Cancer Metastasis

Mark H Smith

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

Abstract: Cancer is the cells that grow out of control. Cancer cells can also invade other tissues. Growing out of control and invading other tissues are what makes a cell a cancer cell. Involved in more than 100 diseases, the cancer can cause serious illness and death. Normally, the cells become cancer cells because of DNA damage. This material is a literature collection of the researches on the cancer merastasis.
[Smith MH. **Cancer Metastasis.** *Cancer Biology* 2012;2(4):274-339]. (ISSN: 2150-1041). http://www.cancerbio.net. 5

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

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

Aikawa, T., J. Gunn, et al. (2006). "Connective tissue growth factor-specific antibody attenuates tumor growth, metastasis, and angiogenesis in an orthotopic mouse model of pancreatic cancer." Mol Cancer Ther **5**(5): 1108-16.

Connective tissue growth factor (CTGF) plays an important role in fibrosis by modulating cell migration and cell growth but may also modify tumor growth and metastasis. Because CTGF is overexpressed in pancreatic ductal adenocarcinoma, we investigated the in vitro effects of CTGF on the proliferation and invasiveness of PANC-1 pancreatic cancer cells and examined the consequences of its in vivo inhibition on the growth and metastasis of these cells using a fully human CTGF-specific monoclonal antibody (FG-3019) in an orthotopic nude mouse model. Although PANC-1 cells expressed relatively high levels of endogenous CTGF mRNA, the addition of CTGF to conditioned medium increased the proliferation and invasiveness of PANC-1 cells. Moreover, transforming growth factor-beta1 caused a further increase in CTGF expression in these cells. In vivo, the twice weekly i.p. administration of FG-3019 decreased tumor growth and metastasis and attenuated tumor angiogenesis and cancer cell proliferation. FG-3019 did not enhance apoptosis and did not attenuate the inhibitory effects of gemcitabine on tumor growth and metastasis. These findings suggest that CTGF may contribute to aberrant autocrine and paracrine pathways that promote pancreatic cancer cell growth, invasion, metastasis, and angiogenesis. Therefore, blocking CTGF actions with FG-3019 may represent a novel therapeutic approach in pancreatic ductal adenocarcinoma.

Allan, A. L., R. George, et al. (2006). "Role of the integrin-binding protein osteopontin in lymphatic metastasis of breast cancer." Am J Pathol **169**(1): 233-46.

Although a primary route of breast cancer metastasis is believed to be via lymphatics, the molecular factors involved are poorly understood. We hypothesized that one such factor may be the integrin-binding protein osteopontin (OPN), and we investigated this clinically and experimentally. In breast cancer patients undergoing sentinel lymph node biopsy, OPN levels were significantly higher in lymph node metastases than in the primary tumor ($P < 0.001$). To test the functional contribution of OPN to lymphatic metastasis and to determine whether the RGD (Arg-Gly-Asp) integrin-binding sequence of OPN is important for this process, we transfected wild-type OPN or mutant OPN (lacking the RGD sequence) into MDA-MB-468 human breast cancer cells. In vitro, cells overexpressing OPN demonstrated increased anchorage-independent growth in soft agar ($P = 0.001$) and increased RGD-dependent adhesion ($P = 0.045$). Following mammary fat pad injection of nude mice, cells overexpressing OPN showed increased lymphovascular invasion, lymph node metastases, and lung micrometastases at earlier time points ($P = 0.024$). Loss of the RGD region partially abrogated this effect in the lymphatics ($P = 0.038$). These novel findings indicate that OPN is a key molecular player involved in lymphatic metastasis of breast cancer, potentially by affecting RGD-mediated

adhesive interactions and by enhancing the establishment/persistence of tumor cells in the lymphatics.

Arboleda, M. J., J. F. Lyons, et al. (2003). "Overexpression of AKT2/protein kinase Bbeta leads to up-regulation of beta1 integrins, increased invasion, and metastasis of human breast and ovarian cancer cells." Cancer Res **63**(1): 196-206.

To determine how AKT2 might contribute to tumor cell progression, a full-length, wild-type, human AKT2/protein kinase B (PKB)beta cDNA was transfected into a panel of eight human breast and ovarian cancer cells. AKT2 transfectants demonstrated increased adhesion and invasion through collagen IV because of up-regulation of beta1 integrins. In addition, AKT2 cells were more metastatic than control cells in vivo. Increased invasion by AKT2 was blocked by preincubation with an anti-beta1 integrin function blocking antibody, exposure to wortmannin, and by expression of phosphatase and tensin homologue tumor suppressor (PTEN). Confocal microscopy performed on transfected human breast cancer cells showed that unlike AKT1, AKT2 protein predominantly localized adjacent to the collagen IV matrix during cellular attachment. Overexpression of AKT2, but not AKT1 or AKT3, was sufficient to duplicate the invasive effects of phosphoinositide 3-OH kinase (PI3-K) transfected in breast cancer cells. Furthermore, expression of kinase dead AKT2(181 amino acid methionine [M]), and not kinase dead AKT1(179M) or AKT3(177M), was capable of blocking invasion induced by either human epidermal growth factor receptor-2 (HER-2) overexpression or by activation of PI3-K. Taken together, these data indicate that AKT2 mediates PI3-K-dependent effects on adhesion, motility, invasion, and metastasis in vivo.

Asanuma, K., T. Yoshikawa, et al. (2007). "Protein C inhibitor inhibits breast cancer cell growth, metastasis and angiogenesis independently of its protease inhibitory activity." Int J Cancer **121**(5): 955-65.

Protein C inhibitor (PCI) regulates the anticoagulant protein C pathway and also inhibits urinary plasminogen activator (uPA), a mediator of tumor cell invasion. In the present study, we evaluated the effect of human PCI and its inactive derivatives on tumor growth and metastasis of human breast cancer (MDA-231) cells, and on angiogenesis in vivo. The invasiveness of MDA-231 cells was inhibited by recombinant intact PCI, but not by reactive site-modified PCI (R354APCI) or by the N-terminal fragment of protease-cleaved PCI (NTPCI). The in vitro invasiveness of MDA-231 cells expressing intact PCI (MDA-PCI) was significantly decreased as compared to MDA-231 cells expressing R354APCI (MDA-R354APCI) or NTPCI (MDA-NTPCI). Further, in vivo growth and metastatic potential of MDA-PCI, MDA-R354APCI and MDA-NTPCI cells in severe combined immunodeficient (SCID) mice were significantly decreased as compared to MDA-Mock cells. Angiogenesis was also significantly decreased in Matrigel implant containing MDA-PCI, MDA-R354APCI or MDA-NTPCI cells as compared to that containing MDA-Mock cells. In vivo angiogenesis in rat cornea and in vitro tube formation were also inhibited by recombinant intact PCI, R354APCI and NTPCI. Furthermore, the anti-angiogenic activity of PCI was strong as cleaved antithrombin (AT), and slightly stronger than that of plasminogen activator inhibitor (PAI)-1 and pigment epithelium-derived factor (PEDF). Overall, this study showed that, in addition to a reactive site-dependent mechanism, PCI may also regulate tumor growth and metastasis independently of its protease inhibitory activity by inhibiting angiogenesis.

Atreya, I., C. C. Schimanski, et al. (2007). "The T-box transcription factor eomesodermin controls CD8 T cell activity and lymph node metastasis in human colorectal cancer." Gut **56**(11): 1572-8.

BACKGROUND/AIMS: An efficient cytolytic T cell function is essential for immune mediated rejection of colorectal cancer. However, the molecular mechanisms driving T cell mediated cancer rejection are still poorly understood. Here, we assessed the relevance of the T-box transcription factor eomesodermin in colorectal cancer. METHODS/ RESULTS: By analysing tissue probes from 88 different colorectal tumours, a significant ($p<0.02$) inverse correlation between eomesodermin expression in colorectal cancers and the presence of lymph node metastases could be shown, whereas no such correlation was noted for the master transcription factor of regulatory T cells, FoxP3 and CD8 alpha expression. To evaluate whether this effect might be due to effects of eomesodermin on tumour infiltrating CD8 T cells, we subsequently analysed the regulated expression and function of this transcription factor in human T cells. Whereas overexpression of this factor induced perforin but not granzyme expression, siRNA mediated suppression of eomesodermin expression led to significantly reduced IFN-gamma production, perforin levels and cytolytic activity of CD8 T cells. Furthermore, TGF-beta and IL4 could be identified as important inducer of eomesodermin expression. CONCLUSION: These data define for the first time a regulatory role of eomesodermin for CD8 T cell activity in humans. Our findings are consistent with a model in which eomesodermin expression in tumour infiltrating T cells regulates cytolytic functions of

CD8 T cells via perforin expression. These data provide novel insights into control mechanisms governing the functional activity of human CD8 T lymphocytes via T-box transcription factors in cancer.

Babyatsky, M., J. Lin, et al. (2009). "Trefoil factor-3 expression in human colon cancer liver metastasis." Clin Exp Metastasis **26**(2): 143-51.

Deaths from colorectal cancer are often due to liver metastasis. Trefoil factor-3 (TFF3) is expressed by normal intestinal epithelial cells and its expression is maintained throughout the colon adenoma-carcinoma sequence. Our previous work demonstrated a correlation between TFF3 expression and metastatic potential in an animal model of colon cancer. The aim of this study was to determine whether TFF3 is expressed in human colon cancer liver metastasis (CCLM) and whether inhibiting TFF3 expression in colon cancer cells would alter their invasive potential in vitro. Human CCLMs were analyzed at the mRNA and protein level for TFF3 expression. Two highly metastatic rat colon cancer cell lines that either natively express TFF3 (LN cells) or were transfected with TFF3 (LPCRI-2 cells), were treated with two rat TFF3 siRNA constructs (si78 and si365), and analyzed in an in vitro invasion assay. At the mRNA and protein level, TFF3 was expressed in 17/17 (100%) CCLMs and 10/11 (91%) primary colon cancers, but not in normal liver tissue. By real time PCR, TFF3 expression was markedly inhibited by both siRNA constructs in LN and LPCRI-2 cells. The si365 and si78 constructs inhibited invasion by 44% and 53%, respectively, in LN cells, and by 74% and 50%, respectively, in LPCRI-2 cells. These results provide further evidence that TFF3 contributes to the malignant behavior of colon cancer cells. These observations may have relevance for designing new diagnostic and treatment approaches to colorectal cancer.

Baker, D. L., Y. Fujiwara, et al. (2006). "Carba analogs of cyclic phosphatidic acid are selective inhibitors of autotaxin and cancer cell invasion and metastasis." J Biol Chem **281**(32): 22786-93.

Autotaxin (ATX, nucleotide pyrophosphate/phosphodiesterase-2) is an autocrine motility factor initially characterized from A2058 melanoma cell-conditioned medium. ATX is known to contribute to cancer cell survival, growth, and invasion. Recently ATX was shown to be responsible for the lysophospholipase D activity that generates lysophosphatidic acid (LPA). Production of LPA is sufficient to explain the effects of ATX on tumor cells. Cyclic phosphatidic acid (cPA) is a naturally occurring analog of LPA in which the sn-2 hydroxy group forms a 5-membered ring with the sn-3 phosphate. Cellular responses to cPA generally oppose those of LPA despite activation of apparently overlapping receptor populations, suggesting that cPA also activates cellular targets distinct from LPA receptors. cPA has previously been shown to inhibit tumor cell invasion in vitro and cancer cell metastasis in vivo. However, the mechanism governing this effect remains unresolved. Here we show that 3-carba analogs of cPA lack significant agonist activity at LPA receptors yet are potent inhibitors of ATX activity, LPA production, and A2058 melanoma cell invasion in vitro and B16F10 melanoma cell metastasis in vivo.

Bandyopadhyay, A., Y. Zhu, et al. (1999). "A soluble transforming growth factor beta type III receptor suppresses tumorigenicity and metastasis of human breast cancer MDA-MB-231 cells." Cancer Res **59**(19): 5041-6.

