework of American ginseng as an anti-cancer agent.

Lv, W., C. Zhang, et al. (2007). "RNAi-mediated gene silencing of vascular endothelial growth factor inhibits growth of colorectal cancer." Cancer Biother Radiopharm **22**(6): 841-52.

Vascular endothelial growth factor (VEGF) is overexpressed in colorectal cancer (CRCs) cells and plays a critical role in angiopoiesis and cell proliferation, making it a potential target for cancer therapy. We developed a system that blocks VEGF in the human colorectal cancer cell line, HCT116, using RNA interference. By transfecting CRCs with the small interfering RNA (siRNA) that targets human VEGF, we were able to establish a stable clones in which VEGF expression was significantly downregulated ($p<0.01$). This resulted in the decreased proliferation of HCT116 cells in vitro and suppressed the size of subcutaneous (s.c.) tumors and the microvessel density in an HCT116 s.c. nude mouse xenograft model in vivo ($p<0.01$). These results suggest that a strategy based on siRNA targeting of VEGF may build the foundation to the clinical management of CRC.

Ma, Y. L., J. Y. Peng, et al. (2009). "Heterogeneous nuclear ribonucleoprotein A1 is identified as a potential biomarker for colorectal cancer based on differential proteomics technology." J Proteome Res **8**(10): 4525-35.

Colorectal cancer (CRC) is the third most common cancer worldwide and has poor prognosis. To identify the proteins involved in colorectal carcinogenesis, we employed 2-DE and MALDI-TOF/TOF-based proteomics approach to study the differentially expressed proteins in tumor and adjacent nontumor tissue samples. Samples from 10 colorectal patients were analyzed. Of the 7 significantly and consistently altered proteins identified, hnRNP A1 was one of the most significantly altered proteins and its overexpression was confirmed using RT-PCR and Western blot analyses. Immunohistochemical examination showed that the enhanced expression of hnRNP A1 was correlated with the increasing severity of colorectal tissue and the progression of the colorectal cancer, as well as UICC (International Union against Cancer) staging, histo-differentiation, recurrence and decreased survival. By developing a highly sensitive immunoassay, hnRNP A1 could be detected in human serum and was significantly elevated in CRC patients compared with healthy volunteers. We proposed that hnRNP A1 could be considered as a novel serum tumor marker for CRC that may have significance in the detection and in the management of patients with this disease. Knockdown of hnRNP A1 expression by RNA interference led to the significant suppression of the cell growth in colorectal cancer SW480 cells in vitro. These data suggested that hnRNP A1 may be a potential biomarker for early diagnosis, prognosis, and monitoring in the therapy of colorectal cancer. Further studies are needed to fully assess the potential clinical value of this biomarker candidate.

Majumdar, A. P., U. Kodali, et al. (2004). "Chemopreventive role of folic acid in colorectal cancer." Front Biosci **9**: 2725-32.

Mortality from colorectal cancer, a leading cause of death in the U.S.A. and other western countries, has remained unchanged over the past 45 years. Therefore, the search for strategies to prevent the development and progression of colorectal cancer has markedly intensified. Chemoprevention is one such strategy. Accumulating evidence suggests that folic acid, a water soluble vitamin, could be an effective chemopreventive agent for colorectal cancer. Results from several studies have demonstrated that a diet deficient in folic acid may be associated with an increased risk of colonic neoplasia, whereas dietary supplementation of this nutrient may be chemopreventive. Although the mechanisms by which folic acid exerts its chemopreventive role in colorectal carcinogenesis remain to be fully elucidated, supplemental folic acid has been shown to arrest the loss of heterozygosity (LOH) of the tumor suppressor gene DCC (deleted in colorectal cancer) and to stabilize its protein in normal appearing rectal mucosa of patients with colorectal adenomas. Data from in

vitro studies utilizing colon cancer cell lines suggest that supplemental folic acid or its metabolite 5-methyltetrahydrofolate (5-MTF) attenuates the expression and activation of EGF-receptor (EGFR) as well as proliferation of cells. The folic acid mediated reduction of EGFR function could partly be the result of suppression of EGFR gene through increased methylation of CpG sequences within its promoter.

Martinez-Balibrea, E., C. Plasencia, et al. (2009). "A proteomic approach links decreased pyruvate kinase M2 expression to oxaliplatin resistance in patients with colorectal cancer and in human cell lines." Mol Cancer Ther **8**(4): 771-8.

We aimed to gain further understanding of the molecular mechanisms involved in oxaliplatin resistance in colorectal cancer by using a proteomic approach. A 5-fold oxaliplatin-resistant cell line, HTOXAR3, was compared with its parental cell line, HT29, using two-dimensional PAGE. Mass spectrometry, Western blot, and real-time quantitative PCR confirmed the down-regulation of pyruvate kinase M2 (PK-M2) in HTOXAR3 cells. In a panel of eight colorectal cancer cell lines, we found a negative correlation between oxaliplatin resistance and PK-M2 mRNA levels (Spearman r=-0.846, P=0.008). Oxaliplatin exposure in both HT29 and HTOXAR3 led to PK-M2 mRNA up-regulation. PK-M2 mRNA levels were measured by real-time quantitative PCR in 41 tumors treated with oxaliplatin/5-fluorouracil. Tumors with the lowest PK-M2 levels attained the lowest response rates (20% versus 64.5%, P=0.026). High PK-M2 levels were associated with high p53 levels (P=0.032). In conclusion, the data provided clearly link PK-M2 expression and oxaliplatin resistance mechanisms and further implicate PK-M2 as a predictive marker of response in patients with oxaliplatin-treated colorectal cancer.

Matos, P., C. Oliveira, et al. (2008). "B-Raf(V600E) cooperates with alternative spliced Rac1b to sustain colorectal cancer cell survival." Gastroenterology **135**(3): 899-906.

BACKGROUND & AIMS: In colorectal tumors, activating BRAF mutations occur alternative to KRAS oncogenic mutations, but in cell culture possess a much lower transforming capacity. Rac1b, a hyperactive Rac1 spliced variant, is over expressed in some colorectal tumors and activates the transcription factor nuclear factor-kappaB, which initiates a transcriptional response that promotes cell cycle progression and inhibits apoptosis. The aim of this study was to determine whether Rac1b overexpression is associated with B-Raf(V600E) in primary colorectal tumors and whether a functional cooperation between these 2 proteins exists in colorectal cells with a wild-type KRAS genotype. METHODS: Screening of BRAF and KRAS mutations by direct sequencing and Rac1b mRNA expression analysis by quantitative real-time polymerase chain reaction were conducted in 74 samples (13 normal colonic mucosa, 45 primary colorectal tumors, and 16 colorectal cancer [CRC] cell lines). RNA interference and focus formation assays were used to assess the cooperation between Rac1b and B-Raf(V600E) in cancer cell viability. RESULTS: Rac1b overexpression and B-Raf(V600E) are significantly associated in primary colorectal tumors (P = .008) and colorectal cell lines. The simultaneous suppression of both proteins dramatically decreased CRC cell viability through impaired cell-cycle progression and increased apoptosis. CONCLUSIONS: Our data demonstrate that Rac1b and B-Raf(V600E) functionally cooperate to sustain colorectal cell viability and suggest they constitute an alternative survival pathway to oncogenic K-Ras. These results reveal a novel molecular characteristic of colon tumors containing B-Raf mutations and should help in defining novel targets for cancer therapy.

Matsuyama, R., S. Togo, et al. (2006). "Predicting 5-fluorouracil chemosensitivity of liver metastases from colorectal cancer using primary tumor specimens: three-gene expression model predicts clinical response." Int J Cancer **119**(2): 406-13.

We identified genes related to 5-fluorouracil (5-FU) sensitivity in colorectal cancer and utilized these genes for predicting the 5-FU sensitivity of liver metastases. Eighty-one candidate genes involved in 5-FU resistance in gastric and colon cancer cell lines were previously identified using a cDNA microarray. In this study, the mRNA expression levels of these 81 selected genes and the genes of 5-FU-related enzymes, including thymidylate synthase (TS), dihydropyrimidine dehydrogenase (DPD) and orotate phosphoribosyltransferase (OPRT), were measured using real-time quantitative RT-PCR assays of surgically resected materials from primary colorectal tumors in 22 patients. Clinical responses were estimated by evaluating the effects of 5-FU-based hepatic artery injection (HAI) chemotherapy for synchronous liver metastases. Four genes (TNFRSF1B, SLC35F5, NAG-1 and OPRT) had significantly different expression profiles in 5-FU-nonresponding and responding tumors ($p < 0.05$). A "Response Index" system using three genes (TNFRSF1B, SLC35F5 and OPRT) was then developed using a discriminate analysis; the results were well correlated with the individual chemosensitivities. Among the 11 cases with positive scores in our response index, 9 achieved a reduction in their liver metastases after 5-FU-based chemotherapy,

whereas only 1 of the 11 cases with negative scores responded well to chemotherapy. Our "Response Index" system, consisting of TNFRSF1B, SLC35F5 and OPRT, has great potential for predicting the efficacy of 5-FU-based chemotherapy against liver metastases from colorectal cancer.

Maurer, G. D., J. H. Leupold, et al. (2007). "Analysis of specific transcriptional regulators as early predictors of independent prognostic relevance in resected colorectal cancer." Clin Cancer Res **13**(4): 1123-32.

PURPOSE: Prognostic studies on transcription factors acting at specific promoter elements have never been done so far. However, in tumors with long necessary follow-up, such as colorectal cancer, early-risk predictors would be needed. The invasion-related gene u-PAR is regulated via an activator protein 2 (AP-2)/Sp1 (-152/-135) and an AP-1 binding promoter motif (-190/-171), mediating u-PAR induction by K-Ras and Src. The present study was done to give first evidence for early prognostic relevance of transcription factors differentially bound to the u-PAR promoter, and their molecular inducers, in colorectal cancer. EXPERIMENTAL DESIGN: Tumor/normal tissues of 92 prospectively followed (median = 26.3 months) patients were analyzed for Src activity/protein, K-ras mutations, and transcription factor binding to both u-PAR promoter motifs (in vivo gel shift, kinase assay, and PCR). RESULTS: Kaplan-Meier/Mantel-Cox analysis showed a significant correlation among elevated Sp1/Sp3 binding to region -152/-135 ($P = 0.002$ and $P = 0.006$), the combinations of Sp1/AP-2 and Sp1/AP-1 binding to both motifs ($P = 0.010$ and $P = 0.005$), and Sp1 binding/high Src protein in tumors ($P < 0.001$), with poor survival. Survival decreased with the number of bound transcription factors to both motifs, with binding of three factors defining a high-risk group ($P = 0.021$). In multivariate analysis, elevated Sp1 binding, combinations of Sp1/AP-2 binding and Sp1/AP-1 binding, or Sp1 binding/high Src were independent prognostic variables; u-PAR expression itself being not yet prognostic. A first molecular staging model (CART) was defined, providing novel early high-risk groups (mean survival time as low as for non-curatively resected patients) from these variables. CONCLUSIONS: This study defines transcription factors acting at specific promoter elements of an invasion-related gene, mediating specific signaling, as novel, independent, early predictors of prognosis in colorectal cancer.

McCarty, M. F., R. J. Somcio, et al. (2007). "Overexpression of PDGF-BB decreases colorectal and pancreatic cancer growth by increasing tumor pericyte content." J Clin Invest **117**(8): 2114-22.

We hypothesized that overexpression of PDGF-BB in colorectal cancer (CRC) and pancreatic cancer cells would result in increased pericyte coverage of ECs in vivo, rendering the tumor vasculature more resistant to antiangiogenic therapy. We stably transfected the cDNA for the PDGF-B into HT-29 human CRC and FG human pancreatic cancer cells. Surprisingly, when HT-29 or FG parental and transfected cells were injected into mice (subcutaneously and orthotopically), we observed marked inhibition of tumor growth in the PDGF-BB-overexpressing clones. In the PDGF-BB-overexpressing tumors, we observed an increase in pericyte coverage of ECs. Treatment of PDGF-BB-overexpressing tumors with imatinib mesylate (PDGFR inhibitor) resulted in increased growth and decreased total pericyte content compared with those in untreated PDGF-BB-overexpressing tumors. In vitro studies demonstrated the ability of VSMCs to inhibit EC proliferation by approximately 50%. These data show that increasing the pericyte content of the tumor microenvironment inhibits the growth of angiogenesis-dependent tumors. Single-agent therapy targeting PDGF receptor must be used with caution in tumors when PDGFR is not the target on the tumor cell itself.

McDermott, U., D. B. Longley, et al. (2005). "Effect of p53 status and STAT1 on chemotherapy-induced, Fas-mediated apoptosis in colorectal cancer." Cancer Res **65**(19): 8951-60.

We investigated the role of p53 and the signal transducer and activator of transcription 1 (STAT1) in regulating Fas-mediated apoptosis in response to chemotherapies used to treat colorectal cancer. We found that 5-fluorouracil (5-FU) and oxaliplatin only sensitized p53 wild-type (WT) colorectal cancer cell lines to Fas-mediated apoptosis. In contrast, irinotecan (CPT-11) and tomudex sensitized p53 WT, mutant, and null cells to Fas-mediated cell death. Furthermore, CPT-11 and tomudex, but not 5-FU or oxaliplatin, up-regulated Fas cell surface expression in a p53-independent manner. In addition, increased Fas cell surface expression in p53 mutant and null cell lines in response to CPT-11 and tomudex was accompanied by only a slight increase in total Fas mRNA and protein expression, suggesting that these agents trigger p53-independent trafficking of Fas to the plasma membrane. Treatment with CPT-11 or tomudex induced STAT1 phosphorylation (Ser727) in the p53-null HCT116 cell line but not the p53 WT cell line. Furthermore, STAT1-targeted small interfering RNA (siRNA) inhibited up-regulation of Fas cell surface

expression in response to CPT-11 and tomudex in these cells. However, we found no evidence of altered Fas gene expression following siRNA-mediated down-regulation of STAT1 in drug-treated cells. This suggests that STAT1 regulates expression of gene(s) involved in cell surface trafficking of Fas in response to CPT-11 or tomudex. We conclude that CPT-11 and tomudex may be more effective than 5-FU and oxaliplatin in the treatment of p53 mutant colorectal cancer tumors by sensitizing them to Fas-mediated apoptosis in a STAT1-dependent manner.

Melotte, V., M. H. Lentjes, et al. (2009). "N-Myc downstream-regulated gene 4 (NDRG4): a candidate tumor suppressor gene and potential biomarker for colorectal cancer." J Natl Cancer Inst **101**(13): 916-27.

BACKGROUND: Identification of hypermethylated tumor suppressor genes in body fluids is an appealing strategy for the noninvasive detection of colorectal cancer. Here we examined the role of N-Myc downstream-regulated gene 4 (NDRG4) as a novel tumor suppressor and biomarker in colorectal cancer. METHODS: NDRG4 promoter methylation was analyzed in human colorectal cancer cell lines, colorectal tissue, and noncancerous colon mucosa by using methylation-specific polymerase chain reaction (PCR) and bisulfite sequencing. NDRG4 mRNA and protein expression were studied using real-time-PCR and immunohistochemistry, respectively. Tumor suppressor functions of NDRG4 were examined by colony formation, cell proliferation, and migration and invasion assays in colorectal cancer cell lines that were stably transfected with an NDRG4 expression construct. Quantitative methylation-specific PCR was used to examine the utility of NDRG4 promoter methylation as a biomarker in fecal DNA from 75 colorectal cancer patients and 75 control subjects. All P values are two-sided. RESULTS: The prevalence of NDRG4 promoter methylation in two independent series of colorectal cancers was 86% (71/83) and 70% (128/184) compared with 4% (2/48) in noncancerous colon mucosa (P < .001). NDRG4 mRNA and protein expression were decreased in colorectal cancer tissue compared with noncancerous colon mucosa. NDRG4 overexpression in colorectal cancer cell lines suppressed colony formation (P = .014), cell proliferation (P < .001), and invasion (P < .001). NDRG4 promoter methylation analysis in fecal DNA from a training set of colorectal cancer patients and control subjects yielded a sensitivity of 61% (95% confidence interval [CI] = 43% to 79%) and a specificity of 93% (95% CI = 90% to 97%). An independent test set of colorectal cancer patients and control subjects yielded a sensitivity of 53% (95% CI = 39% to 67%) and a specificity of 100% (95% CI = 86% to 100%). CONCLUSIONS: NDRG4 is a candidate tumor suppressor gene in colorectal cancer whose expression is frequently inactivated by promoter methylation. NDRG4 promoter methylation is a potential biomarker for the noninvasive detection of colorectal cancer in stool samples.

Meynard, D., V. Le Morvan, et al. (2007). "Functional analysis of the gene expression profiles of colorectal cancer cell lines in relation to oxaliplatin and cisplatin cytotoxicity." Oncol Rep **17**(5): 1213-21.

The objective was to relate the gene expression profiles of colorectal cancer cells in culture to the in vitro cytotoxicity of cisplatin and oxaliplatin. We studied the gene expression profiles of six human colorectal cancer cell lines, using the Atlas Plastic Human 8K Microarray from Clontech, and related it to the in vitro cytotoxicities of oxaliplatin and cisplatin obtained by inhibition of exponential growth of cells. We calculated the Pearson's coefficients of correlation (r) between gene expression and drug IC50. A functional analysis was performed using the Gene Ontology Consortium database. Results were validated on a series of representative genes by real-time quantitative PCR. Validation of the significance of the coefficients of correlation was also performed using a leave-one-out analysis. We identified 394 genes whose expression was significantly correlated ($P<0.05$) to oxaliplatin cytotoxicity and 40 with cisplatin cytotoxicity. Three major functions were preferentially involved in oxaliplatin activity: protein synthesis, cell energetics and response to oxidative stress. No significant correlation was observed between oxaliplatin or cisplatin cytotoxicity and the expression of genes involved in DNA repair, cell proliferation or cell adhesion. A strongly significant correlation was found between the microarray and the rt-PCR approaches ($r=0.968$, $P<10(-6)$). The leave-one-out analysis showed that the same functions still appeared significantly involved in the activity of both drugs. Based on the functional analysis, we hypothesized that oxaliplatin would specifically form protein adducts during synthesis, thus exposing their thiol groups, which are known to be especially vulnerable to reactive oxygen species.

Mhaidat, N. M., F. Q. Alali, et al. (2009). "Inhibition of MEK sensitizes paclitaxel-induced apoptosis of human colorectal cancer cells by downregulation of GRP78." Anticancer Drugs **20**(7): 601-6.

Here we report that paclitaxel induces variable degrees of apoptosis in human colorectal cancer cells. Paclitaxel induces multiple arms of the endoplasmic reticulum stress response, including upregulation of the 78-kDa glucose-regulatory protein (GRP78) and eukaryotic initiation factor alpha

phosphorylation. Inhibition of the MEK/ERK pathway sensitized colorectal cancer cells to paclitaxel-induced apoptosis. A similar result was obtained by the inhibition of GRP78 using small interfering RNA molecules. Knockdown of MEK resulted in a significant downregulation of paclitaxel-induced upregulation of GRP78 indicating that activation of GRP78 is a downstream event of MEK/ERK pathway activation. These results indicate that GRP78 might be a novel mechanism underlying the resistance of colorectal cancer cells to microtubule-targeting drugs. A combination of compounds capable of suppressing GRP78 might be a golden approach for improving the effectiveness of taxanes.

Mingxin, Z., L. Yan, et al. (2008). "The antitumor activity of meisoindigo against human colorectal cancer HT-29 cells in vitro and in vivo." J Chemother **20**(6): 728-33.

The study was conducted to examine the antitumor activity of meisoindigo on HT-29 cells in vitro and in vivo. The cytotoxicity of meisoindigo was evaluated by MTT assay. The related genes and proteins were inspected with RT-PCR and western blot assay respectively, and the effects of meisoindigo on the cell cycle were analyzed by flow cytometry. The efficacy of meisoindigo in vivo was evaluated in an HT-29 cell xenograft nude mice model. The results show that meisoindigo effectively inhibits HT-29 cell proliferation (IC(50) 4.3 mmol/L), arrests HT-29 cells in G2/ M phase and induces HT-29 cell apoptosis. The downstream genes and proteins of GSK-3beta(ser(9)) expression level decrease. Meisoindigo significantly inhibits the HT-29 xenograft tumors growth at the dose of 100 mg/kg. The mechanism of meisoindigo activity against HT-29 cells may be related to its inhibition of glycogen synthase kinase-3beta, GSK-3beta(ser(9)) phosphorylation in Wnt signaling pathway. These findings indicate the potential value of meisoindigo for the treatment of colorectal cancer.

Mohr, A., G. Henderson, et al. (2004). "AAV-encoded expression of TRAIL in experimental human colorectal cancer leads to tumor regression." Gene Ther **11**(6): 534-43.

Gene transfer vectors based on the adeno-associated virus (AAV) are used for various experimental and clinical therapeutic approaches. In the present study, we demonstrate the utility of rAAV as a tumoricidal agent in human colorectal cancer. We constructed an rAAV vector that expresses tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL/Apo2L) and used it to transduce human colorectal cancer cells. TRAIL belongs to the TNF superfamily of cytokines that are involved in various immune responses and apoptotic processes. It has been shown to induce cell death specifically in cancer cells. Transduction with AAV.TRAIL gave rise to rapid expression of TRAIL, followed by induction of apoptosis, which could be inhibited by the caspase inhibitor z-VAD.fmk, in several human colon cancer cell lines. The apoptotic mechanism included activation of caspase-3, as well as cytochrome c release from mitochondria. The outgrowth of human colorectal tumors grown in mice was completely blocked by transduction with AAV.TRAIL in vitro, while in vivo transduction significantly inhibited the growth of established tumors. AAV vectors could provide a safe method of gene delivery and offer a novel method of using TRAIL as a therapeutic protein.

Moore, A. E., A. Greenhough, et al. (2009). "HGF/Met signalling promotes PGE(2) biogenesis via regulation of COX-2 and 15-PGDH expression in colorectal cancer cells." Carcinogenesis **30**(10): 1796-804.

Evidence points towards a pivotal role for cyclooxygenase (COX)-2 in promoting colorectal tumorigenesis through increasing prostaglandin E(2) (PGE(2)) levels. PGE(2) signalling is closely associated with the survival, proliferation and invasion of colorectal cancer cells. Recently, a reduction in PGE(2) inactivation, a process mediated by the nicotinamide adenine dinucleotide (NAD+)-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH), has also been shown to promote tumoral PGE(2) accumulation. The hepatocyte growth factor (HGF) receptor, Met, is frequently over-expressed in colorectal tumours and promotes cancer growth, metastasis and resistance to therapy, although the mechanisms for this have not been fully elucidated. Here, we report that HGF/Met signalling can promote PGE(2) biogenesis in colorectal cancer cells via COX-2 up-regulation and 15-PGDH down-regulation at the protein and messenger RNA level. Pharmacological inhibition of MEK and PI3K suggested that both extracellular signal-regulated kinase (ERK) and AKT signalling are required for COX-2 protein up-regulation and 15-PGDH down-regulation downstream of Met. Notably, inhibition of Met with the small molecule inhibitor SU11274 reduced COX-2 expression and increased 15-PGDH expression in high Met-expressing cells. We also show that hypoxia potentiated HGF-driven COX-2 expression and enhanced PGE(2) release. Furthermore, inhibition of COX-2 impeded the growth-promoting effects of HGF, suggesting that the COX-2/PGE(2) pathway is an important mediator of HGF/Met signalling. These data reveal a critical role for HGF/Met signalling in promoting PGE(2) biogenesis in colorectal cancer cells. Targeting the crosstalk between these two

important pathways may be useful for therapeutic treatment of colorectal cancer.

Murai, M., M. Toyota, et al. (2005). "Aberrant methylation and silencing of the BNIP3 gene in colorectal and gastric cancer." Clin Cancer Res **11**(3): 1021-7.

BNIP3 protein is a proapoptotic member of the Bcl-2 family that is expressed in hypoxic regions of tumors. To examine its role in the progression of gastrointestinal cancer, we examined the expression and DNA methylation status of BNIP3 gene in a panel of colorectal and gastric cancer cell lines. BNIP3 was not expressed in 14 of the 24 cell lines tested, and its absence was not caused by gene mutation or by altered expression of hypoxia inducible factor-1, a key transcription factor that regulates BNIP3 expression. On the other hand, methylation of the 5' CpG island of BNIP3 was closely correlated with silencing the gene. Moreover, treating methylated cells with the methyltransferase inhibitor 5-aza-2'-deoxycytidine restored hypoxia-induced expression of BNIP3 mRNA and protein, which in turn led to cell death. Aberrant methylation of BNIP3 was also detected in 66% of primary colorectal and 49% of primary gastric cancers, but not in normal tissue samples collected from areas adjacent to the tumors. Apparently, epigenetic alteration of BNIP3 is a frequent and cancer-specific event, which suggests that inactivation of BNIP3 likely plays a key role in the progression of some gastrointestinal cancers and that it may be a useful molecular target for therapy.

Ng, E. K., W. P. Tsang, et al. (2009). "MicroRNA-143 targets DNA methyltransferases 3A in colorectal cancer." Br J Cancer **101**(4): 699-706.

BACKGROUND: MicroRNAs (miRNAs) are 19-25-nucleotides regulatory non-protein-coding RNA molecules that regulate the expressions of a wide variety of genes, including some involved in cancer development. In this study, we investigated the possible role of miR-143 in colorectal cancer (CRC). METHODS: Expression levels of human mature miRNAs were examined using real-time PCR-based expression arrays on paired colorectal carcinomas and adjacent non-cancerous colonic tissues. The downregulation of miR-143 was further evaluated in colon cancer cell lines and in paired CRC and adjacent non-cancerous colonic tissues by qRT-PCR. Potential targets of miR-143 were defined. The functional effect of miR-143 and its targets was investigated in human colon cancer cell lines to confirm miRNA-target association. RESULTS: Both real-time PCR-based expression arrays and qRT-PCR showed that miR-143 was frequently downregulated in 87.5% (35 of 40) of colorectal carcinoma tissues compared with their adjacent non-cancerous colonic tissues. Using in silico predictions, DNA methyltranferase 3A (DNMT3A) was defined as a potential target of miR-143. Restoration of the miR-143 expression in colon cell lines decreased tumour cell growth and soft-agar colony formation, and downregulated the DNMT3A expression in both mRNA and protein levels. DNMT3A was shown to be a direct target of miR-143 by luciferase reporter assay. Furthermore, the miR-143 expression was observed to be inversely correlated with DNMT3A mRNA and protein expression in CRC tissues. CONCLUSION: Our findings suggest that miR-143 regulates DNMT3A in CRC. These findings elucidated a tumour-suppressive role of miR-143 in the epigenetic aberration of CRC, providing a potential development of miRNA-based targeted approaches for CRC therapy.

Nibbe, R. K. and M. R. Chance (2009). "Approaches to biomarkers in human colorectal cancer: looking back, to go forward." Biomark Med **3**(4): 385-396.

Like all human cancers, colorectal cancer is a complicated disease. While a mature body of research involving colorectal cancer has implicated the putative sequence of genetic alterations that trigger the disease and sustain its progression, there is a surprising paucity of well-validated, clinically useful diagnostic markers of this disease. For prognosis or guiding therapy, single gene-based markers of colorectal cancer often have limited specificity and sensitivity. Genome-wide analyses (microarrays) have been used to propose candidate patterns of gene expression that are prognostic of outcome or predict the tumor's response to a therapy regimen; however, these patterns frequently do not overlap, and this has raised questions concerning their use as biomarkers. The limitation of gene-expression approaches to marker discovery occurs because the change in mRNA expression across tumors is highly variable and, alone, accounts for a limited variability of the phenotype, such as with cancer. More robust and accurate markers of cancer will result from integrating all the information we have about the cell: genomics, proteomics and interactomics. This article will discuss traditional markers in colorectal cancer, both genomic and proteomic, including their respective approaches and limitations, then conclude with examples of systems biology-based approaches for candidate marker discovery, and discuss how this approach is reshaping our view of a biomarker.

Nozawa, T., T. Enomoto, et al. (2004). "Specific enhanced expression of platelet-derived endothelial cell growth factor in submucosa of human colorectal cancer." Dis Colon Rectum **47**(12): 2093-100.

PURPOSE: Platelet-derived endothelial cell growth factor, identified to be an angiogenic factor, has been implicated in metastases of colorectal cancer. This study aimed to clarify the role and localization of platelet-derived endothelial cell growth factor associated with human colorectal cancer invasion. METHODS: Thirty-two patients with colorectal cancer who had undergone surgery were analyzed. Platelet-derived endothelial cell growth factor enzyme activities in the colorectal cancer specimens were measured. Cells that expressed platelet-derived endothelial cell growth factor were identified and localized by immunohistochemical analysis with anti-human platelet-derived endothelial cell growth factor antibody and by in situ hybridization with specific RNA probe. RESULTS: Platelet-derived endothelial cell growth factor enzyme activity increased significantly in cancer tissues compared with normal colonic mucosa at various distances from the cancer. Immunohistochemical analysis and in situ hybridization demonstrated platelet-derived endothelial cell growth factor expression in stromal macrophages and fibroblasts located in cancer tissues and surrounding noncancerous tissues, although the tumor cells and normal colonic mucosa were negative. The value of platelet-derived endothelial cell growth factor expression was highest at the border of the colorectal cancer (35.3 +/- 8.9 percent), followed by the cancer nest (15.2 +/- 9.2 percent) and normal mucosa (7.7 +/- 3.4 percent). In the border area, the highest value of platelet-derived endothelial cell growth factor expression was observed in the submucosa (35.3 +/- 8.9 percent), followed by the muscular propria (21.9 +/- 7.7 percent) and the subserosa (14.9 +/- 5.5 percent). CONCLUSIONS: Stromal macrophages and fibroblasts are responsible for elevated platelet-derived endothelial cell growth factor activity in colorectal cancer. The significance of enhanced expression of platelet-derived endothelial cell growth factor in the submucosa at the cancer border remains unclear. Cancer stroma may be an important factor for cancer angiogenesis and may serve as a treatment target through specific modulation of angiogenic factors.