Transforming growth factor beta (TGF-beta) can promote late stage tumor progression in a number of model systems. In the present study, we have examined whether expression of a truncated soluble extracellular domain of TGF-beta type III receptor (sRIII) in human breast cancer MDA-MB-231 cells can antagonize the tumor-promoting activity of TGF-beta by sequestering active TGF-beta isoforms that are produced by the cancer cells. The secretion of sRIII reduced the amount of active TGF-beta1 and TGF-beta2 in the conditioned medium. This led to a significant reduction of the growth-inhibitory activity of the medium conditioned by sRIII-expressing cells on the growth of mink lung epithelial CCL64 cells in comparison with the medium conditioned by the control cells. The tumor incidence and growth rate of all of the three sRIII-expressing clones studied were significantly lower than those of the control cells in athymic nude mice. Four of five control cell-inoculated mice showed spontaneous metastasis in the lung, whereas none of the sRIII-expressing cell-inoculated mice had any lung metastasis. Thus, our results suggest that the sRIII may be used to antagonize the tumor-promoting activity of TGF-beta.

Bandyopadhyay, S., S. K. Pai, et al. (2004). "Role of the putative tumor metastasis suppressor gene Drg-1 in breast cancer progression." Oncogene **23**(33): 5675-81.

The differentiation-related gene-1 (Drg-1) was first identified as a gene strongly upregulated by induction of differentiation in colon carcinoma cells in vitro, and later the same gene was shown to suppress tumorigenicity of human bladder cancer cells in vivo. On the other hand, we and others have demonstrated that the Drg-1 gene suppresses prostate and colon cancer metastases in mouse models. In the context of

such potential organ-specific differential function of the Drg-1 gene, the present study was designed to clarify the expression status, regulation and function of Drg-1 in the case of human breast cancer. We found that the expression of the Drg-1 protein was significantly reduced in breast tumor cells, particularly in patients with lymph node or bone metastasis as compared to those with localized breast cancer. Drg-1 expression also exhibited significant inverse correlation with the disease-free survival rate of patients and emerged as an independent prognostic factor. The downregulation of the Drg-1 gene appeared to be largely at the RNA level, and the DNA methylation inhibitor, 5-Azacytidine, significantly elevated the Drg-1 gene expression in various breast tumor cell lines. Furthermore, we found that overexpression of the Drg-1 gene suppresses the invasiveness of breast cancer cells in vitro, and this suppression was also achieved by treatment of cells with 5-Azacytidine. Together, our results strongly suggest functional involvement of the Drg-1 gene in suppressing the metastatic advancement of human breast cancer.

Bandyopadhyay, S., Y. Wang, et al. (2006). "The tumor metastasis suppressor gene Drg-1 down-regulates the expression of activating transcription factor 3 in prostate cancer." Cancer Res **66**(24): 11983-90.

The tumor metastasis suppressor gene Drg-1 has been shown to suppress metastasis without affecting tumorigenicity in immunodeficient mouse models of prostate and colon cancer. Expression of Drg-1 has also been found to have a significant inverse correlation with metastasis or invasiveness in various types of human cancer. However, how Drg-1 exerts its metastasis suppressor function remains unknown. In the present study, to elucidate the mechanism of action of the Drg-1 gene, we did a microarray analysis and found that induction of Drg-1 significantly inhibited the expression of activating transcription factor (ATF) 3, a member of the ATF/cyclic AMP-responsive element binding protein family of transcription factors. We also showed that Drg-1 attenuated the endogenous level of ATF3 mRNA and protein in prostate cancer cells, whereas Drg-1 small interfering RNA up-regulated the ATF3 expression. Furthermore, Drg-1 suppressed the promoter activity of the ATF3 gene, indicating that Drg-1 regulates ATF3 expression at the transcriptional level. Our immunohistochemical analysis on prostate cancer specimens revealed that nuclear expression of ATF3 was inversely correlated to Drg-1 expression and positively correlated to metastases. Consistently, we have found that ATF3 overexpression promoted invasiveness of prostate tumor cells in vitro, whereas Drg-1 suppressed the invasive ability of these cells. More importantly, overexpression of ATF3 in prostate cancer cells significantly enhanced spontaneous lung metastasis of these cells without affecting primary tumorigenicity in a severe combined immunodeficient mouse model. Taken together, our results strongly suggest that Drg-1 suppresses metastasis of prostate tumor cells, at least in part, by inhibiting the invasive ability of the cells via down-regulation of the expression of the ATF3 gene.

Brakenhielm, E., J. B. Burton, et al. (2007). "Modulating metastasis by a lymphangiogenic switch in prostate cancer." Int J Cancer **121**(10): 2153-61.

Prostate cancer dissemination is difficult to detect in the clinic, and few treatment options exist for patients with advanced-stage disease. Our aim was to investigate the role of tumor lymphangiogenesis during metastasis. Further, we implemented a noninvasive molecular imaging technique to facilitate the assessment of the metastatic process. The metastatic potentials of several human prostate cancer xenograft models, LAPC-4, LAPC-9, PC3 and CWR22Rv-1 were compared. The cells were labeled with luciferase, a bioluminescence imaging reporter gene, to enable optical imaging. After tumor implantation the animals were examined weekly during several months for the appearance of metastases. Metastatic lesions were confirmed by immunohistochemistry. Additionally, the angiogenic and lymphangiogenic profiles of the tumors were characterized. To confirm the role of lymphangiogenesis in mediating metastasis, the low-metastatic LAPC-9 tumor cells were engineered to overexpress VEGF-C, and the development of metastases was evaluated. Our results show CWR22Rv-1 and PC3 tumor cell lines to be more metastatic than LAPC-4, which in turn disseminates more readily than LAPC-9. The difference in metastatic potential correlated with the endogenous production levels of lymphangiogenic growth factor VEGF-C and the presence of tumor lymphatics. In agreement, induced overexpression of VEGF-C in LAPC-9 enhanced tumor lymphangiogenesis leading to the development of metastatic lesions. Taken together, our studies, based on a molecular imaging approach for semiquantitative detection of micrometastases, point to an important role of tumor lymphatics in the metastatic process of human prostate cancer. In particular, VEGF-C seems to play a key role in prostate cancer metastasis.

Bulut, I., M. Meral, et al. (2009). "Analysis of HLA class I and II alleles regarding to lymph node and distant metastasis in patients with non-small cell lung cancer." Lung Cancer **66**(2): 231-6.

The aim of this study was to investigate the relation of HLA alleles in patients with non-small cell lung cancer (NSCLC). The incidence of class I and II HLA alleles of 63 patients with NSCLC were prospectively compared with the incidence of class I and II HLA alleles with 88 healthy controls. The number of cases with stage I and II (early stage) was 12 and there were 51 cases with stage III and IV (advanced stage). Metastasis rates of the regional lymph node in patients were as follow; N(0): n=10; N(1): n=13; N(2): n=26 and N(3): n=14. Lymph node metastasis was detected by pathological staging in 15 cases and by clinical staging in 48 cases. Lymph node metastasis was searched in all patients by a helical thorax CT. All distant metastasis were investigated by thorax CT, abdominal CT, brain CT or MRI and bone scintigraphy, and distant organ metastasis was detected in 25 cases. The patients and healthy controls were typed for HLA class I and II alleles. HLA-A2 was an independent risk factor for both critical lymph node (N(2 and 3)) involvement and distant metastasis. HLA-B44, -CW6 and -CW7 frequencies appear to be significant in controls compared to patients. HLA-A2 frequency was higher in patients with advanced stage than early stage, while HLA-A26, -B35 and -CW4 frequencies were more expressed in patients with early stage than in patients with advanced stage. Compared with controls, frequency of HLA-DRB1*07, -DQ02 and -DQ07 were lower expressed in patients. Compared patients with advanced stage, HLA-DRB1*07 was higher in patients with early stage. HLA-A2 was an independent risk factor for lymph node and distant metastasis, and the allele was significantly higher in patients with critical lymph node for surgery and distant metastasis. HLA-A26 appeared to be a significance protective allele against to metastases.

Burton, J. B., S. J. Priceman, et al. (2008). "Suppression of prostate cancer nodal and systemic metastasis by blockade of the lymphangiogenic axis." Cancer Res **68**(19): 7828-37.

Lymph node involvement denotes a poor outcome for patients with prostate cancer. Our group, along with others, has shown that initial tumor cell dissemination to regional lymph nodes via lymphatics also promotes systemic metastasis in mouse models. The aim of this study was to investigate the efficacy of suppressive therapies targeting either the angiogenic or lymphangiogenic axis in inhibiting regional lymph node and systemic metastasis in subcutaneous and orthotopic prostate tumor xenografts. Both androgen-dependent and more aggressive androgen-independent prostate tumors were used in our investigations. Interestingly, we observed that the threshold for dissemination is lower in the vascular-rich prostatic microenvironment compared with subcutaneously grafted tumors. Both vascular endothelial growth factor-C (VEGF-C) ligand trap (sVEGFR-3) and antibody directed against VEGFR-3 (mF4-31C1) significantly reduced tumor lymphangiogenesis and metastasis to regional lymph nodes and distal vital organs without influencing tumor growth. Conversely, angiogenic blockade by short hairpin RNA against VEGF or anti-VEGFR-2 antibody (DC101) reduced tumor blood vessel density, significantly delayed tumor growth, and reduced systemic metastasis, although it was ineffective in reducing lymphangiogenesis or nodal metastasis. Collectively, these data clarify the utility of vascular therapeutics in prostate tumor growth and metastasis, particularly in the context of the prostate microenvironment. Our findings highlight the importance of lymphangiogenic therapies in the control of regional lymph node and systemic metastasis.

Chen, H. W., J. Y. Lee, et al. (2008). "Curcumin inhibits lung cancer cell invasion and metastasis through the tumor suppressor HLJ1." Cancer Res **68**(18): 7428-38.

Curcumin (diferuloylmethane) is an active component of the spice turmeric and has a diversity of antitumor activities. In this study, we found that curcumin can inhibit cancer cell invasion and metastasis through activation of the tumor suppressor DnaJ-like heat shock protein 40 (HLJ1). Human lung adenocarcinoma cells (CL1-5) treated with curcumin (1-20 mumol/L) showed a concentration-dependent reduction in cell migration, invasion, and metastatic ability, and this was associated with increased HLJ1 expression. Knockdown of HLJ1 expression by siRNA was able to reverse the curcumin-induced anti-invasive and antimetastasis effects in vitro and in vivo. The HLJ1 promoter and enhancer in a luciferase reporter assay revealed that curcumin transcriptionally up-regulates HLJ1 expression through an activator protein (AP-1) site within the HLJ1 enhancer. JunD, one of the AP-1 components, was significantly up-regulated by curcumin (1-20 mumol/L) in a concentration- and time-dependent manner. Knockdown of JunD expression could partially reduce the curcumin-induced HLJ1 activation and diminish the anti-invasive effect of curcumin, indicating that JunD would seem to be involved in curcumin-induced HLJ1 expression. Curcumin was able to induce c-Jun NH(2)-kinase (JNK) phosphorylation, whereas the JNK inhibitor (SP-600125) could attenuate curcumin-induced JunD and HLJ1 expression. Activation of HLJ1 by curcumin further leads to up-regulation of E-cadherin and a suppression of cancer cell invasion. Our results show that curcumin induces HLJ1, through activation of the JNK/JunD pathway, and inhibits lung

cancer cell invasion and metastasis by modulating E-cadherin expression. This is a novel mechanism and supports the application of curcumin in anti-cancer metastasis therapy.

Chen, J., P. M. Stavro, et al. (2002). "Dietary flaxseed inhibits human breast cancer growth and metastasis and downregulates expression of insulin-like growth factor and epidermal growth factor receptor." Nutr Cancer **43**(2): 187-92.