Ogawa, K., T. Utsunomiya, et al. (2005). "Genomic screens for genes upregulated by demethylation in colorectal cancer: possible usefulness for clinical application." Int J Oncol **27**(2): 417-26.

Inactivation of tumor suppressor genes may result in clinically aggressive tumors and poor prognoses for cancer patients. This study was conducted to investigate the clinical significance of genomic screens for genes upregulated by demethylation in colorectal cancer. We performed a comprehensive survey of commonly inactivated genes in colorectal cancer through the functional reactivation of epigenetically silenced genes by 5-Azacytidine, using cDNA microarrays containing 12,814 genes. We then investigated the clinical significance of the identified gene in colorectal cancer patients. Among 41 candidate genes identified by this microarray analysis, 31 (76%) harbored CpG islands, and many of the genes were associated with cancer-testis antigens, Wnt inhibitors, growth factors, and cell cycle regulators. Subsequent analysis by quantitative RT-PCR confirmed the reliability of our microarray strategy. In order to elucidate the potential clinical significance of these identified genes, we selected one of these genes, apolipoprotein D (apo D), and investigated its mRNA expression in 63 colorectal cancer patients using quantitative real-time RT-PCR. The mean expression level of Apo D mRNA was significantly lower in cancerous tissues than in non-cancerous tissues ($p < 0.01$), and a lower expression of Apo D was significantly correlated with lymph node metastasis ($p < 0.05$), advanced stages ($p < 0.05$) and poorer overall survival ($p < 0.05$). These results indicated that a genomic screen for genes upregulated by demethylation may be a useful approach for the identification of genes that are of clinical significance in colorectal cancer patients.

Ogino, S., J. A. Meyerhardt, et al. (2005). "Molecular alterations in tumors and response to combination chemotherapy with gefitinib for advanced colorectal cancer." Clin Cancer Res **11**(18): 6650-6.

PURPOSE: Recently, activating mutations of the epidermal growth factor receptor (EGFR) gene were discovered in non-small cell lung cancers sensitive to gefitinib (ZD1839, an EGFR tyrosine kinase inhibitor) but not in gefitinib-resistant cancers. Abnormalities of EGFR and related pathways may have an effect on responsiveness of advanced colorectal cancer to combination chemotherapy with gefitinib. EXPERIMENTAL DESIGN: We examined patients with previously untreated metastatic colorectal cancer, who were enrolled into two phase I/II trials of combination chemotherapy (irinotecan, leucovorin, and 5-fluorouracil) and daily oral gefitinib. We obtained paraffin tissue blocks of primary tumors from 31 patients, sequenced the EGFR, KRAS, and BRAF genes, and did immunohistochemistry for EGFR, phosphorylated AKT1, p53, p21, and p27. RESULTS: Twelve (39%) of the 31 patients experienced a partial objective response to the therapy. A novel EGFR mutation in exon 18 (c.2170G>A, p.Gly724Ser) was identified in only one patient who did not experience an objective tumor response. EGFR immunohistochemistry was not predictive of responsiveness. In contrast, loss of p21 was associated with a higher response rate to

therapy (P = 0.05). Moreover, the response rate among patients whose tumors maintained p21 expression and possessed a mutation in p53 was only 9% (1 of 11, P = 0.005). Overexpression of phosphorylated AKT1 also seemed to predict a trend towards resistance to the therapy. CONCLUSIONS: p21 expression in colorectal cancer, especially in combination with p53 mutation, is a predictor of resistance to the combination chemotherapy with gefitinib. Activating EGFR mutations are rare in colorectal cancer and do not seem to confer sensitivity to gefitinib and chemotherapy.

Ohmachi, T., F. Tanaka, et al. (2006). "Clinical significance of TROP2 expression in colorectal cancer." Clin Cancer Res **12**(10): 3057-63.

PURPOSE AND EXPERIMENTAL DESIGN: To identify cancer-related genes, the expression profiles of colorectal cancer cells and normal epithelial cells were examined and compared using laser microdissection and cDNA microarray analysis. From these combined techniques, several cancer-related genes, including TROP2, were identified. TROP2 is known as a calcium signal transducer and is highly expressed in several types of tumors. However, no studies have investigated the significance of TROP2 expression in colorectal cancer. Thus, the expression status of TROP2 was investigated in 74 colorectal cancer samples by quantitative real-time reverse transcription-PCR and immunohistochemical studies. RESULTS: Laser microdissection and cDNA microarray analysis showed that there were 84 overexpressed genes in cancer cells. One of the highly overexpressed genes was TROP2. Quantitative real-time reverse transcription-PCR showed that TROP2 expression in cancer samples was significantly higher than in normal samples (P < 0.001). The samples were divided into high (n = 26) and low (n = 48) TROP2 expression groups. The cases with high TROP2 expression showed a higher frequency of liver metastasis (P = 0.005) and more cancer-related death (P = 0.046). Those cases also had an inclination of deeper depth of invasion (P = 0.064) and more lymph node metastasis (P = 0.125). Interestingly, the patients with high TROP2 expression tumors had poorer prognosis (P = 0.0036). Multivariate analysis showed that TROP2 expression status was an independent prognostic factor (relative risk, 2.38; 95% confidence interval, 1.29-4.74; P < 0.01). CONCLUSION: TROP2 is one of the cancer-related genes that correlates with biological aggressiveness and poor prognosis of colorectal cancer. Thus, TROP2 is a possible candidate gene for diagnosis and molecular target therapy of colorectal cancer.

Ohta, M., F. Tanaka, et al. (2005). "The high expression of Fractalkine results in a better prognosis for colorectal cancer patients." Int J Oncol **26**(1): 41-7.

Local and systemic immune responses are impaired in patients with colorectal cancer (CRC) and it is known that the number of tumor infiltrating lymphocytes (TILs) is considerably few. On the other hand, some CRC cases in which many TIL were observed, survived longer than those cases with a small number of TIL. Considerable attention has been recently paid to the relationship between chemokines and tumor cells. Some chemokines recruit lymphocytes for tumor lesions. We made a hypothesis that Fractalkine, a CX3C chemokine, would recruit lymphocytes in the CRC and play an important role in anti-tumor immunity. We analyzed the expression level of Fractalkine in CRC cell lines as well as in clinical samples (n=80). The expression level of Fractalkine was thus found to correlate with the density of TIL ($p<0.05$). The CRC cases with a strong Fractalkine expression (n=50) showed a significantly better prognosis than those with a weak expression (n=30) ($p<0.05$). In addition, the Fractalkine expression was found to be an independent prognostic factor ($p<0.05$). We furthermore clarified that some of the tumor-infiltrating cells were natural killer cells and cytotoxic T cells expressed Fractalkine receptor. These data suggest that Fractalkine expressed in the tumor appears to recruit cytotoxic T cells and NK cells to the tumor site and these cytotoxic cells result in a better prognosis mediated by tumor cell cytotoxicity using a perforin and granzyme B mechanism. The expression level of Fractalkine was an essential biomarker for predicting the prognosis of patients with CRC. Fractalkine is considered to be one of the biomarkers for detecting patients with a high risk for recurrence, and who might therefore benefit from additional therapeutic strategies such as adjuvant therapy.

Ohtsuka, T., X. F. Liu, et al. (2006). "Methylation-induced silencing of ASC and the effect of expressed ASC on p53-mediated chemosensitivity in colorectal cancer." Oncogene **25**(12): 1807-11.

Tumor suppressor p53 is known to play a crucial role in chemosensitivity in colorectal cancer. We previously demonstrated that an apoptosis-associated speck-like protein, ASC, is a p53-target gene which regulates p53-Bax mitochondrial apoptotic pathway. ASC is also known to be a target of methylation-induced gene silencing. An inactivation of ASC might thus cause resistance to chemotherapy, and if this is the case, then the expression of ASC would restore the chemosensitivity. The aim of this study was to clarify this hypothesis. ASC was methylated in 25% of all

resected specimens in patients with colorectal cancer; however, ASC methylation did not always correspond to a lack of ASC protein. When expressed in colon cancer cells, in which ASC is absent due to methylation, ASC was found to enhance the chemosensitivity in a p53-dependent manner. In p53-null cells, ASC increased the p53-mediated cell death induced by p53-expressing adenovirus infection. Our data suggest that the methylation-induced silencing of ASC might cause resistance to p53-mediated chemosensitivity in colorectal cancer. The gene introduction of ASC may thus restore such chemosensitivity, and this modality may therefore be a useful new treatment strategy for colorectal cancer.

Pages, F., A. Kirilovsky, et al. (2009). "In situ cytotoxic and memory T cells predict outcome in patients with early-stage colorectal cancer." J Clin Oncol **27**(35): 5944-51.

PURPOSE: Many patients who present with early-stage colorectal cancer (International Union Against Cancer TNM stages I and II) are nevertheless at high risk of relapse. We hypothesized that intratumoral immune reaction could influence their prognosis. PATIENTS AND METHODS: The intratumoral immune reaction was investigated in 29 tumors by large-scale real-time polymerase chain reaction. Cytotoxic (CD8) and memory (CD45RO) T cells were quantified by immunohistochemical analyses of tissue microarrays from the center (CT) and the invasive margin (IM) of the 602 tumors from two independent cohorts. The results were correlated with tumor recurrence and patient survival. RESULTS: Patients with a strong infiltration of CD45RO(+) cells in the tumor exhibited an increased expression of T-helper 1 and cytotoxicity-related genes. Densities of CD45RO(+) and CD8(+) cells in tumor regions (CT/IM) classified the patients into four distinct prognostic groups based on the presence of high density of each marker in each tumor region. The four groups were associated with dramatic differences in disease-free, disease-specific, and overall survival (all P < .0001). Five years after diagnosis, only 4.8% (95% CI, 0.6% to 8.8%) of patients with high densities of CD8(+) plus CD45RO(+) cells had tumor recurrence, and 86.2% (CI, 79.4% to 93.6%) survived. In contrast, the tumor recurred in 75% (95% CI, 17% to 92.5%) of patients with low densities of these cells, and only 27.5% (95% CI, 10.5% to 72%) survived (all P < .0001). Multivariate analyses showed that the immune criteria had independent effects on the rates of complete remission and survival. CONCLUSION: The combined analysis of CD8(+) plus CD45RO(+) cells in specific tumor regions could provide a useful criterion for the prediction of tumor recurrence and survival in patients with early-stage colorectal cancer.

Parkhurst, M. R., J. Joo, et al. (2009). "Characterization of genetically modified T-cell receptors that recognize the CEA:691-699 peptide in the context of HLA-A2.1 on human colorectal cancer cells." Clin Cancer Res **15**(1): 169-80.

PURPOSE: Carcinoembryonic antigen (CEA) is a tumor-associated protein expressed on a variety of adenocarcinomas. To develop an immunotherapy for patients with cancers that overexpress CEA, we isolated and genetically modified a T-cell receptors (TCRs) that specifically bound a CEA peptide on human cancer cells. EXPERIMENTAL DESIGN: HLA-A2.1 transgenic mice were immunized with CEA:691-699. A CEA-reactive TCR was isolated from splenocytes of these mice and was genetically introduced into human peripheral blood lymphocytes via RNA electroporation or retroviral transduction. Amino acid substitutions were introduced throughout the complementarity determining regions (CDR1, CDR2, and CDR3) of both TCR alpha and beta chains to improve recognition of CEA. RESULTS: Murine lymphocytes bearing the CEA-reactive TCR specifically recognized peptide-loaded T2 cells and HLA-A2.1(+) CEA(+) human colon cancer cells. Both CD8(+) and CD4(+) human lymphocytes expressing the murine TCR specifically recognized peptide-loaded T2 cells. However, only gene-modified CD8(+) lymphocytes specifically recognized HLA-A2.1(+) CEA(+) colon cancer cell lines, and tumor cell recognition was weak and variable. We identified two substitutions in the CDR3 of the alpha chain that significantly influenced tumor cell recognition by human peripheral blood lymphocytes. One substitution, T for S at position 112 (S112T), enhanced tumor cell recognition by CD8(+) lymphocytes, and a second dually substituted receptor (S112T L110F) enhanced tumor cell recognition by CD4(+) T cells. CONCLUSIONS: The modified CEA-reactive TCRs are good candidates for future gene therapy clinical trials and show the power of selected amino acid substitutions in the antigen-binding regions of the TCR to enhance desired reactivities.

Patsos, G., V. Hebbe-Viton, et al. (2009). "O-glycan inhibitors generate aryl-glycans, induce apoptosis and lead to growth inhibition in colorectal cancer cell lines." Glycobiology **19**(4): 382-98.

Our studies provide direct evidence that O-glycosylation pathways play a role in the regulation of cell growth through apoptosis and proliferation pathways. A series of small molecular weight analogs of the GalNAc-alpha-1-O-serine/threonine structure based on 1-benzyl-2-acetamido-2-deoxy-alpha-O-d-

galactopyranoside have been synthesized and tested in the human colorectal cancer cell lines PC/AA/C1/SB10C and HCA7/C29. Three inhibitors, 1-benzyl-2-acetamido-2-deoxy-alpha-O-D-galactopyranoside, and the corresponding 2-azido- and C-glycoside analogs were screened in these colorectal cancer cell lines at 0.5 mM and showed induction of apoptosis and downregulation of proliferation. Treatment of both cell lines with inhibitors led to changes in glycosylation detected with peanut lectin. The inhibition of glycosyltransferase activity in cell homogenates from human colorectal mucosal cells and cultured cell lines could be shown. The competitive action of the inhibitors resulted in the intracellular formation of 28 aryl-glycan products which were identified by MALDI and electrospray mass spectroscopy. The structures showed a differential pattern for each of the inhibitors in both cell lines. Gene array analysis of the glycogenes illustrated a pattern of glycosyltransferases that matched the glycan structures found in glycoproteins and aryl-glycans formed in the PC/AA/C1/SB10C cells; however, there was no action of the three inhibitors on glycogene transcript levels. The inhibitors act at both intermediary metabolic and genomic levels, resulting in altered protein glycosylation and aryl-glycan formation. These events may play a part in growth arrest.

Priego, S., F. Feddi, et al. (2008). "Natural polyphenols facilitate elimination of HT-29 colorectal cancer xenografts by chemoradiotherapy: a Bcl-2- and superoxide dismutase 2-dependent mechanism." Mol Cancer Ther **7**(10): 3330-42.

Colorectal cancer is one of the most common malignancies worldwide. The treatment of advanced colorectal cancer with chemotherapy and radiation has two major problems: development of tumor resistance to therapy and nonspecific toxicity towards normal tissues. Different plant-derived polyphenols show anticancer properties and are pharmacologically safe. In vitro growth of human HT-29 colorectal cancer cells is inhibited (approximately 56%) by bioavailable concentrations of trans-pterostilbene (trans-3,5-dimethoxy-4'-hydroxystilbene; t-PTER) and quercetin (3,3',4',5,6-pentahydroxyflavone; QUER), two structurally related and naturally occurring small polyphenols. I.v. administration of t-PTER and QUER (20 mg/kg x day) inhibits growth of HT-29 xenografts (approximately 51%). Combined administration of t-PTER + QUER, FOLFOX6 (oxaliplatin, leucovorin, and 5-fluorouracil; a first-line chemotherapy regimen), and radiotherapy (X-rays) eliminates HT-29 cells growing in vivo leading to long-term survival (>120 days). Gene expression analysis of a Bcl-2 family of genes and antioxidant enzymes revealed that t-PTER + QUER treatment preferentially promotes, in HT-29 cells growing in vivo, (a) superoxide dismutase 2 overexpression (approximately 5.7-fold, via specificity protein 1-dependent transcription regulation) and (b) down-regulation of bcl-2 expression (approximately 3.3-fold, via inhibition of nuclear factor-kappaB activation). Antisense oligodeoxynucleotides to human superoxide dismutase 2 and/or ectopic bcl-2 overexpression avoided polyphenols and chemoradiotherapy-induced colorectal cancer elimination and showed that the mangano-type superoxide dismutase and Bcl-2 are key targets in the molecular mechanism activated by the combined application of t-PTER and QUER.

Przybylowska, K., J. Szemraj, et al. (2008). "Antigen levels of urokinase-type plasminogen activator receptor and its gene polymorphism related to microvessel density in colorectal cancer." Acta Biochim Pol **55**(2): 357-63.

We determined the distribution of genotypes and frequencies of alleles of the (CA)(n) repeat polymorphism in intron 3 of the urokinase plasminogen activator receptor (uPAR) gene, uPAR antigen levels and microvessel density (MVD) in tumour and distant mucosa samples from 52 patients with colorectal cancer. The uPAR level was higher for patients with high MVD comparing to patients with lower MVD which may suggest that uPAR can be correlated with progression of colorectal cancer. The significant relationship between the high MVD and uPAR antigen level appeared to be independent of the (CA)(n) repeat polymorphism because no differences in the level of uPAR antigen between carriers of alleles were found. The received results, indicate that uPAR might be considered as a target in colorectal cancer patients' therapy.

Reinblatt, M., R. H. Pin, et al. (2004). "Carcinoembryonic antigen directed herpes viral oncolysis improves selectivity and activity in colorectal cancer." Surgery **136**(3): 579-84.

BACKGROUND: G207 is an oncolytic herpes virus whose replicative cycle requires cellular ribonucleotide reductase (RR) for viral DNA synthesis. We attempt to enhance viral cytotoxicity in carcinoembryonic antigen (CEA)-producing colorectal cancer (CRC) cells through CEA-driven RR production. METHODS: CEA enzyme-linked immunosorbent assay was performed on LS174T and HCT-8 human CRC cells. The CEA enhancer-promoter (CEA E-P) was functionally assessed by luciferase assay. CEA E-P was cloned upstream of UL39, the gene encoding the large subunit of RR. Cells were transfected with CEA E-P/UL39 and infected with G207 for cytotoxicity assays. LS174T,

with or without CEA E-P/UL39, were implanted into athymic mouse flanks (n = 28) and treated with G207. RESULTS: CEA levels were 7-fold higher in LS174T versus HCT-8 (P <.00001). CEA E-P increased luciferase expression 7.5-fold in LS174T (P <.01), with no increase in HCT-8. G207 cytotoxicity of'CEA E-P/UL39-transfected LS174T cells increased 69% by day 10 versus nontransfected cells (P <.001), with no significant increase in HCT-8. Combining CEA E-P/UL39 with G207 in LS174T flank tumors resulted in a 65% decrease in tumor volume versus G207, phosphate-buffered saline, or'CEA E-P/UL39 alone (P <.0001). CONCLUSIONS: CEA-driven RR production by CEA-secreting CRC cells significantly improves oncolytic viral cytotoxicity and specificity in vitro, and reduces tumor burden in vivo.

Rho, J. H., S. Qin, et al. (2008). "Proteomic expression analysis of surgical human colorectal cancer tissues: up-regulation of PSB7, PRDX1, and SRP9 and hypoxic adaptation in cancer." J Proteome Res **7**(7): 2959-72.

Colorectal adenocarcinoma is one of the worldwide leading causes of cancer deaths. Discovery of specific biomarkers for early detection of cancer progression and the identification of underlying pathogenetic mechanisms are important tasks. Global proteomic approaches have thus far been limited by the large dynamic range of molecule concentrations in tissues and the lack of selective enrichment of the low-abundance proteome. We studied paired cancerous and normal clinical tissue specimens from patients with colorectal adenocarcinomas by heparin affinity fractionation enrichment (HAFE) followed by 2-D PAGE and tandem mass spectrometric (MS/MS) identification. Fifty-six proteins were found to be differentially expressed, of which 32 low-abundance proteins were only detectable after heparin affinity enrichment. MS/MS was used to identify 5 selected differentially expressed proteins as proteasome subunit beta type 7 (PSB7), hemoglobin alpha subunit (HBA), peroxiredoxin-1 (PRDX1), argininosuccinate synthase (ASSY), and signal recognition particle 9 kDa protein (SRP9). This is the first proteomic study detecting the differential expression of these proteins in human colorectal cancer tissue. Several of the proteins are functionally related to tissue hypoxia and hypoxic adaptation. The relative specificities of PSB7, PRDX1, and SRP9 overexpression in colon cancer were investigated by Western blot analysis of patients with colon adenocarcinomas and comparison with a control cohort of patients with lung adenocarcinomas. Furthermore, immunohistochemistry on tissue sections was used to define the specific locations of PSB7, PRDX1, and SRP9 up-regulation within heterogeneous primary human tumor tissue. Overexpression of the three proteins was restricted to the neoplastic cancer cell population within the tumors, demonstrating both cytoplasmic and nuclear localization of PSB7 and predominantly cytoplasmic localization of PRDX1 and SRP9. In summary, we describe heparin affinity fractionation enrichment (HAFE) as a prefractionation tool for the study of the human primary tissue proteome and the discovery of PSB7, PRDX1, and SRP9 up-regulation as candidate biomarkers of colon cancer.

Richter, S. N., G. Cartei, et al. (2006). "In vitro basis for schedule-dependent interaction between gemcitabine and topoisomerase-targeted drugs in the treatment of colorectal cancer." Ann Oncol **17 Suppl 5**: v20-24.

BACKGROUND: While combination of gemcitabine with anti-topoisomerase poisons is routinely used in oncology, little is known on the biological interactions between these drugs. DESIGN: To understand the cellular basis for this association, we hypothesized an interaction of the two agents at the topoisomerase level. A real-time RT-PCR method was designed to quantify topoisomerase expression after treatment with gemcitabine (GEM) in two human colon adenocarcinoma cell lines. Efficacy of drugs as single agents and in combination was analyzed on the basis of their cytotoxic effects. RESULTS: We showed that a) gemcitabine induces expression of all major eukaryotic topoisomerases (I, II alpha and beta) at definite times after drug administration; b) cytotoxicity was more relevant when cells were treated with GEM and the topoisomerase poison within a short period of time. In particular synergistic effects were found when the anti-topoisomerase II agent was given 3 h after gemcitabine or when the anti-topoisomerase I drug was delivered 3 h before or after the antimetabolite. CONCLUSIONS: These findings help explaining the effectiveness of the combined therapy GEM/topoisomerase poisons and suggest a drug administration protocol for clinical treatment.

Ruan, D. T. and R. S. Warren (2005). "Liver-directed therapies in colorectal cancer." Semin Oncol **32**(1): 85-94.

The liver is the most common site of metastatic colorectal cancer (CRC) and the status of this organ is an important determinant of overall survival in patients with advanced disease. Complete resection of hepatic CRC metastases can provide a long-term cure for some patients, but the majority of liver metastases are not amenable to such surgery. Furthermore, most patients after curative resection ultimately suffer from recurrence, and the majority of such failures occur in the liver. Various ablative

techniques can achieve local control of tumor after incomplete resection or for palliation. Tumor ablation currently has a secondary therapeutic role, as there is no evidence that it can achieve long-term survival comparable to surgical resection. Regional chemotherapy delivers tumoricidal agents in a selective fashion, minimizing systemic toxicity and damage to normal liver cells. Chemotherapy agents delivered through the hepatic artery can extend time to liver recurrence after curative resection and may prolong survival both in the adjuvant setting and when given to patients with unresectable disease. Molecular-based therapies, such as gene delivery and oncolytic viruses, provide promise for curative outcomes in patients with advanced disease.

Sagiv, E., L. Memeo, et al. (2006). "CD24 is a new oncogene, early at the multistep process of colorectal cancer carcinogenesis." Gastroenterology **131**(2): 630-9.

BACKGROUND & AIMS: The aim of this study was to identify genes that play a role in colorectal cancer (CRC) carcinogenesis by analysis of differential gene expression of normal and transformed CRC cell lines. METHODS: Gene expression array analysis ([RG-U34] GeneChip) was performed in normal and transformed rat intestinal epithelial cells before and after exposures to celecoxib. In particular, we were looking for (1) altered gene expression in the transformed cells that reverts to normal following exposure to a selective cyclooxygenase-2 inhibitor, (2) novel genes, and (3) genes encoding membrane receptors or ligands. As a validation of the results and for human patients, immunohistochemistry was performed on 398 biological samples from the gastrointestinal tract (normal, polyps, and adenocarcinomas). Human cancer cell lines were tested for their response to anti-CD24 monoclonal antibodies. RESULTS: A total of 1081 genes were differently expressed following malignant transformation; 71 genes showed altered expression that reverted to normal following treatment with celecoxib, including the CD24 gene. Immunohistochemistry confirmed that increased expression of CD24 is an early event in CRC carcinogenesis. It was expressed in 90.7% of adenomas and 86.3% of CRCs. Very low expression was seen in normal epithelium (16.6%). Human cancer cell lines showed growth inhibition in response to the antibodies, according to their expression levels of CD24 and in dose- and time-dependent manners. These results were repetitive for 3 different antibodies. CONCLUSIONS: CD24 is overexpressed in the colonic mucosa, already at an early stage of carcinogenesis. It may be a useful target for early detection and in CRC therapy.

Sagiv, E., A. Starr, et al. (2008). "Targeting CD24 for treatment of colorectal and pancreatic cancer by monoclonal antibodies or small interfering RNA." Cancer Res **68**(8): 2803-12.

CD24 is a potential oncogene reported to be overexpressed in a large variety of human malignancies. We have shown that CD24 is overexpressed in 90% of colorectal tumors at a fairly early stage in the multistep process of carcinogenesis. Anti-CD24 monoclonal antibodies (mAb) induce a significant growth inhibition in colorectal and pancreatic cancer cell lines that express the protein. This study is designed to investigate further the effects of CD24 down-regulation using mAb or small interfering RNA in vitro and in vivo. Western blot analysis showed that anti-CD24 mAb induced CD24 protein down-regulation through lysosomal degradation. mAb augmented growth inhibition in combination with five classic chemotherapies. Xenograft models in vivo showed that tumor growth was significantly reduced in mAb-treated mice. Similarly, stable growth inhibition of cancer cell lines was achieved by down-regulation of CD24 expression using short hairpin RNA (shRNA). The produced clones proliferated more slowly, reached lower saturation densities, and showed impaired motility. Most importantly, down-regulation of CD24 retarded tumorigenicity of human cancer cell lines in nude mice. Microarray analysis revealed a similar pattern of gene expression alterations when cells were subjected to anti-CD24 mAb or shRNA. Genes in the Ras pathway, mitogen-activated protein kinase, or BCL-2 family and others of oncogenic association were frequently down-regulated. As a putative new oncogene that is overexpressed in gastrointestinal malignancies early in the carcinogenesis process, CD24 is a potential target for early intervention in the prevention and treatment of cancer.

Sandur, S. K., A. Deorukhkar, et al. (2009). "Curcumin modulates the radiosensitivity of colorectal cancer cells by suppressing constitutive and inducible NF-kappaB activity." Int J Radiat Oncol Biol Phys **75**(2): 534-42.

PURPOSE: Radiation therapy is an integral part of the preoperative treatment of rectal cancers. However, only a minority of patients achieve a complete pathologic response to therapy because of resistance of these tumors to radiation therapy. This resistance may be mediated by constitutively active pro-survival signaling pathways or by inducible/acquired mechanisms in response to radiation therapy. Simultaneous inhibition of these pathways can sensitize these tumors to radiation therapy. METHODS AND MATERIALS: Human

colorectal cancer cells were exposed to clinically relevant doses of gamma rays, and the mechanism of their radioresistance was investigated. We characterized the transcription factor nuclear factor-kappaB (NF-kappaB) activation as a mechanism of inducible radioresistance in colorectal cancer and used curcumin, the active ingredient in the yellow spice turmeric, to overcome this resistance. RESULTS: Curcumin inhibited the proliferation and the post-irradiation clonogenic survival of multiple colorectal cancer cell lines. Radiation stimulated NF-kappaB activity in a dose- and time-dependent manner, whereas curcumin suppressed this radiation-induced NF-kappaB activation via inhibition of radiation-induced phosphorylation and degradation of inhibitor of kappaB alpha, inhibition of inhibitor of kappaB kinase activity, and inhibition of Akt phosphorylation. Curcumin also suppressed NF-kappaB-regulated gene products (Bcl-2, Bcl-x(L), inhibitor of apoptosis protein-2, cyclooxygenase-2, and cyclin D1). CONCLUSIONS: Our results suggest that transient inducible NF-kappaB activation provides a prosurvival response to radiation that may account for development of radioresistance. Curcumin blocks this signaling pathway and potentiates the antitumor effects of radiation therapy.

Shankaran, V., K. B. Wisinski, et al. (2008). "The role of molecular markers in predicting response to therapy in patients with colorectal cancer." Mol Diagn Ther **12**(2): 87-98.

Advances in systemic therapy for colorectal cancer have dramatically improved prognosis. While disease stage has traditionally been the main determinant of disease course, several molecular characteristics of tumor specimens have recently been shown to have prognostic significance. Although to date no molecular characteristics have emerged as consistent predictors of response to therapy, retrospective studies have investigated the role of a variety of biomarkers, including microsatellite instability, loss of heterozygosity of 18q, type II transforming growth factor beta receptor, thymidylate synthase, epidermal growth factor receptor, and Kirsten-ras (KRAS). This paper reviews the current literature, ongoing prospective studies evaluating the role of these markers, and novel techniques such as gene profiling, which may help to uncover the more complex molecular interactions that will predict response to chemotherapy in patients with colorectal cancer.

Sheen, A. J., D. J. Sherlock, et al. (2003). "T lymphocytes isolated from patients with advanced colorectal cancer are suitable for gene immunotherapy approaches." Br J Cancer **88**(7): 1119-27.