Recent studies indicate that diets rich in phytoestrogens and n-3 fatty acid have anticancer potential. This study determined the effect of flaxseed (FS), the richest source of lignans and alpha-linolenic acid, on growth and metastasis of established human breast cancer in a nude mice model. Estrogen receptor-negative human breast cancer cells, MDA-MB-435, were injected into the mammary fat pad of mice (Ncr nu/nu) fed a basal diet (BD). At Week 8, mice were randomized into two diet groups, such that the groups had similar tumor size and body weight. One continued on the BD, while the other was changed to BD supplemented with 10% FS, until sacrifice at Week 15. A significant reduction ($P < 0.05$) in tumor growth rate and a 45% reduction ($P = 0.08$) in total incidence of metastasis were observed in the FS group. Lung metastasis incidence was 55.6% in the BD group and 22.2% in the FS group, while the lymph node metastasis incidence was 88.9% in the BD group and 33.3% in the FS group ($P < 0.05$). Mean tumor number (tumor load) of total and lymph node metastasis was significantly lower in the FS than in the BD group ($P < 0.05$). Metastatic lung tumor number was reduced by 82%, and a significantly lower tumor trend ($P < 0.01$) was observed in the FS group. Lung weight, which also reflects metastatic tumor load, in the FS group was reduced by 20% ($P < 0.05$) compared with the BD group. Immunohistochemical study showed that Ki-67 labeling index and expression of insulin-like growth factor I and epithelial growth factor receptor in the primary tumor were lower in the FS ($P < 0.05$) than in the BD group. In conclusion, flaxseed inhibited the established human breast cancer growth and metastasis in a nude mice model, and this effect is partly due to its downregulation of insulin-like growth factor I and epidermal growth factor receptor expression.

Chen, J. H., H. H. Lin, et al. (2008). "Gaseous nitrogen oxide promotes human lung cancer cell line A549 migration, invasion, and metastasis via iNOS-mediated MMP-2 production." Toxicol Sci **106**(2): 364-75.

Gaseous nitrogen oxide (gNO) is an important indoor and outdoor air pollutant. Many studies have indicated gNO causes lung tissue damage by its oxidation properties and free radicals. However, there are considerably few data on the association between lung cancer and gNO exposure. The purpose of this study was to examine whether gNO could contribute to the process of malignant progression of lung cancer. The results of wound-healing assay and in vitro transwell assay revealed that gNO-induced dose and time dependently the migration and invasion of A549 cells, a human lung cancer cell line, under noncytotoxic concentrations. gNO was able to induce release of NO from A549 cells, an effect that was mediated via the activation of inducible nitric oxide synthases (iNOS), but not constitutive isoforms, during the same treatment period. An increased expression of matrix metalloproteinase (MMP) and a coincided reduction in repress tissue inhibitors of metalloprotease-2 were observed upon the treatment of gNO. The gNO-mediated MMP-2 induction appeared to be a consequence of nuclear factor kappa B and activation protein-1 activation, because that their DNA binding activity was enhanced by gNO. All these influences of gNO were efficiently repressed by the pretreatment of a NOS inhibitor (N(G)-nitro-L-arginine methyl ester). Using a mouse model, we showed that gNO promoted A549 metastasis to the lung through a mechanism involving the iNOS-dependent MMP-2 activity. Our data imply that gNO exposure, which in turn led to iNOS activation and the enhancement of MMP-mediated cellular events, was related to lung cancer development.

Chen, Y. J., W. M. Chang, et al. (2009). "A small-molecule metastasis inhibitor, norcantharidin, downregulates matrix metalloproteinase-9 expression by inhibiting Sp1 transcriptional activity in colorectal cancer cells." Chem Biol Interact **181**(3): 440-6.

Norcantharidin (NCTD) is a small-molecule metastasis inhibitor without renal toxicity derived from a renal toxic compound cantharidin, which is found in blister beetles (Mylabris phalerata Pall.), commonly used in traditional Chinese medicine. The anti-metastatic capacity of NCTD is apparently through the downexpression of matrix metalloproteinase-9 (MMP-9) activity. The aim of this study was to clarify the transcriptional regulation of MMP-9 gene by NCTD in colorectal cancer CT-26 cells. NCTD not only downregulated MMP-9 mRNA and protein expression, but also inhibited gelatinase activity in a concentration- and time-dependent manner. In CT26 cells with transfection of cis-element reporter plasmids, NCTD treatment decreased reporter luciferase activity from a Sp1 construct, augmented with a NF-kappaB construct, but this did not occur with an AP-1 construct. Further transfecting with constructs containing wild-type or various mutant

MMP-9 promoters in CT26 cells indicated that Sp1, but not the others, was required for NCTD-inhibition of MMP-9 promoter transactivation. More evidence by electrophoretic mobility shift assay demonstrated that NCTD inhibited the DNA-binding activity of Sp1. In addition, the increase effect of NF-kappaB-luciferase activity by NCTD may include the upexpression of nuclear STAT1 and result in competitive suppression of NF-kappaB-binding activity in MMP-9 promoter. In conclusion, the metastasis inhibitor NCTD downregulates MMP-9 expression by inhibiting Sp1 transcriptional activity in colorectal cancer CT26 cells.

Chien, C. W., S. C. Lin, et al. (2008). "Regulation of CD151 by hypoxia controls cell adhesion and metastasis in colorectal cancer." Clin Cancer Res **14**(24): 8043-51.

PURPOSE: The first step of metastasis is the detachment of cancer cells from the surrounding matrix and neighboring cells; however, how cancer cells accomplish this process remains unclear. Thus, we aimed to investigate the underlying mechanism that controls the early event of metastasis. EXPERIMENTAL DESIGN: One hundred and thirty-seven paired colorectal carcinoma and normal colon tissues were examined by immunohistochemical staining and Western blot for the expression of CD151, a member of the tetraspanin family that plays important roles in cell adhesion and motility. The effect of CD151 on cancer cell adhesion was investigated under normoxia and hypoxia conditions. RESULTS: The level of CD151 was down-regulated in colon cancer compared with the paired normal counterparts. Expression of CD151 was negatively regulated by hypoxia inducible factor-1-dependent hypoxic stress. Suppression of CD151 by hypoxia caused the detachment of cancer cells from the surrounding matrix and neighboring cells whereas restoration of CD151 expression during reoxygenation facilitated the adhesion capacity. Clinical examination further showed that metastasized cancer cells expressed a greater level of CD151 compared with that of primary tumor. CONCLUSION: Regulation of CD151 by oxygen tension may play an important role in cancer metastasis by regulating the detachment from the primary site and homing in the secondary site.

Chien, C. Y., C. Y. Su, et al. (2006). "High expressions of CD105 and VEGF in early oral cancer predict potential cervical metastasis." J Surg Oncol **94**(5): 413-7.

BACKGROUND AND OBJECTIVES: Although elective neck dissection has become a popular treatment modality for early oral cancer among most head and neck surgeons, a large portion of patients revealed pathological N0 postoperatively. Our study is aimed to evaluate the expressions of the angiogenic factors, vascular endothelial growth factor (VEGF), and CD105 (endoglin) on the prediction of neck metastasis for clinical N0 patients in early oral cancer. PATIENTS AND METHODS: Between July 1996 and July 2005, the preoperative biopsy specimens among 176 patients who underwent surgical treatment for early oral cancer were retrieved for the immunohistochemical study for VEGF and CD105. RESULTS: High expressions of CD105 and VEGF significantly correlated with positive nodal metastasis, respectively ($P<0.001$). The expression of CD105 also significantly correlated with that of VEGF ($P<0.001$). Furthermore, the sensitivity and specificity for prediction of cervical metastasis by high expressions of CD105 versus VEGF were 81.8% and 97.7% versus 93.2% and 72%, respectively. Low expression of CD105 and VEGF also significantly correlated with higher survival rate, respectively ($P<0.001$). CONCLUSIONS: Besides the image studies, the expressions of both CD105 and VEGF could be useful to guide the elective treatment for clinical N0 neck in early oral cancer.

Choo, M. K., H. Sakurai, et al. (2008). "A ginseng saponin metabolite suppresses tumor necrosis factor-alpha-promoted metastasis by suppressing nuclear factor-kappaB signaling in murine colon cancer cells." Oncol Rep **19**(3): 595-600.

SC-514, an inhibitor of IkappaB kinase beta (IKKbeta), blocked the TNF-alpha-induced activation of nuclear factor-kappaB (NF-kappaB) as well as the TNF-alpha-promoted metastasis of murine colon adenocarcinoma cells. We investigated the effect of 20-O-beta-D-glucopyranosyl-20(S)-protopanaxadiol (M1), a main intestinal bacterial metabolite of ginseng, on the NF-kappaB-dependent metastasis. M1 was effective in suppressing the TNF-alpha-induced activation of NF-kappaB, expression of matrix metalloprotease-9 (MMP-9), migration and invasion. The TNF-alpha-evoked increase in lung and liver metastasis of colon carcinoma was also abrogated by treatment with M1 in vitro. These results suggest that ginseng has potential to suppress inflammation-related metastasis by downregulating the NF-kappaB signaling pathway.

Choo, M. K., H. Sakurai, et al. (2006). "TAK1-mediated stress signaling pathways are essential for TNF-alpha-promoted pulmonary metastasis of murine colon cancer cells." Int J Cancer **118**(11): 2758-64.

We have recently established a TNF-alpha-promoted metastasis model, in which the ability to metastasize to the lung was enhanced by stimulation

of cultured colon 26 cells with TNF-alpha before intravenous inoculation. To investigate intracellular events in metastatic cascades of TNF-alpha-treated cancer cells, we have focused on the stress signaling pathways to c-Jun N-terminal kinase (JNK) and p38. Treatment with a specific inhibitor, SP600125 or SB203580, in vitro suppressed TNF-alpha-induced migration and pulmonary metastasis. Activation of endogenous TAK1, a mitogen-activated protein kinase (MAP3K) regulating the JNK and p38 MAPK pathways, was induced rapidly by TNF-alpha, and co-transfection of TAK1 with its activator protein TAB1 stimulated activation of JNK and p38 MAPKs, which led to activation of the transcription factor AP-1. The activation of stress signaling pathways by TAK1 resulted in enhanced migration to fibronectin in vitro and metastasis to the lung in vivo without affecting cell proliferation in vitro and tumor growth in vivo. Moreover, knockdown of endogenous TAK1 using small interfering RNA (siRNA) suppressed the TNF-alpha-induced JNK/p38 activation, migration and pulmonary metastasis. These results indicate that TAK1-mediated stress signaling pathways in cancer cells are essential for TNF-alpha-promoted metastasis to the lung.

Cicek, M., R. Fukuyama, et al. (2005). "Breast cancer metastasis suppressor 1 inhibits gene expression by targeting nuclear factor-kappaB activity." Cancer Res **65**(9): 3586-95.

Breast cancer metastasis suppressor 1 (BRMS1) functions as a metastasis suppressor gene in breast cancer and melanoma cell lines, but the mechanism of BRMS1 suppression remains unclear. We determined that BRMS1 expression was inversely correlated with that of urokinase-type plasminogen activator (uPA), a prometastatic gene that is regulated at least in part by nuclear factor-kappaB (NF-kappaB). To further investigate the role of NF-kappaB in BRMS1-regulated gene expression, we examined NF-kappaB binding activity and found an inverse correlation between BRMS1 expression and NF-kappaB binding activity in MDA-MB-231 breast cancer and C8161.9 melanoma cells stably expressing BRMS1. In contrast, BRMS1 expression had no effect on activation of the activator protein-1 transcription factor. Further, we showed that suppression of both constitutive and tumor necrosis factor-alpha-induced NF-kappaB activation by BRMS1 may be due to inhibition of IkappaBalpha phosphorylation and degradation. To examine the relationship between BRMS1 and uPA expression in primary breast tumors, we screened a breast cancer dot blot array of normalized cDNA from 50 breast tumors and corresponding normal breast tissues. There was a significant reduction in BRMS1 mRNA expression in breast tumors compared with matched normal breast tissues (paired t test, $P < 0.0001$) and a general inverse correlation with uPA gene expression ($P < 0.01$). These results suggest that at least one of the underlying mechanisms of BRMS1-dependent suppression of tumor metastasis includes inhibition of NF-kappaB activity and subsequent suppression of uPA expression in breast cancer and melanoma cells.

Cicek, M. and M. J. Oursler (2006). "Breast cancer bone metastasis and current small therapeutics." Cancer Metastasis Rev **25**(4): 635-44.

Patients with advanced breast cancer frequently develop metastasis to bone. Bone metastasis results in intractable pain and a high risk of fractures due to tumor-driven bone loss (osteolysis), which is caused by increased osteoclast activity. Osteolysis releases bone-bound growth factors including transforming growth factor beta (TGF-beta). The widely accepted model of osteolytic bone metastasis in breast cancer is based on the hypothesis that the TGF-beta released during osteolytic lesion development stimulates tumor cell parathyroid hormone related protein (PTHrP), causing stromal cells to secrete receptor activator of NFkappaB ligand (RANKL), thus increasing osteoclast differentiation. Elevated osteoclast numbers results in increased bone resorption, leading to more TGF-beta being released from bone. This interaction between tumor cells and the bone microenvironment results in a vicious cycle of bone destruction and tumor growth. Bisphosphonates are commonly prescribed small molecule therapeutics that target tumor-driven osteoclastic activity in osteolytic breast cancers. In addition to bisphosphonate therapies, steroidal and non-steroidal antiestrogen and adjuvant therapies with aromatase inhibitors are additional small molecule therapies that may add to the arsenal for treatment of osteolytic breast cancer. This review focuses on a brief discussion of tumor-driven osteolysis and the effects of small molecule therapies in reducing osteolytic tumor progression.

Dabrosin, C., J. Chen, et al. (2002). "Flaxseed inhibits metastasis and decreases extracellular vascular endothelial growth factor in human breast cancer xenografts." Cancer Lett **185**(1): 31-7.

Angiogenesis is important in tumor growth, progression and metastatic dissemination. Vascular endothelial growth factor (VEGF) is one key factor in promotion of breast cancer angiogenesis. VEGFs are bioactive in the extracellular space where they become available to the endothelial cells. Phytoestrogens such as lignans have been shown to alter breast cancer incidence and be cancer-protective in rats. We show that supplementation of 10% flaxseed, the richest

source of mammalian lignans, to nude mice with established human breast tumors reduced tumor growth and metastasis. Moreover, flaxseed decreased extracellular levels of VEGF, which may be one mechanistic explanation to the decreased tumor growth and metastasis.

Dai, L., L. Gu, et al. (2009). "TWEAK promotes ovarian cancer cell metastasis via NF-kappaB pathway activation and VEGF expression." Cancer Lett **283**(2): 159-67.

The poor prognosis of human ovarian cancer is partly due to its metastasis and recurrence. It has been demonstrated that tumor necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK)-fibroblast growth factor inducible-14 (Fn14) signaling system may be a potential regulator of human tumorigenesis. The objective of this study was to understand the effect of TWEAK on ovarian cancer metastasis. We recently showed that activation of Fn14 signaling by TWEAK promoted cell migration and invasion in human HO-8910PM cells. Treating HO-8910PM cells with TWEAK resulted in the activation of nuclear factor-kappa B (NF-kappaB) and subsequently the translocation of NF-kappaB from cytoplasm to nucleus. In addition, TWEAK promoted vascular endothelial growth factor (VEGF) protein expression, and this effect was dependent upon NF-kappaB transcriptional activity. Blocking the NF-kappaB pathway with PDTC suppressed TWEAK-induced up-regulation of VEGF protein expression and cell metastasis. Our results suggest that TWEAK-Fn14 functions, in part, through the NF-kappaB signaling pathway to up-regulate VEGF expression to foster ovarian cancer cell metastasis. Targeted therapy against TWEAK-Fn14 signaling system as an adjuvant to surgery may improve clinical management of invasive ovarian cancer cells and advance the outcome of this devastating cancer.

Das Roy, L., L. B. Pathangey, et al. (2009). "Breast-cancer-associated metastasis is significantly increased in a model of autoimmune arthritis." Breast Cancer Res **11**(4): R56.

INTRODUCTION: Sites of chronic inflammation are often associated with the establishment and growth of various malignancies including breast cancer. A common inflammatory condition in humans is autoimmune arthritis (AA) that causes inflammation and deformity of the joints. Other systemic effects associated with arthritis include increased cellular infiltration and inflammation of the lungs. Several studies have reported statistically significant risk ratios between AA and breast cancer. Despite this knowledge, available for a decade, it has never been questioned if the site of chronic inflammation linked to AA creates a milieu that attracts tumor cells to home and grow in the inflamed bones and lungs which are frequent sites of breast cancer metastasis. METHODS: To determine if chronic inflammation induced by autoimmune arthritis contributes to increased breast cancer-associated metastasis, we generated mammary gland tumors in SKG mice that were genetically prone to develop AA. Two breast cancer cell lines, one highly metastatic (4T1) and the other non-metastatic (TUBO) were used to generate the tumors in the mammary fat pad. Lung and bone metastasis and the associated inflammatory milieu were evaluated in the arthritic versus the non-arthritic mice. RESULTS: We report a three-fold increase in lung metastasis and a significant increase in the incidence of bone metastasis in the pro-arthritic and arthritic mice compared to non-arthritic control mice. We also report that the metastatic breast cancer cells augment the severity of arthritis resulting in a vicious cycle that increases both bone destruction and metastasis. Enhanced neutrophilic and granulocytic infiltration in lungs and bone of the pro-arthritic and arthritic mice and subsequent increase in circulating levels of proinflammatory cytokines, such as macrophage colony stimulating factor (M-CSF), interleukin-17 (IL-17), interleukin-6 (IL-6), vascular endothelial growth factor (VEGF), and tumor necrosis factor-alpha (TNF-alpha) may contribute to the increased metastasis. Treatment with anti-IL17 + celecoxib, an anti-inflammatory drug completely abrogated the development of metastasis and significantly reduced the primary tumor burden. CONCLUSIONS: The data clearly has important clinical implications for patients diagnosed with metastatic breast cancer, especially with regards to the prognosis and treatment options.

Dasgupta, S., M. Bhattacharya-Chatterjee, et al. (2006). "Tumor metastasis in an orthotopic murine model of head and neck cancer: possible role of TGF-beta 1 secreted by the tumor cells." J Cell Biochem **97**(5): 1036-51.

In an orthotopic murine model of head and neck cancer, combined subcutaneous and intratumoral vaccination with recombinant vaccinia virus expressing interleukin-2 (rvv-IL-2) induced significant tumor regression early on therapy. However, its efficacy was restricted by recurrent tumor growth and loco-regional metastases. In this study, we explored the mechanism of tumor metastasis. We compared the levels of expression of a number of molecules involved in tumor metastasis, which included transforming growth factor-beta1 (TGF-beta1), E-cadherin, matrix metalloproteinases (MMPs): MT1-MMP, MMP-2, MMP-9, their tissue inhibitors (TIMPs): TIMP-1/TIMP-2, and pro-angiogenic factors

CD31, VEGF-R2, and iNOS between primary and metastatic tumors by real-time RT-PCR and immunohistochemistry. We detected spontaneous lymph node and tongue metastasis. Metastasis was delayed in rvv-IL-2 treated mice. Cultured tumor cells expressed negligible amount of TGF-beta1. Untreated or metastatic tumors, on the other hand, expressed high levels of TGF-beta1 and secreted TGF-beta1 in the sera of tumor-bearing mice. Levels of TGF-beta1 in the sera suddenly jumped at the time when tumor metastasis started. In the metastatic tumors, levels of MT1-MMP, MMP-2, and MMP-9 were significantly elevated ($P < 0.001$), while levels of TIMP-1/TIMP-2 and E-cadherin were decreased ($P < 0.001$) compared to control or primary tumors. Levels of CD31, VEGF-R2, and iNOS were also significantly elevated in the metastatic lesions ($P < 0.001$). The concurrence of high levels of TGF-beta1 in the sera, expression of proteins involved in metastasis and initiation of metastasis suggested possible role of TGF-beta1 in on setting the metastatic cascade in this model.

DiMeo, T. A., K. Anderson, et al. (2009). "A novel lung metastasis signature links Wnt signaling with cancer cell self-renewal and epithelial-mesenchymal transition in basal-like breast cancer." Cancer Res **69**(13): 5364-73.

The establishment of metastasis depends on the ability of cancer cells to acquire a migratory phenotype combined with their capacity to recreate a secondary tumor in a distant tissue. In epithelial cancers, such as those of the breast, the epithelial-mesenchymal transition (EMT) is associated with basal-like breast cancers, generates cells with stem-like properties, and enables cancer cell dissemination and metastasis. However, the molecular mechanism(s) that connects stem cell-like characteristics with EMT has yet to be defined. Using an orthotopic model of human breast cancer metastasis to lung, we identified a poor prognosis gene signature, in which several components of the wnt signaling pathway were overexpressed in early lung metastases. The wnt genes identified in this signature were strongly associated with human basal-like breast cancers. We found that inhibiting wnt signaling through LRP6 reduced the capacity of cancer cells to self-renew and seed tumors in vivo. Furthermore, inhibition of wnt signaling resulted in the reexpression of breast epithelial differentiation markers and repression of EMT transcription factors SLUG and TWIST. Collectively, these results provide a molecular link between self-renewal, EMT, and metastasis in basal-like breast cancers.

Freudenberg, J. A., Q. Wang, et al. (2009). "The role of HER2 in early breast cancer metastasis and the origins of resistance to HER2-targeted therapies." Exp Mol Pathol **87**(1): 1-11.

The HER2 gene encodes the receptor tyrosine kinase HER2 and is often over-expressed or amplified in breast cancer. Up-regulation of HER2 contributes to tumor progression. Many aspects of tumor growth are favorably affected through activation of HER2 signaling. Indeed, HER2 plays a role in increasing proliferation and survival of the primary tumor and distant lesions which upon completion of full transformation cause metastases. P185(HER2/neu) receptors and signaling from them and associated molecules increase motility of both intravasating and extravasating cells, decrease apoptosis, enhance signaling interactions with the microenvironment, regulate adhesion, as well as a multitude of other functions. Recent experimental and clinical evidence supports the view that the spread of incompletely transformed cells occurs at a very early stage in tumor progression. This review concerns the identification and characterization of HER2, the evolution of the metastasis model, and the more recent cancer stem cell model. In particular, we review the evidence for an emerging mechanism of HER2(+) breast cancer progression, whereby the untransformed HER2-expressing cell shows characteristics of stem/progenitor cell, metastasizes, and then completes its final transformation at the secondary site.

Fu, H., Z. Hu, et al. (2009). "TGF-beta promotes invasion and metastasis of gastric cancer cells by increasing fascin1 expression via ERK and JNK signal pathways." Acta Biochim Biophys Sin (Shanghai) **41**(8): 648-56.

Transforming growth factor-beta (TGF-beta) is involved in actin cytoskeleton reorganization and tumor progression. Fascin1, an actin-binding protein, increases cell invasiveness and motility in various transformed cells. To determine whether fascin1 is an important mediator of the tumor response to TGF-beta, we applied the small interfering RNA (siRNA) technique to silence fascin1 in gastric cancer (GC) cells MKN45. Results showed that the effects of TGF-beta1 on GC cells invasion and metastasis were mediated by tumor production of fascin1; furthermore, it was found that TGF-beta1- induced fascin1 expression was suppressed by the specific inhibitors of JNK and ERK pathways, SP6001125 and PD98059, respectively, but not by transient transfection of Smad2 and Smad4 siRNA. Our data for the first time demonstrated that fascin1 is an important mediator of TGF-beta1-induced invasion and metastasis of GC cells, which involves JNK and ERK signaling pathways.

Fukunaga, S., K. Maeda, et al. (2006). "Association between expression of vascular endothelial growth factor C, chemokine receptor CXCR4 and lymph node metastasis in colorectal cancer." Oncology **71**(3-4): 204-11.