Despite improvements in treatment, the 5-year survival for metastatic colorectal cancer remains poor. Novel approaches such as gene immunotherapy are being investigated to improve treatment. Retroviral gene transfer methods have been shown to transduce primary human T lymphocytes effectively resulting in the expression of therapeutic genes. However, a number of defects have been identified in T lymphocytes isolated from patients bearing tumour, which may have critical implications for the development of gene-targeted T cells as an anticancer therapy. To address this issue, primary T lymphocytes were isolated from patients with advanced colorectal cancer and tested for their ability to be transduced and to express subsequently a chimeric immune receptor consisting of a single-chain antibody fragment antigen-binding moiety specific for carcinoembryonic antigen (CEA) fused to the T cell receptor (TCR) CD3zeta chain. In 10 out of 10 patients, T lymphocytes were transduced, expanded in the absence of selection and tested for functional activity against CEA-expressing tumour cells. In each case, functional-specific cytotoxic activity was observed. Negligible activity was found in control cultures. This study highlights the feasibility of patient-derived T lymphocytes as a source of immune cells for autologous gene immunotherapy approaches.

Shimizu, D., T. Ishikawa, et al. (2005). "Prediction of chemosensitivity of colorectal cancer to 5-fluorouracil by gene expression profiling with cDNA microarrays." Int J Oncol **27**(2): 371-6.

5-Fluorouracil (5-FU) is the most widely used anticancer agent for gastrointestinal cancers. Because many tumors show primary resistance, it is clinically meaningful to predict tumor sensitivity to the drug before treatment. cDNA microarrays containing 21,168 clones were used to identify genes associated with sensitivity to 5-FU. Gene expression profiling of 3 colorectal cancer cell lines (DLD-1, HT-29 and NUGC-3) and the corresponding 5-FU-resistant sublines (DLD-1/FU, HT-29/FU and NUGC-3/5FU/L) showed 81 genes that were differentially expressed. The gene set thus identified successfully predicted the sensitivities of 5 other colorectal cancer cell lines and could also separate 5-FU resistant clinical samples from sensitive ones.

Shin, S. W., H. C. Kwon, et al. (2009). "Clinical significance of chicken ovalbumin upstream promoter-transcription factor II expression in human colorectal cancer." Oncol Rep **21**(1): 101-6.

Chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII) plays an essential role in angiogenesis and development. A previous study showed that the expression of COUP-TFII

enhanced invasiveness of human lung carcinoma cells. However, no published data are available concerning the biological and clinical significance of COUP-TFII expression in colorectal cancer. Thus, our objective was to explore the expression of COUP-TFII in colorectal cancer as well as its association with clinicopathological features, and to evaluate the role of COUP-TFII as a prognostic indicator in colorectal cancer. We investigated the presence of COUP-TFII in human colorectal cancer tissues and adjacent normal tissues from 95 primary colorectal cancer patients by immunohistochemistry. The correlation between the expression of COUP-TFII and clinicopathologic features was investigated. The 3-year disease-free survival (DFS) and overall survival (OS) of patients with tumors expressing different levels of COUP-TFII were evaluated by the Kaplan-Meier method. No significant correlation was found between COUP-TFII expression and age at surgery, gender, histopathologic differentiation, vessel invasion, carcinoembryonic antigen (CEA), or nodal involvement. However, survival analysis showed that the COUP-TFII-positive group had a significantly better OS compared to COUP-TFII-negative group (80.4% vs. 57.7%, P=0.0491). Based on our results, COUP-TFII may represent a biomarker for good prognosis in colorectal cancer.

Simiantonaki, N., U. Kurzik-Dumke, et al. (2007). "Reduced expression of TLR4 is associated with the metastatic status of human colorectal cancer." Int J Mol Med **20**(1): 21-9.

Signaling mediating colorectal cancer (CRC) progression is incompletely understood. Previously, we identified lipopolysaccharide (LPS), an endotoxin of ubiquitously existing colonic bacteria, as a pivotal stimulus increasing the metastatic potential of human CRC. Since the ubiquitous colonic bacteria release large amounts of LPS this observation could be of enormous relevance for the progression of CRC. In this study we present data contributing to the elucidation of its mode of action. Since both receptors CD14 and TLR4 act as LPS mediators, we determined their expression in various CRC cell lines and in 115 non-metastatic, lymphogenous-metastatic and haematogenous-metastatic CRC specimens. Here we showed that CD14 was not expressed in normal colon epithelium, in non-metastatic and metastatic CRC. Furthermore, we showed that diverse CRC cell lines did not express CD14 under normal conditions and after LPS stimulation. Thus, CD14 can be ruled out as a mediator of LPS-induced signaling related to CRC progression. In contrast, we found that normal colon epithelium and CRC cell lines were positive for TLR4. Furthermore, both lymphogenous and haematogenous metastatic cases showed either loss of expression or strong downregulation of TLR4 as compared to normal tissue and to non-metastatic tumors. We found that LPS stimulation resulted in significant TLR4 upregulation in cells expressing lower constitutive TLR4 levels such as CaCo2, whereas no significant response to LPS was observed in cells characterized by relatively high amounts of constitutive TLR4. Our data suggest that TLR4 expression may be associated with mechanisms preventing CRC progression.

Stahtea, X. N., A. E. Roussidis, et al. (2007). "Imatinib inhibits colorectal cancer cell growth and suppresses stromal-induced growth stimulation, MT1-MMP expression and pro-MMP2 activation." Int J Cancer **121**(12): 2808-14.

Tumor progress depends on the proliferation of cancer cells, their interactions with stroma and the proteolytic action of enzymes. Colon cancer is c-kit positive and responsive to the specific tyrosine kinase inhibitor imatinib. We investigated the effect of imatinib on the proliferation of a panel of epithelial colon cancer cell lines in presence and absence of the antimetabolite 5-FU, and the effect of conditioned media (CM) derived from colon stromal fibroblasts with and without previous exposure to imatinib. The effects of imatinib on gene expression of MMPs and TIMPs were also studied. Imatinib effectively inhibited the proliferation of all cell lines, showing IC(50) from 0.3 to 3 microM. Its combination with 5-FU significantly enhances the growth inhibition of the highly tumourigenic HT-29 cells. CM derived from stromal fibroblasts induced the proliferation of the HT-29 cells; this stimulatory effect was abolished upon treatment with CM obtained after exposure of fibroblasts to imatinib. Gene expression of MT1-, MT2-MMP and MMP-7 was also inhibited depending on the cell line, whereas that of TIMP-2 was not affected. CM stimulated MT1-MMP protein expression by HT-29; this stimulatory effect was suppressed in the presence of imatinib. Activation of pro-MMP2 to MMP2 in culture medium of HT-29 treated with CM was increased and this activity was inhibited in presence of imatinib. The obtained data showed that imatinib is a powerful inhibitor of human colon cancer cell growth and effectively suppresses the stromal-induced stimulation of cancer cell growth and activation of proMMP2. Further studies are warranted to evaluate the in vivo effects.

Sun, Z. Q., C. S. Deng, et al. (2008). "Antitumor bioactivity of adenovirus-mediated p27mt in colorectal cancer cell line SW480." World J Gastroenterol **14**(38): 5827-33.

AIM: To explore the antitumor bioactivity of adenovirus-mediated mutant type p27(kip1) gene in a

colorectal cancer cell line SW480. METHODS: We constructed recombinant adenovirus vector expressing a mutant type p27(kip1) gene (ad-p27mt), with mutation of Thr-187/Pro-188 (ACGCCC) to Met-187/Ile-188 (ATGATC), and transduced into SW480 cells. Then we detected expression of p27, Bcl-2 and Bax protein in the transductants by Western blotting, cell cycle of transductants by a digital flow cytometric system, migrating potential with Boyden Chamber and SW480 tumor cell growth inhibition in vitro and in vivo. RESULTS: We found that a recombinant adenovirus vector of expressing ad-p27mt, with mutation of Thr-187/Pro-188 (ACGCCC) to Met-187/Ile-188 (ATGATC) has potent inhibition of SW480 tumor cell growth in vitro and in vivo. Furthermore, ad-p27mt induced cell apoptosis via regulating bax and bcl-2 expressions, and G(1)/S arrest in SW480 cells and inhibited cell migration. CONCLUSION: ad-p27mt has a strong anti-tumor bioactivity and has the potential to develop into new therapeutic agents for colorectal cancer.

Takagi, K., Y. Sowa, et al. (2008). "CDK inhibitor enhances the sensitivity to 5-fluorouracil in colorectal cancer cells." Int J Oncol **32**(5): 1105-10.

Thymidylate synthase (TS) is a dNTP synthetic enzyme and is also a target enzyme of 5-fluorouracil (5-FU). 5-FU is one of the anticancer agents most frequently used for the treatment of colorectal cancers. However, the clinical rate of response to its use as a single agent is not exceptionally high. Therefore, various combination chemotherapies have been devised. The elevated expression of TS in cancer cells is a serious obstacle in the clinical use of 5-FU. In the present study, TS expression was up-regulated by the knockout of the p21WAF1/CIP1 gene in human colorectal cancer HCT116 cells, suggesting that TS expression is mediated through the inhibition of cyclin-dependent kinase (CDK). Based on these findings, we tested whether the CDK inhibitor (CDKI) SU9516, acted as a suppressor of TS. SU9516 effectively reduced the expression of TS in a dose-dependent manner. Furthermore, the reduction of TS expression resulted in enhancement of the sensitivity to 5-FU in human colon cancer DLD-1 cells. Thus, SU9516 might be a promising compound for combination chemotherapy with 5-FU.

Takeuchi, T., M. Hisanaga, et al. (2004). "The membrane-anchored matrix metalloproteinase (MMP) regulator RECK in combination with MMP-9 serves as an informative prognostic indicator for colorectal cancer." Clin Cancer Res **10**(16): 5572-9.

PURPOSE: RECK, a membrane-anchored regulator of matrix metalloproteinases (MMPs), is widely expressed in healthy tissue, whereas it is expressed at lower levels in many tumor-derived cell lines. Studies in mice and cultured cells have shown that restoration of RECK expression inhibits tumor invasion, metastasis, and angiogenesis. However, the clinical relevance of these findings remains to be fully documented. Here we examined the expression of RECK and one of its targets, MMP-9, in colorectal cancer tissue. EXPERIMENTAL DESIGN: The RECK and MMP-9 expression levels in colorectal cancer samples from 53 patients were determined by immunohistochemical techniques. The expression level of each protein was scored, and the patients were divided into two groups based on these scores. In 33 cases, we performed gelatin zymography to estimate the degree of MMP-2 and MMP-9 activation. Microvessel density and vascular endothelial growth factor (VEGF) expression were also evaluated histologically. RESULTS: RECK protein was detected in 30 of 53 (56.6%) specimens. Importantly, patients with tumors expressing relatively high levels of RECK (high-RECK group) had a significantly lower risk of recurrence than did patients with tumors expressing relatively low levels of RECK (low-RECK group; P = 0.011). Moreover, RECK-dominant (RECK score > or = MMP-9 score) patients showed a significantly lower incidence of recurrence than did MMP-9-dominant patients (P = 0.0003). Multivariate analysis revealed that the RECK/MMP-9 balance was an independent prognostic factor (P = 0.0122). The expression of VEGF and microvessel density were inversely correlated with the level of RECK expression. CONCLUSIONS: RECK/MMP-9-balance is an informative prognostic indicator for colorectal cancer. Our data also suggest that RECK suppresses tumor angiogenesis, probably by limiting the availability of VEGF in tumor tissues.

Talieri, M., K. Mathioudaki, et al. (2009). "Clinical significance of kallikrein-related peptidase 7 (KLK7) in colorectal cancer." Thromb Haemost **101**(4): 741-7.

Human tissue kallikrein-related peptidases are a family of 15 secreted serine proteases, located at chromosome 19q13.4. Most of them have been reported to be potential biomarkers for several carcinomas and other diseases. Human tissue kallikrein-related peptidase 7 (KLK7) has been purified from human stratum corneum and resembles a chymotryptic endopeptidase originally called stratum corneum chymotryptic enzyme (SCCE). In this study, we examined for the first time, the prognostic value of KLK7 mRNA expression, using a semi-quantitative RT-PCR method, in 105 colorectal cancer tissues for 54 of which, paired normal colonic mucosa were available. Furthermore, we analysed the expression of KLK7 in 10 adenomas, in 18 biopsies of

inflamed colon mucosa, as well as in 22 human cancer cell lines of various origin, four of them being of colon. A defined number of colon cancer samples were also examined by immunohistochemistry. KLK7 expression was higher in cancerous than in normal tissues. Less differentiated tumors of more advanced stage showed higher KLK7 expression. Follow-up analysis revealed that KLK7 was significantly associated with shorter overall survival (OS) and disease-free survival (DFS). In addition, selected colon cancer samples highly expressing KLK7 gene, showed intense immunohistochemical staining for KLK7, enhancing RT-PCR results. Present data suggest that KLK7 gene is up-regulated in colon cancer and its expression predicts poor prognosis for colon cancer patients.

Tanami, H., H. Tsuda, et al. (2005). "Involvement of cyclin D3 in liver metastasis of colorectal cancer, revealed by genome-wide copy-number analysis." Lab Invest **85**(9): 1118-29.

The question of whether any genetic differences exist between primary and colorectal cancers (CRCs) and their metastatic foci is controversial. To look for genetic aberrations involved in metastasis of CRCs to the liver, we performed subtractive comparative genomic hybridization (CGH) experiments using paired samples from 20 CRC patients with primary tumors and synchronous or metachronous liver metastases. Relatively frequent gains in DNA copy number were detected at 6p, suggesting the presence of one or more metastasis-related genes in the region. Analysis of 11 CRC cell lines using array-based CGH (CGH-array) revealed one 6p candidate gene, CCND3. Quantitative reverse transcriptase-polymerase chain reaction experiments showed that CCND3 was significantly upregulated in liver-metastatic lesions compared with primary lesions ($P<0.0152$). In addition, immunohistochemical analysis of 120 primary CRC tumors demonstrated that cyclin D3 expression in the region of rolled edge was significantly associated with total recurrence, especially hematogenous recurrence ($P=0.0307$). The results implied involvement of cyclin D3 in liver metastasis of CRC, and the data may contribute to the development of a novel therapy or diagnostic agent for this currently intractable disease. Our experiments also confirmed the power of subtractive CGH and CGH-array analysis for identifying cancer-related genes.

Tang, J. T. and J. Y. Fang (2009). "MicroRNA regulatory network in human colorectal cancer." Mini Rev Med Chem **9**(8): 921-6.

Epigenetic modifications include DNA methylation, histone modifications, and noncoding RNAs containing microRNAs (miRNA). miRNAs are small noncoding RNAs that are 21 to 25 nt in length; they downregulate gene expression during cell development, cell proliferation, cell differentiation, and apoptosis. They play a critical role in human carcinogenesis. Presently, evidences show that miRNAs participate as oncogenic miRNAs or tumor suppressors in the developmental and physiological processes of human colorectal cancer (CRC). Disturbed miRNA expression may be attributable to a mechanism involving multiple factors. In this review, we focus on the colorectal miRNA expression profile and further discuss the miRNA regulatory network involved in the tumorigenesis of human CRC. We, thus, hope to open up new avenues for anticancer therapy based on the epigenetic regulation of miRNA.

Thottassery, J. V., L. Westbrook, et al. (2006). "c-Abl-independent p73 stabilization during gemcitabine- or 4'-thio-beta-D-arabinofuranosylcytosine-induced apoptosis in wild-type and p53-null colorectal cancer cells." Mol Cancer Ther **5**(2): 400-10.