OBJECTIVES: Lymph node metastasis is one of the determining factors of a poor prognosis for colorectal cancer. Recent studies have reported that cancer cells can promote lymphangiogenesis and that chemokine receptors expressed by cancer cells might play a role in metastasis. In this study, we examined the correlation between the expression of vascular endothelial growth factor (VEGF) C, the chemokine receptor CXCR4 and lymph node metastasis in colorectal cancer. METHODS: One hundred and sixty-one consecutive patients who underwent resection at our department were studied. Lymph node metastasis was observed in 69 cases (43%) and lymphatic involvement was present in 105 cases (65%). Immunohistochemical staining was performed using antibodies for VEGF-C and CXCR4. Moreover, lymphatic vessel density (LVD) was evaluated within the tumor by immunostaining with a D2-40 antibody. RESULTS: VEGF-C expression was found in 81 cases (50%) and CXCR4 expression in 87 cases (54%). Regarding the correlation between nodal metastasis and the expression of CXCR4 and VEGF-C, the incidence of nodal metastasis was significantly ($p < 0.01$) higher in patients with CXCR4-positive tumors than in those with CXCR4-negative tumors. In addition, a significant correlation was observed between CXCR4 and VEGF-C expression and lymphatic invasion ($p < 0.01$). LVD was significantly higher in VEGF-C-positive tumors compared with VEGF-C-negative tumors. However, there was no significant correlation between LVD and CXCR4 expression. Using multivariate analysis, VEGF-C, CXCR4, lymphatic invasion and wall invasion were found to be independent risk factors for lymph node metastasis. CONCLUSIONS: This study suggests that although the mechanism that promoted lymph node metastasis was different between VEGF-C and CXCR4, both VEGF-C and CXCR4 contributed to lymphatic involvement and nodal metastasis in colorectal cancer.

Gomes, R. R., Jr., P. Buttke, et al. (2009). "Osteosclerotic prostate cancer metastasis to murine bone are enhanced with increased bone formation." Clin Exp Metastasis **26**(7): 641-51.

Spontaneous development of osteoblastic lesions of prostate cancer (PCa) in mice is modeled by orthotopic (intraprostatic) deposition of neoplastic cells followed by an extremely long latency associated with low incidence of spontaneous bone metastasis. Intracardial injection results in overt bone metastases only with osteoclastic PCa cells (i.e., PC-3). Herein, we report that androgen independent osteoblastic PCa cells readily colonize bone when in a high remodeling state. SCID/Beige mice were subjected to periods of intermittent human parathyroid hormone 1-34 (hPTH) exposure, followed by an intracardiac infusion of osteoblastic C4-2 PCa cells. At the time of PCa infusion, analysis of bone turnover markers from mice treated with hPTH revealed significant increases in osteocalcin (55.06 +/- 7.5 vs. 74.01 +/- 18.5 ng/ml) and TRAcP-5b (3.3 +/- 0.6 vs. 4.81 +/- 0.8 U/l), but no change in type I collagen C-terminal teleopeptide levels relative to control mice. Analysis of femoral cancellous bone architecture revealed significant increases in bone mineral density, trabecular thickness (0.056 +/- 0.002 vs. 0.062 +/- 0.001 mm) and porosity, but significant decreases in connectivity density and trabecular number in hPTH treated mice relative to controls. By 8 weeks post-infusion, 70% of mice pre-treated with hPTH demonstrated detectable serum prostate specific antigen (PSAs) ranging between 2 and 18.8 ng/ml. Immuno-histochemical labeling of femurs for PSA and pan-Cytokeratin revealed the presence of significant tumor cell nests in marrow and trabecular spaces. These results suggest that: (1) local bone physiology is an important factor for developing osteoblastic/sclerotic PCa bone metastases in murine hosts; (2) the establishment of osteosclerotic PCa bone metastases in mice is enhanced by alterations that drive bone formation.

Guo, Y., A. P. Mazar, et al. (2002). "An antiangiogenic urokinase-derived peptide combined with tamoxifen decreases tumor growth and metastasis in a syngeneic model of breast cancer." Cancer Res **62**(16): 4678-84.

Expression of urokinase (uPA) and its receptor (uPAR) is associated with increased tumor-cellinvasion and metastasis in several malignancies including breast cancer. An 8-mer peptide derived from the nonreceptor-binding domain of urokinase (A6) has been shown to have antiangiogenic and proapoptotic effects to block the progression of breast cancer in vivo. In the present study, we evaluated the effects of A6 and the antiestrogen tamoxifen (TAM) alone and in combination on estrogen-receptor-positive Mat B-III rat breast cancer cells in vitro and in vivo. Treatment of Mat B-III cells with A6 and TAM resulted in a dose-dependent decrease in tumor-cell invasion through Matrigel; these effects were more marked when A6 and TAM were tested in combination. In addition, treatment of Mat B-III cells with either A6 or TAM resulted in a significant reduction of vascular endothelial growth factor receptor (flk-1) expression and in transforming growth factor beta activity, effects that were significantly

higher after combined treatment with A6 and TAM. For in vivo studies, female Fischer rats were inoculated with Mat B-III cells (1 x 10(6)) into the mammary fat pad. These orthotopic tumors were staged to 30-40 mm(3) in volume and then treatment was initiated with A6 (75 mg/kg/day) and TAM (3 mg/kg/day) alone or in combination. Both A6 and TAM caused a significant reduction in tumor volume; however, these antitumor effects were significantly greater in animals receiving both A6 and TAM, which demonstrated a 75% reduction in tumor growth as compared with control animals. The number of macroscopic tumor foci was significantly reduced in A6-treated animals, whereas TAM failed to exhibit any antimetastatic effects. Histological analysis of primary tumors from different groups showed a decrease in new blood-vessel density and increased tumor-cell death in A6- and TAM-treated animals, and these effects were greater in experimental animals receiving A6 and TAM in combination. Collectively, these studies demonstrate that the addition of novel antiangiogenic/antimetastatic agents like A6 to hormone therapy can enhance the antitumor effects of hormone therapy through increased inhibition of angiogenesis and induction of tumor-cell death.

Halder, S. K., G. Rachakonda, et al. (2008). "Smad7 induces hepatic metastasis in colorectal cancer." Br J Cancer **99**(6): 957-65.

Although Smad signalling is known to play a tumour suppressor role, it has been shown to play a prometastatic function also in breast cancer and melanoma metastasis to bone. In contrast, mutation or reduced level of Smad4 in colorectal cancer is directly correlated to poor survival and increased metastasis. However, the functional role of Smad signalling in metastasis of colorectal cancer has not been elucidated. We previously reported that overexpression of Smad7 in colon adenocarcinoma (FET) cells induces tumorigenicity by blocking TGF-beta-induced growth inhibition and apoptosis. Here, we have observed that abrogation of Smad signalling by Smad7 induces liver metastasis in a splenic injection model. Polymerase chain reaction with genomic DNA from liver metastases indicates that cells expressing Smad7 migrated to the liver. Increased expression of TGF-beta type II receptor in liver metastases is associated with phosphorylation and nuclear accumulation of Smad2. Immunohistochemical analyses have suggested poorly differentiated spindle cell morphology and higher cell proliferation in Smad7-induced liver metastases. Interestingly, we have observed increased expression and junctional staining of Claudin-1, Claudin-4 and E-cadherin in liver metastases. Therefore, this report demonstrates, for the first time, that blockade of TGF-beta/Smad pathway in colon cancer cells induces metastasis, thus supporting an important role of Smad signalling in inhibiting colon cancer metastasis.

Havens, A. M., Y. Jung, et al. (2006). "The role of sialomucin CD164 (MGC-24v or endolyn) in prostate cancer metastasis." BMC Cancer **6**: 195.

BACKGROUND: The chemokine stromal derived factor-1 (SDF-1 or CXCL12) and its receptor CXCR4 have been demonstrated to be crucial for the homing of stem cells and prostate cancers to the marrow. While screening prostate cancers for CXCL12-responsive adhesion molecules, we identified CD164 (MGC-24) as a potential regulator of homing. CD164 is known to function as a receptor that regulates stem cell localization to the bone marrow. RESULTS: Using prostate cancer cell lines, it was demonstrated that CXCL12 induced both the expression of CD164 mRNA and protein. Functional studies demonstrated that blocking CD164 on prostate cancer cell lines reduced the ability of these cells to adhere to human bone marrow endothelial cells, and invade into extracellular matrices. Human tissue microarrays stained for CD164 demonstrated a positive correlation with prostate-specific antigen levels, while its expression was negatively correlated with the expression of androgen receptor. CONCLUSION: Our findings suggest that CD164 may participate in the localization of prostate cancer cells to the marrow and is further evidence that tumor metastasis and hematopoietic stem cell trafficking may involve similar processes.

Hayashi, S., I. Yokoyama, et al. (1999). "Inhibitory effect on the establishment of hepatic metastasis by transduction of the tissue plasminogen activator gene to murine colon cancer." Cancer Gene Ther **6**(4): 380-4.

Hepatic metastasis is a major factor in limiting the prognosis of patients with colon carcinoma. Recent investigations indicate a correlation between plasminogen activator profiles and hepatic metastasis. We examined the effectiveness of tissue plasminogen activator (tPA) gene therapy using a hepatic metastasis model of murine colon carcinoma. Murine colon carcinoma Colon 26 cells transduced with an MFGtPA retroviral vector (Colon 26/tPA) or an MFGLacZ retroviral vector (Colon 26/LacZ) were injected into the liver via the superior mesenteric vein of BALB/c mice, whose survival rates were checked daily. The mean survival rate of mice with hepatic metastasis induced by Colon 26/LacZ was 23.1 days, whereas that of mice with Colon 26/tPA was >100 days. The in vitro proliferation of Colon 26/tPA was comparable with that of Colon 26/LacZ, and antitumor immunity to wild-type Colon 26 cells was not induced

after an intrahepatic injection of Colon 26/tPA. We suggest that transduction of the tPA gene to murine colon cancer is useful against the establishment of hepatic metastasis.

Hazan, R. B., G. R. Phillips, et al. (2000). "Exogenous expression of N-cadherin in breast cancer cells induces cell migration, invasion, and metastasis." J Cell Biol **148**(4): 779-90.

E- and N-cadherin are calcium-dependent cell adhesion molecules that mediate cell-cell adhesion and also modulate cell migration and tumor invasiveness. The loss of E-cadherin-mediated adhesion has been shown to play an important role in the transition of epithelial tumors from a benign to an invasive state. However, recent evidence indicates that another member of the cadherin family, N-cadherin, is expressed in highly invasive tumor cell lines that lacked E-cadherin expression. These findings have raised the possibility that N-cadherin contributes to the invasive phenotype. To determine whether N-cadherin promotes invasion and metastasis, we transfected a weakly metastatic and E-cadherin-expressing breast cancer cell line, MCF-7, with N-cadherin and analyzed the effects on cell migration, invasion, and metastasis. Transfected cells expressed both E- and N-cadherin and exhibited homotypic cell adhesion from both molecules. In vitro, N-cadherin-expressing cells migrated more efficiently, showed an increased invasion of Matrigel, and adhered more efficiently to monolayers of endothelial cells. All cells produced low levels of the matrix metalloproteinase MMP-9, which was dramatically upregulated by treatment with FGF-2 only in N-cadherin-expressing cells. Migration and invasion of Matrigel were also greatly enhanced by this treatment. When injected into the mammary fat pad of nude mice, N-cadherin-expressing cells, but not control MCF-7 cells, metastasized widely to the liver, pancreas, salivary gland, omentum, lung, lymph nodes, and lumbar spinal muscle. The expression of both E- and N-cadherin was maintained both in the primary tumors and metastatic lesions. These results demonstrate that N-cadherin promotes motility, invasion, and metastasis even in the presence of the normally suppressive E-cadherin. The increase in MMP-9 production by N-cadherin-expressing cells in response to a growth factor may endow them with a greater ability to penetrate matrix protein barriers, while the increase in their adherence to endothelium may improve their ability to enter and exit the vasculature, two properties that may be responsible for metastasis of N-cadherin-expressing cells.

He, B. P., J. J. Wang, et al. (2006). "Differential reactions of microglia to brain metastasis of lung cancer." Mol Med **12**(7-8): 161-70.

The brain is a common metastatic site for various types of cancers, especially lung cancer. Patients with brain metastases have a poor prognosis in spite of radiotherapy and/or chemotherapy. It is postulated that immune cells in the brain may play a major role in cancer metastasis, dormancy, and relapse. Although microglia may serve as a major component in the brain immune system, the interaction between metastatic cancer cells and microglia is still largely unknown and remains to be elucidated. In this study, we have investigated microglial reactions in brain tissues with metastatic lung cancer cells and evaluated the cytotoxic effects of lipopolysaccharide (LPS)-activated microglia on metastatic lung cancer cells in vitro. In the vicinity of metastatic lung cancer mass in the brain, microglia showed signs of significant activation. There was an obvious increase in the number of microglia labeled with ionized calcium binding adaptor molecule 1 (Iba-1) antibody, a specific marker of microglia. The microglia were observed to form a clear boundary between the tumor mass and normal brain tissue. In the region where the tumor mass was situated, only a few microglia expressed inducible nitric oxide synthase (iNOS) and tumor necrosis factor-alpha (TNF-alpha), indicating differential activation in those microglia. The supernatant from LPS-activated microglia induced apoptosis of metastatic lung cancer cells in vitro in a dose- and time-dependent manner. However, at lower concentrations of activated microglial supernatant, trophic effects on cancer cells were observed, some lung cancer cells being insensitive to microglial cytotoxicity. Together with the observation that TNF-alpha alone induced proliferation of the tumor cells, the findings provide possible clues to the mechanism involved in metastasis of lung cancer cells to the brain.

Helbig, G., K. W. Christopherson, 2nd, et al. (2003). "NF-kappaB promotes breast cancer cell migration and metastasis by inducing the expression of the chemokine receptor CXCR4." J Biol Chem **278**(24): 21631-8.

Metastasis of cancer cells is a complex process involving multiple steps including invasion, angiogenesis, and trafficking of cancer cells through blood vessels, extravasations, organ-specific homing, and growth. While matrix metalloproteinases, urokinase-type plasminogen activator, and cytokines play a major role in invasion and angiogenesis, chemokines such as stromal derived factor-1alpha (SDF-1alpha) and their receptors such as CXCR4 are thought to play a critical role in motility, homing, and

proliferation of cancer cells at specific metastatic sites. We and others have previously reported that the extracellular signal-activated transcription factor NF-kappaB up-regulates the expression of matrix metalloproteinases, urokinase-type plasminogen activator, and cytokines in highly metastatic breast cancer cell lines. In this report, we demonstrate that NF-kappaB regulates the motility of breast cancer cells by directly up-regulating the expression of CXCR4. Overexpression of the inhibitor of kappaB (IkappaB) in breast cancer cells with constitutive NF-kappaB activity resulted in reduced expression of CXCR4 and a corresponding loss of SDF-1alpha-mediated migration in vitro. Introduction of CXCR4 cDNA into IkappaB-expressing cells restored SDF-1alpha-mediated migration. Electrophoretic mobility shift assays and transient transfection assays revealed that the NF-kappaB subunits p65 and p50 bind directly to sequences within the -66 to +7 region of the CXCR4 promoter and activate transcription. We also show that the cell surface expression of CXCR4 and the SDF-1alpha-mediated migration are enhanced in breast cancer cells isolated from mammary fat pad xenografts compared with parental cells grown in culture. A further increase in CXCR4 cell surface expression and SDF-1alpha-mediated migration was observed with cancer cells that metastasized to the lungs. Taken together, these results implicate NF-kappaB in the migration and the organ-specific homing of metastatic breast cancer cells.

Hibi, T., T. Mori, et al. (2009). "Synuclein-gamma is closely involved in perineural invasion and distant metastasis in mouse models and is a novel prognostic factor in pancreatic cancer." Clin Cancer Res **15**(8): 2864-71.

PURPOSE: Perineural invasion is associated with the high incidence of local recurrence and a dismal prognosis in pancreatic cancer. We previously reported a novel perineural invasion model and distinguished high- and low-perineural invasion groups in pancreatic cancer cell lines. This study aimed to elucidate the molecular mechanism of perineural invasion. EXPERIMENTAL DESIGN: To identify key biological markers involved in perineural invasion, differentially expressed molecules were investigated by proteomics and transcriptomics. Synuclein-gamma emerged as the only up-regulated molecule in high-perineural invasion group by both analyses. The clinical significance and the biological property of synuclein-gamma were examined in 62 resected cases of pancreatic cancer and mouse models. RESULTS: Synuclein-gamma overexpression was observed in 38 (61%) cases and correlated with major invasive parameters, including perineural invasion and lymph node metastasis ($P < 0.05$). Multivariate analyses revealed synuclein-gamma overexpression as the only independent predictor of diminished overall survival [hazard ratio, 3.4 (95% confidence interval, 1.51-7.51)] and the strongest negative indicator of disease-free survival [2.8 (1.26-6.02)]. In mouse perineural invasion and orthotopic transplantation models, stable synuclein-gamma suppression by short hairpin RNA significantly reduced the incidence of perineural invasion ($P = 0.009$) and liver/lymph node metastasis ($P = 0.019$ and $P = 0.020$, respectively) compared with the control. CONCLUSIONS: This is the first study to provide in vivo evidence that synuclein-gamma is closely involved in perineural invasion/distant metastasis and is a significant prognostic factor in pancreatic cancer. Synuclein-gamma may serve as a promising molecular target of early diagnosis and anticancer therapy.

Hinton, C. V., S. Avraham, et al. (2008). "Contributions of integrin-linked kinase to breast cancer metastasis and tumourigenesis." J Cell Mol Med **12**(5A): 1517-26.

Metastasis contributes to more than 90% of mortality in breast cancer. Critical stages in the development of aggressive breast cancer include growth of the primary tumours, and their abilities to spread to distant organs, colonize and establish an independent blood supply. The integrin family of cell adhesion receptors is essential to breast cancer progression. Furthermore, integrin-linked kinase can 'convert' localized breast cancer cells into invasive and metastatic cells. Upon stimulation by growth factors and chemokine ligands, integrin-linked kinase mediates the phosphorylation of Akt Ser473, and glycogen synthase kinase-3. The current notion is that overexpression of integrin-linked kinase resulted in an invasive, metastatic phenotype in several cancer model systems in vivo and in vitro, thus, implicating a role for integrin-linked kinase in oncogenic transformation, angiogenesis and metastasis. Here, we will review the role of integrin-linked kinase in breast cancer metastasis. Elucidation of signalling events important for breast tumour metastasis should provide insights into successful breast cancer therapies.

Holmes, C. E., J. E. Levis, et al. (2009). "Activated platelets enhance ovarian cancer cell invasion in a cellular model of metastasis." Clin Exp Metastasis **26**(7): 653-61.

Increased platelet counts and systemic coagulation activation are associated with ovarian cancer progression. Platelet activation occurs in the tumor microenvironment and may influence local invasion and metastasis. We used a cellular model of tumor invasion to investigate the effect of activated platelets on the human ovarian cancer cell line,

SKOV3. SKOV3 cells were exposed to washed, thrombin receptor activating peptide (TRAP)-activated or TRAP-naive platelets under various experimental conditions, and tumor cell invasion was assayed in Matrigel chambers. The effect of platelets on the content of urokinase plasminogen activator (uPA) and VEGF in SKOV3 cell conditioned medium was measured using an ELISA assay. TRAP-activated platelets stimulated a dose-dependent increase in SKOV3 cell invasion. Exposure to activated platelet membranes and to soluble proteins contained in activated platelet releasate both contributed to the observed increase in invasion. The inhibition of platelet activation with prostaglandin E1 (PGE(1)) attenuated the invasive capacity of SKOV3 cells. Exposure to platelets resulted in significantly increased uPA and VEGF content of SKOV3 cell conditioned medium. Activated platelets enhance SKOV3 human ovarian cancer cell invasion through Matrigel and increase the amount of uPA and VEGF secreted into SKOV3 cell conditioned medium. If generalizable to additional cell lines and human disease, this observation may partially explain the adverse prognosis associated with thrombocytosis in ovarian cancer. Platelets, therefore, may represent a potential target for therapeutic intervention in human ovarian cancer.

Horiguchi, T., S. Tachikawa, et al. (2000). "Usefulness of serum carboxy-terminal telopeptide of type I collagen (ICTP) as a marker of bone metastasis from lung cancer." Jpn J Clin Oncol **30**(4): 174-9.

BACKGROUND: Serum pyridinoline cross-linked carboxy-terminal telopeptide of type I collagen (ICTP) is a metabolite of type I collagen comprising 90% or more of organic substances in bone. Its usefulness as a marker of bone metastasis from malignant tumors is expected. METHOD: We measured ICTP to evaluate its clinical usefulness for diagnosis of bone metastasis in 140 patients with lung cancer. For comparison, serum carcinoembryonic antigen (CEA), cytokeratin 19 fragment (CYFRA 21-1), gastrin-releasing peptide precursor (ProGRP), alkaline phosphatase and calcium were simultaneously measured. ICTP was measured by double-antibody radioimmunoassay. RESULTS: ICTP was significantly higher in patients with bone metastasis from lung cancer than in the group without bone metastasis, patients with other pulmonary diseases or healthy control subjects and showed excellent sensitivity and specificity, indicating that this marker is highly useful for complementary diagnosis of bone metastasis from lung cancer. Moreover, the survival duration was significantly shorter in the ICTP-positive group than in the ICTP-negative group, suggesting that ICTP can be a prognostic factor in lung cancer. CONCLUSION: It is suggested that measurement of ICTP is worthwhile as a serological diagnostic method of bone metastasis from lung cancer. Moreover, since repeated measurements are possible, this measure was considered very helpful in complementary diagnosis of bone metastasis and also as a standard to determine the timing of examinations such as bone scintigraphy.

Hsu, P. I., H. L. Hsieh, et al. (2009). "Loss of RUNX3 expression correlates with differentiation, nodal metastasis, and poor prognosis of gastric cancer." Ann Surg Oncol **16**(6): 1686-94.

BACKGROUND: RUNX3 is a major growth regulator of gastric epithelial cells that is involved in gastric tumorigenesis in both humans and mice. In this study, we investigated the involvement of RUNX3 in tumor progression, and in the prognosis of human gastric cancer. METHODS: We analyzed the extent of RUNX3 protein expression by immunohistochemistry in 95 primary gastric adenocarcinomas, and correlated expression levels with clinicopathological parameters. We examined the effects of pFlag/RUNX3 on cell growth, apoptosis, and caspase-3 expression in AGS and SNU1 gastric cancer cell lines by colony-forming assay, terminal deoxynucleotidyl transferase (TdT)-mediate deoxyuridine triphosphatase (dUTP) nick-end labeling (TUNEL) assay, and Western blot analysis, respectively. The pFlag/RUNX3 effects on AGS invasion and migration potentials were also evaluated. RESULTS: RUNX3 expression was lost in 37 (39%) cases of gastric cancer. The expression of RUNX3 in diffuse- and mixed-type cancers was less frequent than expression in intestinal-type cancer ($P < 0.001$ and $P = 0.001$, respectively). In addition, the loss of RUNX3 expression was associated with lymph node metastasis ($P = 0.02$), and correlated with poor gastric cancer survival ($P = 0.018$). The growth of gastric cancer cells was suppressed after pFlag/RUNX3 transfection. The re-expression of RUNX3 resulted in the upregulation of caspase-3 and promoted apoptosis. Furthermore, Re-expression of RUNX3 induced significant inhibitions of AGS cell invasion and migration in vitro. CONCLUSIONS: This work shows that loss of RUNX3 expression is highly associated with lymph node metastasis and poor prognosis of gastric cancer. The re-expression of RUNX3 may induce apoptosis and inhibit the growth as well as invasion/migration of cancer cells. These results indicate that the targeting of the RUNX3 pathway could represent a potential modality for treating gastric cancer.