Nucleoside anticancer drugs like gemcitabine (2'-deoxy-2',2'-difluorocytidine) are potent inducers of p53, and ectopic expression of wild-type p53 sensitizes cells to these agents. However, it is also known that nucleosides are efficient activators of apoptosis in tumor cells that do not express a functional p53. To clarify this issue, we examined the effects of gemcitabine and 4'-thio-beta-d-arabinofuranosylcytosine (T-ara-C) on p73, a structural and functional homologue of p53, whose activation could also account for nucleoside-induced apoptosis because no functionally significant mutations of p73 have been reported in cancers. Acute treatment of HCT 116 colon carcinoma cells with gemcitabine or T-ara-C induced marked cytotoxicity and cleavage of caspase-3 and poly(ADP-ribose) polymerase. T-ara-C and gemcitabine markedly induced p53 accumulation as well as increased levels of phospho-p53 (Ser15/Ser20/Ser46) and induced its binding to a consensus p53 response element. Despite robust activation of p53 by T-ara-C and gemcitabine, we found that wild-type and p53-/- HCT 116 cells exhibited almost equivalent sensitivity towards these nucleosides. Examination of p73 revealed that T-ara-C and gemcitabine markedly increased p73 protein levels and p73 DNA-binding activities in both p53-/- and wild-type cells. Furthermore, T-ara-C- and gemcitabine-induced increases in p73 levels occur due to a decrease in p73 protein turnover. RNA interference studies show that nucleoside-induced p73 increases are independent of c-Abl, a nucleoside-activated kinase recently implicated in p73 stabilization. HCT 116 lines, wherein the downstream p53/p73 targets Bax and PUMA (p53 up-regulated

modulator of apoptosis) were deleted, were less sensitive to T-ara-C and gemcitabine. Together, these studies indicate that c-Abl-independent p73 stabilization pathways could account for the p53-independent mechanisms in nucleoside-induced apoptosis.

Tuynman, J. B., M. P. Peppelenbosch, et al. (2004). "COX-2 inhibition as a tool to treat and prevent colorectal cancer." Crit Rev Oncol Hematol **52**(2): 81-101.

The cyclooxygenase-2 (COX-2) enzyme has a fundamental role in the carcinogenesis of colorectal cancer. The anticarcinogenic mechanisms of NSAIDs are not completely understood and appear to be only partially dependent on inhibition tumoral COX-2. Moreover, the mechanisms of NSAIDs depend on the concentration. In experimental setting, at low levels NSAIDs downregulate the COX-2 gene in colorectal cancer cells, whereas at clinical relevant concentrations the production of prostaglandin E2 by enzymatic activity of COX-2 is diminished resulting in inhibition of the tumor angiogenesis. At higher levels NSAIDs and especially some selective COX-2 inhibitors are capable of COX-2 independent effects, such as apoptosis induction of tumor cells. In animal models, NSAIDs administration results in inhibition of angiogenesis and proliferation, induction apoptosis and prevention of metastasis. In clinical setting, NSAIDs and selective COX-2 inhibitors have the capacity to prevent the development of colorectal adenomas. We have summarized data regarding the role of COX-2 in CRC and discuss the multiple targets of NSAIDs in their anticarcinogenic action. However, the translation of these anticarcinogenic effects of NSAIDs to its clinical application as adjuvant therapy in CRC is hampered by a lack of randomized clinical trials with long-term follow-up.

Van Geelen, C. M., E. G. de Vries, et al. (2004). "Lessons from TRAIL-resistance mechanisms in colorectal cancer cells: paving the road to patient-tailored therapy." Drug Resist Updat **7**(6): 345-58.

Colorectal cancer is one of the leading causes of cancer-related deaths worldwide. Intrinsic, as well as acquired, resistance to chemotherapy remains a major problem in the treatment of this disease. It is, therefore, of great importance to develop new, patient-tailored, treatment strategies for colorectal cancer patients. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) acts through the pro-apoptotic DR4 and DR5 receptors in tumor cells without harming normal cells and will soon be tested in clinical trials as a novel anti-cancer agent. However, not all human colon cancer cell lines are sensitive to TRAIL due to intrinsic or acquired TRAIL-resistance. This review discusses the mechanisms and modulation of TRAIL-resistance in colon cancer cells. Cell sensitivity to TRAIL can be affected by TRAIL-receptor expression at the cell membrane, DR4/DR5 ratio and functionality of TRAIL-receptors. Additional intracellular factors leading to TRAIL-resistance affect the caspase 8/c-FLIP ratio, such as loss of caspase 8 and caspase 10 due to mutations or gene methylation, CARP-dependent degradation of active caspase 8 and changes in caspase 8 or c-FLIP expression levels. Further downstream in the TRAIL apoptotic pathway, Bax mutations, or increased expression of IAP family members, in particularly XIAP and survivin, also cause resistance. Chemotherapeutic drugs, NSAIDs, interferon-gamma and proteasome inhibitors can overcome TRAIL-resistance by acting on TRAIL-receptor expression or changing the expression of pro- or anti-apoptotic proteins.

Walther, A., E. Johnstone, et al. (2009). "Genetic prognostic and predictive markers in colorectal cancer." Nat Rev Cancer **9**(7): 489-99.

Despite many studies of the likely survival outcome of individual patients with colorectal cancer, our knowledge of this subject remains poor. Until recently, we had virtually no understanding of individual responses to therapy, but the discovery of the KRAS mutation as a marker of probable failure of epidermal growth factor receptor (EGFR)-targeted therapy is a first step in the tailoring of treatment to the individual. With the application of molecular analyses, as well as the ability to perform high-throughput screens, there has been an explosive increase in the number of markers thought to be associated with prognosis and treatment outcome in this disease. In this Review, we attempt to summarize the sometimes confusing findings, and critically assess those markers already in the public domain.

Wang, T. L., L. A. Diaz, Jr., et al. (2004). "Digital karyotyping identifies thymidylate synthase amplification as a mechanism of resistance to 5-fluorouracil in metastatic colorectal cancer patients." Proc Natl Acad Sci U S A **101**(9): 3089-94.

Resistance to chemotherapy is a major cause of mortality in advanced cancer patients. In this study, digital karyotyping was used to search for genomic alterations in liver metastases that were clinically resistant to 5-fluorouracil (5-FU). In two of four patients, we identified amplification of an approximately 100-kb region on 18p11.32 that was of particular interest because it contained the gene encoding thymidylate synthase (TYMS), a molecular target of 5-FU. Analysis of TYMS by fluorescence in situ hybridization identified TYMS gene amplification

in 23% of 31 5-FU-treated cancers, whereas no amplification was observed in metastases of patients that had not been treated with 5-FU. Patients with metastases containing TYMS amplification had a substantially shorter median survival (329 days) than those without amplification (1,021 days, P <0.01). These data suggest that genetic amplification of TYMS is a major mechanism of 5-FU resistance in vivo and have important implications for the management of colorectal cancer patients with recurrent disease.

Wang, Z., T. Cook, et al. (2004). "Adenoviral gene transfer of the human inducible nitric oxide synthase gene enhances the radiation response of human colorectal cancer associated with alterations in tumor vascularity." Cancer Res **64**(4): 1386-95.

Nitric oxide is a potent radiosensitizer of tumors, but its use clinically is limited by serious side effects when administered systemically. We have demonstrated previously that gene transfer of the inducible nitric oxide synthase gene (iNOS) into colorectal cancer cells enhances radiation-induced apoptosis in vitro. The objectives of this study were to further characterize the effects of iNOS gene transfer on the radiosensitivity of human colorectal cancer cells in vitro and tumors grown in athymic nude mice. Adenoviral gene transfer of iNOS (AdiNOS) into human colorectal cancer cell lines (HCT-116 and SNU-1040 cells) significantly enhanced the effects of radiation with sensitizing enhancement ratios (0.1) of 1.65 and 1.6, respectively. The radiation enhancement induced by iNOS was associated with increased iNOS expression and nitric oxide production and prevented by L-NIO, an enzymatic inhibitor of iNOS. AdiNOS treatment of HCT-116 tumors combined with radiation (2 Gy x three fractions) led to a 3.4-fold greater (P < 0.005) tumor growth delay compared with radiation (RT) alone. AdiNOS plus RT also caused significant (P < 0.01) tumor regression with 63% of tumors regressing compared with only 6% of tumors treated with RT. AdiNOS plus RT significantly (P < or = 0.001) increased the percentage of apoptotic cells (22 +/- 4%) compared with either tumors treated with control vector plus RT (9 +/- 1%), AdiNOS alone (9 +/- 3%), or no treatment (2 +/- 1%). These radiosensitizing effects of AdiNOS occurred at low infection efficiency (4% of tumor infected), indicating a significant bystander effect.

Wang, Z. X., H. B. Bian, et al. (2009). "Adenovirus-mediated suicide gene therapy under the control of Cox-2 promoter for colorectal cancer." Cancer Biol Ther **8**(15): 1480-8.

Colorectal cancer is a most frequent type of gastrointestinal tract cancers. The prognosis of patients with colorectal cancer remains poor despite intensive interventions. Tumor specific promoter-directed gene therapy and adenoviral technology can be promising strategies for such advanced disease. This study was conducted to explore the possible therapeutic approach of Cox-2 promoter-directed suicide gene therapy with herpes simplex virus thymidine kinase (HSV-tk) in combination with adenoviral technology for advanced colorectal cancer. Firstly, the activity of Cox-2 promoter was assessed by dual luciferase and enhanced green fluorescent protein reporter gene assays in colorectal cancer cell lines and normal human intestinal epithelial cell line. Then, the expression of coxsackievirus and adenovirus receptor (CAR) was detected in colorectal cancer cell lines. The Cox-2 promoter-directed HSV-tk/ganciclovir (GCV) system mediated by adenovirus (Ad-Cp-TK) was developed (Ad-CMVp-TK, Ad-null and no Ad as controls). In vitro cytoxicity, colony formation and apoptosis assays were performed using Ad-Cp-TK. An animal study was carried out in which BALB/C nude mice bearing tumors were treated with Ad-Cp-TK and GCV treatments. Results showed that Cox-2 promoter possessed high transcriptional activity in a tumor-specific manner. All colorectal cancer cells were detected CAR-positive. In vitro cytotoxic and colony formation assays showed that colorectal cancer cells infected with Ad-Cp-TK became more sensitive to GCV but the sensitivity of normal cells infected with Ad-Cp-TK to GCV were not altered. Moreover, the Ad-Cp-TK system combined with GCV treatment could significantly induce apoptosis of colorectal cancer cells but not normal intestinal epithelial cells. Furthermore, this system also significantly inhibited the growth of subcutaneous tumors and prolonged survival of mice. Thus, adenovirus primary receptor was positive in colorectal cancer cells and adenovirus-mediated suicide gene therapy under the control of Cox-2 promoter could provide a promising treatment modality for advanced colorectal cancer with tumor specificity.

Wehler, T. C., K. Frerichs, et al. (2008). "PDGFRalpha/beta expression correlates with the metastatic behavior of human colorectal cancer: a possible rationale for a molecular targeting strategy." Oncol Rep **19**(3): 697-704.

As new multi-target tyrosine kinase inhibitors are emerging in the therapy of various malignancies, our aim was to define the co-expression pattern of receptor-tyrosine-kinase platelet-derived growth factor receptors alpha and beta (PDGFRalpha/beta) in human colorectal cancer. The co-expression pattern of PDGFRalpha/beta was analyzed by RT-PCR in 99 histologically confirmed human colorectal carcinomas and five colorectal cancer cell lines. In addition,

immunohistochemical (IHC) staining was applied for confirmation of expression and analysis of receptor tyrosine kinase (RTK) localisation. The colorectal cancer cell lines that were analysed revealed varying expression intensities of PDGFRalpha and PDGFRbeta. The majority of human colorectal cancer specimens revealed a PDGFRalpha (83%) or PDGFRbeta (60%) expression. While PDGFRalpha showed a predominantly cytoplasmic staining in tumor cells as well as in stromal pericytes, PDGFRbeta was restricted to stromal pericytes only. Furthermore, PDGFRalpha expression significantly correlated with lymph node metastasis (P=0.0082) and advanced UICC stages III/IV (P=0.018) in older patients (P=0.043). PDGFRbeta expression only revealed a trend towards lymphatic dissemination (P=0.099). Co-expression of PDGFRalpha/beta occurred in 57% of the colorectal cancer samples, whereas another 29% of the samples depicted mono-expression of PDGFRalpha or PDGFRbeta. Notably, PDGFRalpha/beta expression significantly correlated with lymphatic metastasis (P=0.007) and advanced UICC stages III/IV (P=0.017) in older patients (P=0.03). In summary, our results revealed that PDGFRalpha/beta expression significantly correlates with lymphatic dissemination and therefore encourages application of PDGFRalpha/beta RTK-inhibitors within a combination therapy.

Whitehead, R. P., C. Rankin, et al. (2009). "Phase II trial of romidepsin (NSC-630176) in previously treated colorectal cancer patients with advanced disease: a Southwest Oncology Group study (S0336)." Invest New Drugs **27**(5): 469-75.

INTRODUCTION: Patients with metastatic colorectal cancer who progress on standard chemotherapy have limited treatment options. New and effective drugs are needed for these patients. Romidepsin is a histone deacetylase inhibitor that can alter chromatin structure and gene transcription leading to multiple changes in cellular protein production. This may result in cell cycle arrest and tumor growth inhibition. Romidepsin has shown anti-proliferative activity in vitro against multiple mouse and human tumor cell lines and in vivo in human tumor xenograft models. PATIENTS AND METHODS: Patients were required to have pathologically verified, measurable, metastatic or locally advanced colorectal cancer that was surgically unresectable. They must have failed either one or two prior chemotherapy regimens, had performance status of 0-1, adequate bone marrow, renal and hepatic function, and no significant cardiac disease. Patients were treated with romidepsin at a dose of 13 mg/m(2) as a 4-h iv infusion on days 1, 8, and 15 of a 28-day cycle. The study had a two stage design. The primary objective of the study was to determine the confirmed response probability in this group of patients treated with romidepsin. RESULTS: Twenty-eight patients were registered to the study, two of whom were ineligible. One eligible patient refused all treatment and was not analyzed. For the 25 remaining patients, performance status was 0 in 16 patients and 1 in nine patients. Ten patients had received one prior chemotherapy regimen and fifteen 2 prior regimens. Out of the 25 eligible and analyzable patients accrued in the first stage of the protocol, no objective responses were observed and the study was permanently closed. Four patients had stable disease as the best response. Twenty-five patients were assessed for toxicity. No grade 4 or greater toxicities were seen. Fourteen of the 25 patients experienced grade 3 toxicities the most common of which were fatigue or anorexia. CONCLUSION: Romidepsin at this dose and schedule is ineffective in the treatment of patients with metastatic colorectal cancer after prior chemotherapy. Future trials might evaluate combinations of romidepsin with chemotherapeutic or other agents.

Wilson, T. R., M. McEwan, et al. (2009). "Combined inhibition of FLIP and XIAP induces Bax-independent apoptosis in type II colorectal cancer cells." Oncogene **28**(1): 63-72.