Huang, W. C., D. Wu, et al. (2006). "beta2-microglobulin is a signaling and growth-promoting factor for human prostate cancer bone metastasis." Cancer Res **66**(18): 9108-16.

The protein factor beta2-microglobulin (beta2M), purified from the conditioned medium of human prostate cancer cell lines, stimulated growth and enhanced osteocalcin (OC) and bone sialoprotein (BSP) gene expression in human prostate cancer cells by activating a cyclic AMP (cAMP)-dependent protein kinase A signaling pathway. When beta2M was overexpressed in prostate cancer cells, it induced explosive tumor growth in mouse bone through increased phosphorylated cAMP-responsive element binding protein (CREB) and activated CREB target gene expression, including OC, BSP, cyclin A, cyclin D1, and vascular endothelial growth factor. Interrupting the beta2M downstream signaling pathway by injection of the beta2M small interfering RNA liposome complex produced an effective regression of previously established prostate tumors in mouse bone through increased apoptosis as shown by immunohistochemistry and activation of caspase-9, caspase-3, and cleavage of poly(ADP-ribose) polymerase. These results suggest that beta2M signaling is an attractive new therapeutic target for the treatment of lethal prostate cancer bone metastasis.

Ichiki, K., N. Mitani, et al. (2000). "Regulation of activator protein-1 activity in the mediastinal lymph node metastasis of lung cancer." Clin Exp Metastasis **18**(7): 539-45.

Orthotopic implantation of a metastatic cell line of Lewis lung carcinoma (LLC-MLN), which was isolated by an in vivo selection method, resulted in greater metastatic growth in mediastinal lymph nodes as compared with that of the original LLC cells. LLC-MLN cells also had increased invasive ability and activator protein-1 (AP-1) transcriptional activity as compared with the original LLC cells. This is well consistent with the previously reported finding that overexpression of AP-1 is associated with lymphatic metastasis in lung cancer patients. Oral administration of curcumin, which downregulates AP-1 transcription, significantly inhibited the mediastinal lymph node metastasis of orthotopically implanted LLC cells in a dose-dependent manner, but did not affect the tumor growth at the implantation site. Combined treatment with curcumin and an anti-cancer drug, cis-diamine-dichloroplatinum (CDDP), resulted in a marked inhibition of tumor growth at the implanted site and of lymphatic metastasis, and a significant prolongation of the survival time. The downregulation of transcriptional AP-1 activity by curcumin as seen in the dual luciferase assay caused inhibition of LLC cell invasion through the repression of expression of the mRNAs for urokinase-type plasminogen activator (u-PA) and its receptor (u-PAR). Inhibition of AP-1 transcriptional activity may offer improved therapeutic efficacy for lung cancer patients with lymphatic metastasis.

Ide, H., K. Hatake, et al. (2008). "Serum level of macrophage colony-stimulating factor is increased in prostate cancer patients with bone metastasis." Hum Cell **21**(1): 1-6.

Recent evaluation of human prostate tissues has shown predominantly high expression of the macrophage colony-stimulating factor receptor in prostatic intra-epithelial neoplasia or prostate cancer. However, the expression of its ligand, the macrophage colony-stimulating factor (M-CSF), and the biological role of this signaling in prostate cancer has not been analyzed. In this research we determined the relationship of serum M-CSF level to clinical parameters of prostate cancer progression. We measured the serum level of M-CSF in 170 patients with histologically confirmed prostatic adenocarcinoma and in 54 patients in whom prostate cancer was not detected. We also investigated the M-CSF expression in prostate cancer tissues by immunohistochemistry. The serum levels of M-CSF in bone metastatic prostate cancer patients was significantly higher than those in non-metastatic patients, while M-CSF did not differ with regards to histological grade, Gleason score or local tumor progression. M-CSF expression was detected in prostate cancer cells themselves by immunohistochemistry. These results suggest that M-CSF may have a functional role in prostate cancer progression.

Ikeguchi, M., T. Taniguchi, et al. (2000). "Reduced E-cadherin expression and enlargement of cancer nuclei strongly correlate with hematogenic metastasis in colorectal adenocarcinoma." Scand J Gastroenterol **35**(8): 839-46.

BACKGROUND: Synchronous and metachronous hematogenic metastases are frequently detected in patients with colorectal carcinoma. Once these metastases have developed, the prognoses of patients are poor. Previously, we reported that enlargement of cancer nuclei significantly correlated with metastatic potential of gastric cancer and hepatocellular carcinoma. Moreover, recently it has been reported that reduced expression of E-cadherin is associated with tumor metastasis. To evaluate the correlation between nuclear area (NA) of cancer cells and expression of E-cadherin, and to elucidate whether these factors correlate with clinical outcome in patients with colorectal carcinoma, 105 consecutive patients were investigated. METHODS: In each case, the NAs of 600 cancer nuclei were analyzed by means of a computer-assisted image analysis system and E-cadherin expression was detected

immunohistochemically by an anti-E-cadherin monoclonal antibody. The expression levels of E-cadherin were divided into three groups according to the percentages of E-cadherin-positive cells (level 0: positive cells < or = 50%, level 1: 50% < positive cells < or = 80%, level 2: positive cells > 80%). RESULTS: The mean NA of cancer cells in 105 tumors was 57 microm2. The NAs of cancer cells enlarged in proportion to the decrease of E-cadherin expression levels (level 0, n = 48, 62 microm2; level 1, n = 35, 57 microm2, level 2, n = 22, 46 microm2, P = 0.002). The 10-year survival rates decreased in proportion to the reduced E-cadherin expression levels (80% in level 2, 64% in level 1, and 42% in level 0, P = 0.004). Moreover, the 10-year survival rate of 54 patients with large NA tumors (< or = 54 microm2, 36%) was significantly poorer than that of 51 patients with small NA tumors (< 54 microm2, 80%, P < 0.001). The NA of cancer cells was recognized as an important predictor for prognosis and hematogenic metastasis. Although reduced E-cadherin expression was not recognized as the risk factor for hematogenic metastasis, 80% of patients who developed hematogenic metastasis had tumors with both enlarged cancer nuclei and reduced E-cadherin expression. CONCLUSIONS: Detection of NA of cancer cells and E-cadherin expression in patients with colorectal carcinoma may reveal important information for hematogenic metastasis.

Ilan, N., M. Elkin, et al. (2006). "Regulation, function and clinical significance of heparanase in cancer metastasis and angiogenesis." Int J Biochem Cell Biol **38**(12): 2018-39.

Heparanase is an endoglycosidase which cleaves heparan sulfate (HS) and hence participates in degradation and remodeling of the extracellular matrix (ECM). Heparanase is preferentially expressed in human tumors and its over-expression in tumor cells confers an invasive phenotype in experimental animals. The enzyme also releases angiogenic factors from the ECM and thereby induces an angiogenic response in vivo. Heparanase upregulation correlates with increased tumor vascularity and poor post-operative survival of cancer patients. Heparanase is synthesized as a 65 kDa inactive precursor that undergoes proteolytic cleavage, yielding 8 and 50 kDa protein subunits that heterodimerize to form an active enzyme. Human heparanase is localized primarily within late endosomes and lysosomes and occasionally on the cell surface and within the cell nucleus. Transcriptional activity of the heparanase promoter is stimulated by demethylation, early growth response 1 (EGR1) transcription factor, estrogen, inflammatory cytokines and inactivation of p53. N-acetylated glycol-split species of heparin as well as siRNA heparanase gene silencing inhibit tumor metastasis and angiogenesis in experimental models. These observations and the unexpected identification of a single functional heparanase, suggest that the enzyme is a promising target for anti-cancer and anti-inflammatory drug development. Heparanase exhibits also non-enzymatic activities, independent of its involvement in ECM degradation and changes in the extracellular microenvironment. For example, cell surface expression of heparanase elicits a firm cell adhesion, reflecting an involvement in cell-ECM interaction. Heparanase enhances Akt signaling and stimulates PI3K- and p38-dependent endothelial cell migration and invasion. It also promotes VEGF expression via the Src pathway. The enzyme may thus activate endothelial cells and elicits angiogenic and survival responses. Studies with heparanase over-expressing transgenic mice revealed that the enzyme functions in normal processes involving cell mobilization, HS turnover, tissue vascularization and remodeling. In this review, we summarize the current status of heparanase research, emphasizing molecular and cellular aspects of the enzyme, including its mode of processing and activation, control of heparanase gene expression, enzymatic and non-enzymatic functions, and causal involvement in cancer metastasis and angiogenesis. We also discuss clinical aspects and strategies for the development of heparanase inhibitors.

Inoue, M., S. Matsumoto, et al. (2008). "Intraperitoneal administration of a small interfering RNA targeting nuclear factor-kappa B with paclitaxel successfully prolongs the survival of xenograft model mice with peritoneal metastasis of gastric cancer." Int J Cancer **123**(11): 2696-701.

Activation of nuclear factor-kappa B (NF-kappaB) has been detected in various malignant tumors, including gastric carcinoma, and is associated with tumor growth, metastasis, resistance to chemotherapeutic agents and poor prognosis. Therefore, NF-kappaB is a potential target for antitumor therapy. In this study, we used a small interfering RNA (siRNA) to knockdown NF-kappaB p65 expression and determined whether intraperitoneal administration of NF-kappaB p65 siRNA and paclitaxel was effective for treating peritoneal metastasis of gastric cancer. Western blot analysis revealed that NF-kappaB p65 expression was diminished by NF-kappaB p65 siRNA. Apoptotic cells were increased after transfection of NF-kappaB p65 siRNA compared with control siRNA in the treatment with paclitaxel. In a murine xenograft model, abundant fluorescence was observed on the surface of intraperitoneal nodules of gastric cancer after siRNA administration. Moreover, intraperitoneal

administration of NF-kappaB p65 siRNA reduced NF-kappaB expression in intraperitoneal nodules of gastric cancer. Finally, mice treated by intraperitoneal administration of NF-kappaB p65 siRNA and paclitaxel survived for a significantly longer time than mice treated by intraperitoneal administration of paclitaxel alone ($p = 0.0002$). Taken together, the present results demonstrate that intraperitoneal administration of NF-kappaB p65 siRNA and paclitaxel inhibited cancer growth in mice with peritoneal metastasis of gastric cancer. Therefore, intraperitoneal administration of NF-kappaB p65 siRNA and paclitaxel may provide a breakthrough in the treatment of peritoneal metastasis of gastric cancer.

Inoue, M., T. Sawada, et al. (2005). "Plasminogen activator inhibitor-1 (PAI-1) gene transfection inhibits the liver metastasis of pancreatic cancer by preventing angiogenesis." Oncol Rep **14**(6): 1445-51.

Plasminogen activator inhibitor-1 (PAI-1) is a unique type of serine protease inhibitor and one of the key regulators of tumor invasion and metastasis. The purpose of this study was to elucidate the effect of PAI-1 gene transfection on liver metastasis and its mechanism by using the human high liver metastasis pancreatic cancer cell line, SW1990. PAI-1-transfected SW1990 (SW/PAI-1) produced a significantly higher level of PAI-1 in supernatant than parental cells. While no difference was observed for the production of u-PA and u-PA activity in the supernatant, cell proliferation of SW/PAI-1 was slightly suppressed on the 7th day of incubation compared to parental cells. Cellular invasion, in vivo tumorigenesis in xenograft and liver metastasis were significantly suppressed in SW/PAI-1 cells compared to parental cells. The angiogenesis of xenograft by detecting microvascular density and the production of metastasis-related factors, such as VEGF and TGF-beta1, were also decreased in SW/PAI-1 cells. These findings suggested that PAI-1 gene transfection might have the ability to prevent the liver metastasis of pancreatic cancer by modulating angiogenesis.