Death receptors can directly (type I cells) or indirectly induce apoptosis by activating mitochondrial-regulated apoptosis (type II cells). The level of caspase 8 activation is thought to determine whether a cell is type I or II, with type II cells less efficient at activating this caspase following death receptor activation. FLICE-inhibitory protein (FLIP) blocks death receptor-mediated apoptosis by inhibiting caspase 8 activation; therefore, we assessed whether silencing FLIP could convert type II cells into type I. FLIP silencing-induced caspase 8 activation in Bax wild-type and null HCT116 colorectal cancer cells; however, complete caspase 3 processing and apoptosis were only observed in Bax wild-type cells. Bax-null cells were also more resistant to chemotherapy and tumor necrosis factor-related apoptosis inducing ligand and, unlike the Bax wild-type cells, were not sensitized to these agents by FLIP silencing. Further analyses indicated that release of second mitochondrial activator of caspases from mitochondria and subsequent inhibition of X-linked inhibitor of apoptosis protein (XIAP) was required to induce full caspase 3 processing and apoptosis following FLIP silencing. These results indicate that silencing FLIP does not necessarily bypass the requirement for mitochondrial involvement in type II cells. Furthermore, targeting FLIP and XIAP may represent a therapeutic strategy for the treatment of

colorectal tumors with defects in mitochondrial-regulated apoptosis.

Wu, J. T., S. Kakar, et al. (2005). "Prognostic significance of DCC and p27Kip1 in colorectal cancer." Appl Immunohistochem Mol Morphol **13**(1): 45-54.

The progression of colorectal cancer is a multistage process associated with specific molecular alterations. The stepwise accumulation of these multiple genetic mutations progressively results in the acquisition of neoplastic cell behavior. The genetic abnormalities associated with the expression of metastatic phenotype, therefore, may be of prognostic significance in the clinical treatment of colorectal cancer patients. In this study, the immunohistochemical expression of the deleted in colorectal cancer gene (DCC) and p27Kip1 was assessed in 168 paraffin-embedded, formalin-fixed tumors of patients with stage II and III colorectal cancer. Kaplan-Meier survival curves and log-rank statistics were used to analyze survival times after curative primary tumor resection, and Cox proportional hazards models were used to adjust the assessment of demographic and clinical covariates. Loss of DCC or p27Kip1 expression had no influence on survival in patients with stage II or III colorectal cancer. The 5-year survival rates of DCC-positive and DCC-negative tumors were 51.8% and 35.7% (P=0.40), respectively. The 5-year survival rate of patients with p27Kip1-positive tumors was 47.9%, whereas the rate for patients with p27Kip1-negative tumors was 38.8% (P=0.68). After adjustment for all evaluated variables, neither DCC or p27Kip1 was found to be a predictor of survival (risk ratio for DCC, 0.98; 95% confidence interval, 0.66-1.56; P=0.92; risk ratio for p27Kip1, 0.87; 95% confidence interval, 0.58-1.29; P=0.49). The present study demonstrated that the expression of neither DCC nor p27Kip1 was predictive in poor survival outcome in patients with stage II or III colorectal cancer.

Xi, Y., A. Formentini, et al. (2008). "Validation of biomarkers associated with 5-fluorouracil and thymidylate synthase in colorectal cancer." Oncol Rep **19**(1): 257-62.

Previous studies from our laboratory have identified a number of genes associated with chemosensitivity to 5-fluorouracil (5-FU) using an in vitro colon cancer cell line model. In this study, the in vivo significance of several marker genes in terms of prognostic potential was evaluated using colorectal cancer patient samples. Eight marker genes were selected based on their functional roles and significant fold changes in expression. They are SERTA domain containing 1 (SEI1), ribonucleotide reductase M2 polypeptide (RRM2), origin recognition complex, subunit 6 homolog-like (ORC6L), eukaryotic translation initiation factor 4E (EIF4E), thymidylate synthase (TS), SET and MYND domain containing 3 (SMYD3), Dickkopf homolog 4, and methyl-CpG binding domain protein 4 (MBD4). Forty-eight snap frozen clinical colorectal samples (24 normal and 24 paired colorectal cancer patient samples) were selected with detailed clinical follow-up information. cDNAs were synthesized and the expression levels of marker genes were quantified via qRT-PCR analysis. The statistical significance of these markers for disease prognosis was evaluated using the two-tailed paired Wilcoxon test. Survival curves were plotted according to the method of Kaplan-Meier and compared using the log-rank test. Based on the quantitative expression analysis, RRM2 (p=0.0001; 95% CI, 2.0-4.5), ORC6L (p=0.0001; 95% CI, 1.8-4.6), EIF4E (p=0.0002; 95% CI, 0.3-0.9), TS (p=0.0005; 95% CI, 0.7-2.2) and SMYD3 (p=0.0001; 95% CI, 0.8-1.5) were overexpressed in tumor tissues. However, the expression of SEI1 was decreased in tumors (p=0.02; 95% CI, 0.1-1.3), consistent with the function of SEI1 as a potential tumor suppressor. Kaplan-Meier survival analysis indicated that MBD4 is a significant prognostic factor for patient survival (p=0.03). MBD4 was a key protein involved in DNA methylation. The expression of TS was associated with tumor stage as it had a significantly higher expression level in UICC stage I and II compared to stage IV patients (p=0.03). MBD4 may be a potential novel prognostic marker for predicting patient survival for colorectal cancer.

Xiong, H., Z. G. Zhang, et al. (2008). "Inhibition of JAK1, 2/STAT3 signaling induces apoptosis, cell cycle arrest, and reduces tumor cell invasion in colorectal cancer cells." Neoplasia **10**(3): 287-97.

Abnormalities in the STAT3 pathway are involved in the oncogenesis of several cancers. However, the mechanism by which dysregulated STAT3 signaling contributes to the progression of human colorectal cancer (CRC) has not been elucidated, nor has the role of JAK, the physiological activator of STAT3, been evaluated. To investigate the role of both JAK and STAT3 in CRC progression, we inhibited JAK with AG490 and depleted STAT3 with a SiRNA. Our results demonstrate that STAT3 and both JAK1 and 2 are involved in CRC cell growth, survival, invasion, and migration through regulation of gene expression, such as Bcl-2, p1(6ink4a), p21(waf1/cip1), p27(kip1), E-cadherin, VEGF, and MMPs. Importantly, the FAK is not required for STAT3-mediated regulation, but does function downstream of JAK. In addition, our data show that proteasome-mediated proteolysis promotes

dephosphorylation of the JAK2, and consequently, negatively regulates STAT3 signaling in CRC. Moreover, immunohistochemical staining reveals that nuclear staining of phospho-STAT3 mostly presents in adenomas and adenocarcinomas, and a positive correlation is found between phospho-JAK2 immunoreactivity and the differentiation of colorectal adenocarcinomas. Therefore, our findings illustrate the biologic significance of JAK1, 2/STAT3 signaling in CRC progression and provide novel evidence that the JAK/STAT3 pathway may be a new potential target for therapy of CRC.

Xu, X. M., C. He, et al. (2003). "Tumor necrosis factor-related apoptosis-inducing ligand gene on human colorectal cancer cell line HT29." World J Gastroenterol **9**(5): 965-9.

AIM: To evaluate the therapeutic efficiency of Tumor Necrosis Factor-related Apoptosis-inducing Ligand (TRAIL) gene on human colorectal cancer cell line HT29. METHODS: Human embryonal kidney cells transformed by introducing sheared fragments of Ad5 DNA (293 cell) were used for amplification of adenoviral vectors: Ad/GT-TRAIL,Ad/GT-Bax, Ad/GT-LacZ and Ad/PGK-GV16. Human colorectal cancer cell line HT29 was transfected with binary adenovirus-mediated TRAIL gene. Bax gene was used as positive control, LacZ gene was used as the vector control,and cells treated with PBS only were used as a mock control. The morphological changes, cell growth and apoptosis were measured by reversmicroscope, MTT method and flow cytometry. RESULTS: All adenoviral vectors titer determined by optical absorbency at A260nm were 1X10(10) viral particle/ml(vp/ml). Obviously morphological changes of HT29 cells were observed when infected with Ad/GT-TRAIL, and these changes were much more obviously when Ad/PGK-GV16 was coinfected. The cell suppression percentage and the percentage of apoptotic cells were 52.5 % and 16.5 % respectively when infected with Ad/GT-TRAIL alone, while combining with Ad/PGK-GV16, the growth of HT29 was suppressed by 85.2 % and the percentage of apoptotic cells was 35.9 %. It showed a significantly enhanced therapeutic efficiency with binary system ($P<0.05$). CONCLUSION: A binary adenoviral vector system provides an effective approach to amplify viral vectors that express potentially toxic gene, TRAIL. Ad/GT-TRAIL showed a significantly enhanced therapeutic efficiency for HT29 when coinfected with Ad/PGK-GV16. Ad/GT-TRAIL could induce apoptosis of HT29 and inhibit its growth.

Yaacob, N. S., H. M. Darus, et al. (2008). "Modulation of cell growth and PPARgamma expression in human colorectal cancer cell lines by ciglitazone." Exp Toxicol Pathol **60**(6): 505-12.

Studies have shown that ligand activation of peroxisome proliferator-activated receptor gamma (PPARgamma) can induce differentiation and inhibit proliferation of several cancer cells. The present study was performed to investigate the effects of the PPARgamma ligand, ciglitazone, and the involvement of PPARgamma in modulating the growth of human colorectal cancer cells. Lactate dehydrogenase release assay showed that ciglitazone potently inhibited HT-29 (well-differentiated) and COLO-205 (poorly differentiated) colorectal adenocarcinoma cell growth. Measurement of apoptosis by flow cytometry using a fluorescein-conjugated monoclonal antibody against cytokeratin 18 revealed a high induction of apoptosis by ciglitazone in a time-dependent fashion. The expression of PPARgamma1 but not PPARgamma2 mRNA was significantly downregulated as measured by real-time quantitative PCR, and the PPARgamma protein levels were decreased as determined by Western blot analysis. We conclude that ciglitazone treatment suppressed colon cancer cell growth via induction of apoptosis. However, the anticancer effects of ciglitazone may not depend solely on PPARgamma activation.

Yamada, Y., N. Hamajima, et al. (2003). "Association of a polymorphism of the phospholipase D2 gene with the prevalence of colorectal cancer." J Mol Med (Berl) **81**(2): 126-31.

Phospholipase D plays an important role in transmembrane signaling in a variety of cell types and its activity is increased in certain cancers, suggesting that it also contributes to tumorigenesis. A C-->T transition at nucleotide 1814 of the human phospholipase D(2) gene, which results in a Thr-->Ile substitution at amino acid 577, was noted in the GenBank database. The relationship of this polymorphism to the prevalence of cancer of the esophagus, stomach, colon-rectum, lung, and breast in Japanese was investigated in a case-control study. The genotype of the phospholipase D(2) gene was determined by the polymerase chain reaction with confronting two-pair primers. Multivariate logistic regression analysis with adjustment for age, gender, and smoking status revealed that the frequency of the T allele of the 1814C-->T polymorphism was significantly higher in individuals with colorectal cancer than in controls. A significant association of the polymorphism with the prevalence of colorectal cancer was found in analyses assuming either dominant (TT+CT vs. CC) or additive (CT vs. CC) effects of the T allele, but the T allele was not associated with the prevalence of esophageal, gastric, lung, or breast cancer. The activities of phospholipase

D in cell lysates or membrane fractions did not differ between cells transfected with cDNAs encoding the Thr-577 or Ile-577 variants of phospholipase D(2). These results suggest that the phospholipase D(2) gene is a susceptibility locus for colorectal cancer in Japanese individuals, although a functional effect of the 1814C-->T (Thr577Ile) polymorphism was not detected.

Yamaguchi, K., S. H. Lee, et al. (2006). "A novel peroxisome proliferator-activated receptor gamma ligand, MCC-555, induces apoptosis via posttranscriptional regulation of NAG-1 in colorectal cancer cells." Mol Cancer Ther **5**(5): 1352-61.

Apoptosis and/or differentiation induction caused by the peroxisome proliferator-activated receptor gamma (PPARgamma) ligand is a promising approach to cancer therapy. The thiazolidinedione derivative MCC-555 has an apoptotic activity in human colorectal cancer cells, accompanied by up-regulation of a proapoptotic nonsteroidal anti-inflammatory drug-activated gene (NAG-1) in a PPARgamma-independent manner. Treatment with MCC-555 resulted in the induction of NAG-1 expression and apoptosis in HCT-116 cells. Down-regulation of NAG-1 by small interfering RNA suppressed MCC-555-induced apoptosis. MCC-555 was found to affect NAG-1 mRNA stability. To further define the underlying mechanism of RNA stability affected by MCC-555, we cloned the 3'-untranslated region (3'UTR) of human NAG-1 mRNA, which contains four copies of an AU-rich element (ARE), downstream from the luciferase gene. The reporter activity was reduced to approximately 70% by inserting the 3'UTR. In addition, deletion of ARE sequences in the 3'UTR or MCC-555 treatment substantially restored activity. This effect of MCC-555 on the ARE-mediated mRNA degradation was inhibited by extracellular signal-regulated kinase (ERK) pathway inhibitors. Subsequently, rapid phosphorylation of ERK1/2 by MCC-555 treatment was detected. Moreover, ERK small interfering RNA suppressed MCC-555-induced NAG-1 expression. These results suggest that ARE sequences in the 3'UTR of the NAG-1 gene contribute to mRNA degradation and ERK1/2 phosphorylation is responsible for the stabilization of NAG-1 mRNA. These findings may provide a novel explanation for the antitumorigenic and/or proapoptotic action of MCC-555 in human colorectal cancer and the ability of pharmacologic approaches to be used against diseases caused by alterations of RNA stability.

Yamaguchi, K., J. L. Liggett, et al. (2006). "Anti-proliferative effect of horehound leaf and wild cherry bark extracts on human colorectal cancer cells." Oncol Rep **15**(1): 275-81.

Marubium vulgare (horehound) and Prunus serotina (wild cherry) have been traditionally used for the treatment of inflammatory-related symptoms such as cold, fever, and sore throat. In this report, we show that extracts of anti-inflammatory horehound leaves and wild cherry bark exhibit anti-proliferative activity in human colorectal cancer cells. Both horehound and wild cherry extracts cause suppression of cell growth as well as induction of apoptosis. We found that horehound extract up-regulates pro-apoptotic non-steroidal anti-inflammatory drug-activated gene (NAG-1) through transactivation of the NAG-1 promoter. In contrast, wild cherry extract decreased cyclin D1 expression and increased NAG-1 expression in HCT-116 and SW480 cell lines. Treatment with wild cherry extract resulted in the suppression of beta-catenin/T cell factor transcription, as assessed by TOP/FOP reporter constructs, suggesting that suppressed beta-catenin signaling by wild cherry extract leads to the reduction of cyclin D1 expression. Our data suggest the mechanisms by which these extracts suppress cell growth and induce apoptosis involve enhanced NAG-1 expression and/or down-regulation of beta-catenin signaling, followed by reduced cyclin D1 expression in human colorectal cancer cells. These findings may provide mechanisms for traditional anti-inflammatory products as cancer chemopreventive agents.

Yamaguchi, Y., E. Miyahara, et al. (2003). "Locoregional immunotherapy of malignant effusion from colorectal cancer using the streptococcal preparation OK-432 plus interleukin-2: induction of autologous tumor-reactive CD4+ Th1 killer lymphocytes." Br J Cancer **89**(10): 1876-84.

In total, 16 patients with cytologically proven malignant effusion from colorectal cancer were treated by locoregional administration of the streptococcal preparation OK-432 alone or OK-432 plus the T-cell growth factor interleukin (IL)-2, and the action mechanism of the treatment was studied. A positive clinical response, showing a cytologic disappearance of cancer cells and decrease of effusion, was observed in nine of 11 (82%) patients treated with OK-432 alone and in all five patients treated with OK-432 plus IL-2. Flow cytometric analysis revealed that OK-432 plus IL-2 locally induced acute inflammation-like responses, including serial cellular infiltrations of granulocyte migration within a matter of hours, and activation of macrophages and T lymphocyte involvement within the following days, and that a predominant expansion of CD3+CD4+ lymphocytes (CD: cluster of differentiation) was induced by in vitro stimulation with IL-2 of locoregional cells after the

OK-432 administration (OK/IL-2AK cells). The OK/IL-2AK cells produced tumour necrosis factor-alpha and interferon-gamma, but these cells did not produce IL-4 and IL-6. The OK/IL-2AK cells expressed potent killing activity against autologous tumour cells. This activity was abrogated by treatment of the lymphocytes with anti-CD3, -CD4, -TCRalphabeta antibody, and by the treatment of target cells with anti-human leukocyte antigen (HLA)-DR antibody. The OK/IL-2AK cells expressed Fas-L gene, and flow cytometric analysis demonstrated HLA-DR expression in approximately 75% of CEA+ or cytokeratin+ effusion cells. TCRVbeta gene analysis of the OK/IL-2AK cells showed an oligoclonal usage of TCRbeta20, which was also involved in the cytotoxic mechanism of the OK/IL-2AK cells. Single-strand conformational polymorphism analysis demonstrated the clonotypes for the TCRVbeta20 gene, and the CDR3s of the gene were sequenced. The clonotypic PCR using the TCRVbeta20-CDR3 sequences could detect the CDR3-identical TCRs in effusion lymphocytes from the other patients. Taken together, it is suggested that locoregional administration of OK-432 plus IL-2 is highly effective for the management of malignant effusion from colorectal cancer. OK-432 plus IL-2 induces autologous tumour-reactive CD4+ Th1 killer lymphocytes, which recognise tumour antigen(s) presented with HLA class II molecules on effusion tumour cells by means of preferential usage of TCRVbeta20. The clonotypic PCR using the TCRVbeta20-CDR3 sequences may be informative for treating malignant effusion from colorectal cancer using OK-432 plus IL-2.

Yang, M., X. Cao, et al. (2008). "Potent antitumor efficacy of ST13 for colorectal cancer mediated by oncolytic adenovirus via mitochondrial apoptotic cell death." Hum Gene Ther **19**(4): 343-53.

ST13 is a cofactor of heat shock protein 70 (Hsp70). To date, all data since the discovery of ST13 in 1993 until more recent studies in 2007 have proved that ST13 is downregulated in tumors and it was proposed to be a tumor suppressor gene, but no work reported its antitumor effect and apoptotic mechanism. In the work described in this paper, ST13 was inserted into ZD55, an oncolytic adenovirus with the E1B 55-kDa gene deleted, to form ZD55-ST13, which exerts an excellent antitumor effect in vitro and in an animal model of colorectal carcinoma SW620 xenograft. ZD55-ST13 inhibited tumor cells 100-fold more than Ad-ST13 and ZD55-EGFP in vitro. However, ZD55-ST13 showed no damage of normal fibroblast MRC5 cells. In exploring the mechanism of ZD55-ST13 in tumor cell killing, we found that ZD55-ST13-infected SW620 cells formed apoptotic bodies and presented obvious apoptosis phenomena. ZD55-ST13 induced the upregulation of Hsp70, the downregulation of antiapoptotic gene Bcl-2, and the release of cytochrome c. Cytochrome c triggered apoptosis by activating caspase-9 and caspase-3, which cleave the enzyme poly(ADP-ribose) polymerase in ZD55-ST13-infected SW620 cells. In summary, overexpressed ST13 as mediated by oncolytic adenovirus could exert potent antitumor activity via the intrinsic apoptotic pathway and has the potential to become a novel therapeutic for colorectal cancer gene therapy.

Yokoo, S., S. Masuda, et al. (2008). "Significance of organic cation transporter 3 (SLC22A3) expression for the cytotoxic effect of oxaliplatin in colorectal cancer." Drug Metab Dispos **36**(11): 2299-306.

The effect of oxaliplatin against colorectal cancer is superior to that of cisplatin, but the molecular mechanism(s) involved is not clear. We found previously that oxaliplatin, but not cisplatin, was transported by human (h) and rat organic cation transporter 3 (OCT3)/SLC22A3. In the present study, we examined whether hOCT3 was significantly involved in the oxaliplatin-induced cytotoxicity and accumulation of platinum in colorectal cancer. The level of hOCT3 mRNA in the colon was 9.7-fold higher in cancerous than in normal tissues in six Japanese patients ($P = 0.0247$). In human colorectal cancer-derived cell lines, the mRNA of hOCT3 was highly expressed compared with that of other organic cation transporters. The release of lactate dehydrogenase (LDH) and accumulation of platinum with oxaliplatin treatment were increased in SW480 cells transfected with hOCT3 cDNA compared with empty vector-transfected cells. T84 and SW837 cells, with high levels of hOCT3, released more LDH and accumulated more platinum after oxaliplatin treatment than low hOCT3-expressing cells such as SW480, HCT116, HT29, and Lovo. However, the amount of platinum accumulated after cisplatin treatment did not differ among these six cell lines. The levels of hOCT3 expression in colon and rectum were also higher in cancerous than in normal tissues in Caucasian patients as determined by dot blotting. In conclusion, the hOCT3-mediated uptake of oxaliplatin into the cancers was suggested to be important for its cytotoxicity, and hOCT3 expression may be a marker for cancer chemotherapy including oxaliplatin.

Young, G. P., Y. Hu, et al. (2005). "Dietary fibre and colorectal cancer: a model for environment--gene interactions." Mol Nutr Food Res **49**(6): 571-84.

As environmental factors are clearly associated with risk for colorectal cancer, we set out to model how dietary fibre, or the effects of its ingestion, might impact upon the complex events that

characterise colorectal oncogenesis. The diverse nature of dietary fibre and its resultant fate in the gut is outlined. The evidence indicates that different types of fibre create different conditions in different regions of the gut. This is reflected in different effects on oncogenesis especially in animal models. Data from animal models show that insoluble fibre is protective. Evidence from human studies are not consistent, especially considering the interventional studies. However, all such studies have been dependent on biomarkers short of cancer formation, for measurement of an effect. The biological and molecular events characteristic of colorectal oncogenesis are reviewed in an effort to identify how fibre ingestion might regulate oncogenesis. While several mechanisms might account for protection, the results of fermentation and especially butyrate production provide examples of how genomic instability might be controlled. Activation of apoptosis and cell cycle arrest seem likely to be mechanisms that would enable correction of genomic events that drive oncogenesis. Butyrate itself can regulate gene expression by both epigenetic and direct effects.

Zhang, B., X. X. Liu, et al. (2006). "Polyamine depletion by ODC-AdoMetDC antisense adenovirus impairs human colorectal cancer growth and invasion in vitro and in vivo." J Gene Med **8**(8): 980-9.

BACKGROUND: Polyamine biosynthesis is controlled primarily by ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (AdoMetDC). Polyamine concentrations are elevated in colorectal cancer. Depletion of polyamine content in colorectal cancer by chemotherapy is related to tumor regression and impaired tumorigenicity. The current study evaluates the therapeutic effects of antisense ODC and AdoMetDC sequences on colorectal cancer in vitro and in vivo. METHODS: Antisense ODC and AdoMetDC sequences were cloned into an adenoviral vector (Ad-ODC-AdoMetDCas). The human colon cancer cell lines, HT-29 and Caco-2, were infected with Ad-ODC-AdoMetDCas as well as with control vector. Viable cell counting, determination of polyamine concentrations, cell cycle analysis, and Matrigel invasion assays were performed in order to assess properties of tumor growth and invasiveness. Furthermore, the antitumor effects of Ad-ODC-AdoMetDCas were also evaluated in vivo in a nude mouse tumor model. RESULTS: Our study demonstrated that adenovirus-mediated ODC and AdoMetDC antisense expression inhibits tumor cell growth through a blockade of the polyamine synthesis pathway. This inhibitory effect cannot be reversed by the administration of putrescine. Tumor cells were arrested at the G1 phase of the cell cycle after gene transfer and had reduced invasiveness. The adenovirus also induced tumor regression in established tumors in nude mice. CONCLUSIONS: Our study suggests that Ad-ODC-AdoMetDCas has antitumor activity and therapeutic potential for the treatment of colorectal cancer.

Zhang, L. H., B. Tian, et al. (2006). "Dominant expression of 85-kDa form of cortactin in colorectal cancer." J Cancer Res Clin Oncol **132**(2): 113-20.

PURPOSE: Cortactin is commonly expressed in several human cancers, which may alter their invasive or metastatic properties. Eighty five kilodalton form (p85) and 80-kDa form (p80) of cortactin are two separate bands in SDS-PAGE representing different conformational states. The objective of this study was to investigate cortactin expression in colorectal cancer (CRC). EXPERIMENTAL DESIGN: Cortactin expression was studied in an eight paired laser capture microdissection (LCM) CRC tissues and matched non-cancerous epithelia by immunoblotting. The expression in 58 CRC and two cell lines, HCT8 and HCT116, was studied respectively by immunohistochemistry and confocal laser scanning immunofluorescence. RESULTS: Dominant expression of p85 was identified in LCM-procured CRC tissues compared with equal intensity of p85 and p80 forms in non-cancerous tissues, while the amount of total cortactin was approximate. Immunohistochemistry analysis demonstrated that cortactin located in the cytoplasm of tumor cells and adjacent non-cancerous cells, and its expression was negatively correlated with TNM staging and lymphatic invasion status. However, the invasion fronts in 3 of 58 primary tumors and 28 of 39 available lymph node metastases were intensively stained. Further, immunofluorescence analysis showed that cortactin was distributed in cytoplasm and enriched in the front of the extending lamellipodia at adhering side of cultured cancer cells. CONCLUSIONS: Our results demonstrated the dominant expression of p85 form of cortactin in CRC for the first time. The enrichment of cortactin in the invasion front of some tumor cells and in the extending lamellipodia of cultured cancer cells suggests that cortactin may help cancer cell movement.

Zhang, W., M. Gordon, et al. (2007). "FCGR2A and FCGR3A polymorphisms associated with clinical outcome of epidermal growth factor receptor expressing metastatic colorectal cancer patients treated with single-agent cetuximab." J Clin Oncol **25**(24): 3712-8.

PURPOSE: Cetuximab, a chimeric immunoglobulin G 1 (IgG1) anti-epidermal growth factor receptor (EGFR) monoclonal antibody (mAb), has shown efficacy in 10% of patients with metastatic colorectal cancer (CRC). Recent studies demonstrate antibody-dependent cell-mediated cytotoxicity (ADCC) is one of the modes of action for rituximab and trastuzumab. Fragment c (Fc) portion of IgG1 mAb has shown to induce ADCC. Fragment c gamma receptors (FcgammaR) play an important role in initiating ADCC. Studies have shown that two IgG FcgammaR polymorphisms (FCGR2A-H131R and FCGR3A-V158F) independently predict response to rituximab in patients with follicular lymphoma. We tested the hypothesis of whether these two polymorphisms are associated with clinical outcome in metastatic CRC patients treated with single-agent cetuximab. PATIENTS AND METHODS: Thirty-nine metastatic CRC patients were enrolled onto the ImClone0144 trial. Using an allele-specific polymerase chain reaction (PCR) -based method, gene polymorphisms of FCGA2A-H131R and FCGA3A-V158F were assessed from genomic DNA extracted from peripheral blood samples. RESULTS: FCGR2A-H131R and FCGR3A-V158F polymorphisms were independently associated with progression-free survival (PFS; P = .037 and .055, respectively; log-rank test). Combined analysis of these two polymorphisms showed that patients with the favorable genotypes (FCGR2A, any histidine allele, and FCGR3A, any phenylalanine allele) showed a median PFS of 3.7 months (95% CI, 2.4 to 4.4 months), whereas patients with any two unfavorable genotypes (FCGR2A arginine/arginine or valine/valine) had a PFS of 1.1 months (95% CI, 1.0 to 1.4 months; P = .004; log-rank test). CONCLUSION: Our preliminary data suggest that these two polymorphisms may be useful molecular markers to predict clinical outcome in metastatic CRC patients treated with cetuximab and that they may indicate a role of ADCC of cetuximab.

Zieglschmid, V., C. Hollmann, et al. (2007). "Tumor-associated gene expression in disseminated tumor cells correlates with disease progression and tumor stage in colorectal cancer." Anticancer Res **27**(4A): 1823-32.

BACKGROUND: A possible correlation of disease progression and tumor stage in colorectal cancer patients with tumor-associated gene expression in disseminated tumor cells (DTC) was evaluated. Detection of DTC and expression of tumor-associated genes might be of clinical value with respect to individual patient prognosis, monitoring of therapy and as a surrogate tumor staging parameter. PATIENTS AND METHODS: In a multicenter study, a total of 196 peripheral blood samples were collected from 76 patients with tumor stage Dukes' A to D and analyzed using a DTC detection assay consisting of immunomagnetic selection and expression analysis of the tumor-associated genes CEA, EGFR and GA733-2. DTC detection rates were assessed prior to surgery and post surgery in patients with tumor stage Dukes' A, B and C, and compared with results in metastatic patients. CEA serum protein levels were determined and compared with DTC and CEA expression, respectively. RESULTS: In a comparison analysis, EGFR and CEA expression was detected in 88% ($p = 0.001$) and 0% ($p = 0.002$) prior to surgery, in 66% ($p = 0.001$) and 20% ($p = 0.002$) post surgery, as well as in 15% ($p < 0.0001$) and 66% ($p < 0.0001$) of blood samples collected from metastatic patients, respectively. Expression of tumor-associated genes in DTC prior to surgery and in follow-up samples indicated an ongoing metastatic process. DTC detection rates in patients with Dukes' A (14%), Dukes' B (13%) and Dukes' C (40%) prior to surgery correlated statistically with the expected recurrence rate. There was no correlation between DTC expressing CEA and elevation of CEA serum protein levels. CONCLUSION: EGFR and CEA gene expression correlated with disease progression and tumor stage. Detection of CEA expression in DTC might have a predictive value in colorectal cancer and may help to identify patients at a greater risk of relapse. DTC in peripheral blood collected prior to surgery as well as in follow-up samples have a prognostic clinical value.

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