Ishii, H., T. Yazawa, et al. (2004). "Enhancement of pleural dissemination and lymph node metastasis of intrathoracic lung cancer cells by vascular endothelial growth factors (VEGFs)." Lung Cancer **45**(3): 325-37.

The expression of vascular endothelial growth factors (VEGFs) in tumors including lung cancer is considered to be associated with tumor development via capillary and lymph vessel neogenesis. Dissemination of the tumor cells to the pleura or regional lymph nodes is a critical poor prognostic factor for lung cancer patients. To investigate how VEGFs expressed in the intrathoracic infiltrating lung cancer cells participate in disease progression, we established stably VEGF-A-, VEGF-C-, VEGF-D-, VEGF-A and VEGF-C-, and VEGF-A and VEGF-D-expressing large cell lung cancer clones (TKB5/VEGF-A, TKB5/VEGF-C, TKB5/VEGF-D, TKB5/VEGF-A/C, and TKB5/VEGF-A/D), orthotopically inoculated these into the right thoracic cavity (i.t.) of nude mice, and evaluated the subsequent development of lung lesion, pleural effusion, pleural dissemination, and lymph node metastasis. While there were no significant differences either in culture or in subcutaneous tumor cell growth between the empty vector-transfected group (TKB5/empty) and each transfectant, the i.t. model demonstrated significantly different biological properties between the transfectants. TKB5/empty-inoculated mice frequently developed a large tumor on the pleura without pleural effusion, dissemination, or lymph node (LN) metastasis. In contrast, VEGF-A promoted a bloody pleural effusion (6/14), and VEGF-A and VEGF-D frequently generated pleural dissemination (11/14 and 9/11, respectively). Although both VEGF-C and VEGF-D generated LN metastasis (6/10 and 8/11, respectively), the locations of the metastasized LNs were quite different. TKB5/VEGF-C metastasized on the same side of axillary LNs as i.t. (right axillary LNs), whereas TKB5/VEGF-D metastasized to the mediastinal and left axillary and/or cervical LNs. Since the TKB5/VEGF-A/C or TKB5/VEGF-A/D co-transfectants revealed overlapping tumor progression patterns of VEGF-A and VEGF-C or VEGF-D, the metastatic LNs had abundant new capillaries and were larger than those of TKB5/VEGF-C or TKB5/VEGF-D-inoculated mice. Our results clearly demonstrate that VEGF-A secreted from intrathoracic lung cancer cells plays important roles in producing pleural effusion, dissemination, and capillary neogenesis, that VEGF-C is involved in LN metastasis, and VEGF-D in pleural dissemination and LN metastasis. It is most likely, however, that the mechanisms by which VEGF-C promotes LN metastasis are different from those of VEGF-D. The regulation of the expression of VEGFs in intrathoracic lung cancer cells might be a useful therapeutic approach to inhibiting tumor development and improving patient prognosis.

Iwasaki, T., M. Mukai, et al. (2002). "Ipriflavone inhibits osteolytic bone metastasis of human breast cancer cells in a nude mouse model." Int J Cancer **100**(4): 381-7.

Osteolytic bone metastasis is a frequent problem in the treatment of cancer. Ipriflavone, a synthetic isoflavone that inhibits osteoclastic bone resorption, has been used for the treatment of osteoporosis in some countries. Some other

isoflavones also exhibit an antitumor effect in vitro and in vivo. Here, we studied the effects of ipriflavone on osteolytic bone metastasis of MDA-231 human breast cancer cells injected intracardially into athymic nude mice (ICR-nu/nu). Daily oral administration of ipriflavone at 12 mg/mouse significantly inhibited the development of new osteolytic bone metastases ($p < 0.05$) and the progression of established osteolytic lesions ($p = 0.01$), prolonging the life of tumor-bearing mice ($p = 0.01$ vs. control). In addition, ipriflavone reduced the number of osteoclasts at the bone-cancer interface with no severe adverse effects on the host. In vitro, ipriflavone inhibited the proliferation and DNA synthesis of MDA-231 cells and blocked the ligand-induced phosphorylation of Tyr(845) of the EGFR. Ipriflavone did not promote apoptosis of MDA-231 cells. Our results show that ipriflavone not only directly inhibits the growth of cancer cells but also reduces osteoclasts to prevent the soft tissue tumor burden and osteolytic bone metastases. These findings raise the possibility that ipriflavone may be of use as a therapeutic agent against osteolytic bone metastasis.

Iwasaki, T., K. Yamashita, et al. (2002). "Interleukin-18 inhibits osteolytic bone metastasis by human lung cancer cells possibly through suppression of osteoclastic bone-resorption in nude mice." J Immunother **25 Suppl 1**: S52-60.

Interleukin (IL)-18 exhibits antitumor as well as antiosteoclastogenic activities. These findings suggest that IL-18 is a potential tool for the treatment of cancers with associated osteolytic bone metastasis. We have previously shown that systemic daily administration of recombinant (r) IL-18 inhibits the development of osteolytic bone metastasis by human breast cancer cells. Here we demonstrate that systemic daily administration of rIL-18 (1 microg/mouse/d) for 21 days significantly inhibited the number and the total area of osteolytic bone metastasis by RWGT2 human lung cancer cells in nude mice. No severe adverse effects were observed. Natural killer (NK) cells did not increase in splenocytes from rIL-18-treated mice, and the in vitro NK activity of splenocytes against RWGT2 cells was only weakly enhanced in the presence of IL-18. The administration of rIL-18 made no difference in the growth of subcutaneous tumors, histologic indices (mitotic index, apoptotic index, and Ki-67-labeling index) of subcutaneous tumors or metastatic bone foci, or in the number of osteoclasts along the bone surface adjacent to tumors. Moreover, serum levels of cytokines including interferon-gamma, IL-1alpha, IL-6, tumor necrosis factor-alpha, and granulocyte/macrophage colony-stimulating factor, which regulate bone-resorbing activity of osteoclasts, were evaluated. Among them, IL-6 was remarkably downregulated in rIL-18-treated mice. These findings suggest that IL-18 inhibits osteolytic bone metastasis possibly through suppression of osteoclastic bone-resorption mediated in part by IL-6.

Jackson, P., M. O. Grimm, et al. (2002). "Relationship between expression of KAI1 metastasis suppressor gene, mRNA levels and p53 in human bladder and prostate cancer cell lines." Urol Oncol **7**(3): 99-104.

The molecular basis for the loss of KAI1 expression in invasive and metastatic tumors and tumor cell lines is not understood. Recently, identification of a sequence with homology to the consensus p53-binding motif in the promoter of the KAI1 metastasis suppressor gene, has led to a proposal that transcriptional regulation by p53 controls expression of KAI1, and that a dramatic down-regulation of KAI1 mRNA levels in invasive tumors and many tumor cell lines, is directly due to loss of p53 function. We have tested this hypothesis by assessing KAI1 mRNA levels in a series of 22 cell lines derived from bladder and prostate cancers, in which we confirmed the p53 gene sequence and characterized the functional status of the endogenous p53 protein. We anticipated that cell lines expressing p53 capable of transactivation should express high levels of KAI1 mRNA compared with cell lines expressing defective p53, or which were p53-null. KAI1 mRNA levels were determined by northern analysis using a full-length KAI1 cDNA probe, and varied widely between cell lines examined. However, there was no association between these levels and p53 status. Furthermore, transfection of representative cell lines with wild-type p53, or exposure to DNA damaging agents, had no effect on KAI1 mRNA levels. Our data suggest that p53 is not a major factor regulating levels of KAI1 mRNA in bladder and prostate cancer cell lines.

Jallal, H., M. L. Valentino, et al. (2007). "A Src/Abl kinase inhibitor, SKI-606, blocks breast cancer invasion, growth, and metastasis in vitro and in vivo." Cancer Res **67**(4): 1580-8.

The central role of Src in the development of several malignancies, including breast cancer, and the accumulating evidence of its interaction with receptor tyrosine kinases, integrins, and steroid receptors have identified it as an attractive therapeutic target. In the current study, we have evaluated the effect of a Src/Abl kinase inhibitor, SKI-606, on breast cancer growth, migration, invasion, and metastasis. Treatment of human breast cancer cells MDA-MB-231 with SKI-606 caused a marked inhibition of cell proliferation, invasion, and migration by inhibiting mitogen-activated protein kinase and Akt

phosphorylation. For in vivo studies, MDA-MB-231 cells transfected with the plasmid encoding green fluorescent protein (GFP; MDA-MB-231-GFP) were inoculated into the mammary fat pads of female BALB/c nu/nu mice. Once tumor volume reached 30 to 50 mm(3), animals were randomized and treated with vehicle alone or 150 mg/kg SKI-606 by daily oral gavage. Experimental animals receiving SKI-606 developed tumors of significantly smaller volume (45-54%) compared with control animals receiving vehicle alone. Analysis of lungs, liver, and spleen of these animals showed a significant decrease in GFP-positive tumor metastasis in animals receiving SKI-606 at a dose that was well tolerated. Western blot analysis and immunohistochemical analysis of primary tumors showed that these effects were due to the ability of SKI-606 to block tumor cell proliferation, angiogenesis, growth factor expression, and inhibition of Src-mediated signaling pathways in vivo. Together, the results from these studies provide compelling evidence for the role of Src inhibitors as therapeutic agents for blocking breast cancer growth and metastasis.

Janowska-Wieczorek, A., M. Wysoczynski, et al. (2005). "Microvesicles derived from activated platelets induce metastasis and angiogenesis in lung cancer." Int J Cancer **113**(5): 752-60.

The role of platelets in tumor progression and metastasis has been recognized but the mechanism of their action remains unclear. Five human lung cancer cell lines (A549, CRL 2066, CRL 2062, HTB 183, HTB 177) and a murine Lewis lung carcinoma (LCC) cell line (for an in vivo model of metastasis) were used to investigate how platelet-derived microvesicles (PMV), which are circular fragments shed from the surface membranes of activated platelets, and exosomes released from platelet alpha-granules, could contribute to metastatic spread. We found that PMV transferred the platelet-derived integrin CD41 to most of the lung cancer cell lines tested and stimulated the phosphorylation of mitogen-activated protein kinase p42/44 and serine/threonine kinase as well as the expression of membrane type 1-matrix metalloproteinase (MT1-MMP). PMV chemoattracted 4 of the 5 cell lines, with the highly metastatic A549 cells exhibiting the strongest response. In A549 cells, PMV were shown to stimulate proliferation, upregulate cyclin D2 expression and increase trans-Matrigel chemoinvasion. Furthermore, in these cells, PMV stimulated mRNA expression for angiogenic factors such as MMP-9, vascular endothelial growth factor, interleukin-8 and hepatocyte growth factor, as well as adhesion to fibrinogen and human umbilical vein endothelial cells. Intravenous injection of murine PMV-covered LLC cells into syngeneic mice resulted in significantly more metastatic foci in their lungs and LLC cells in bone marrow than in control animals injected with LCC cells not covered with PMV. Based on these findings, we suggest that PMV play an important role in tumor progression/metastasis and angiogenesis in lung cancer.

Joo, Y. H., C. K. Jung, et al. (2009). "Relationship between vascular endothelial growth factor and Notch1 expression and lymphatic metastasis in tongue cancer." Otolaryngol Head Neck Surg **140**(4): 512-8.

OBJECTIVE: To determine the role of angiogenesis in lymph node metastasis and the depth of invasion in early tongue cancer. STUDY DESIGN: Retrospective analysis. SUBJECTS AND METHODS: The study included 51 subjects with tongue cancer. Immunohistochemical staining for vascular endothelial growth factor, Notch1, and Notch3 was performed. Microvessel density was evaluated by counting the number of CD34-stained microvessels in each pathologic specimen. RESULTS: Significant correlations were found between vascular endothelial growth factor and Notch1 expression and cervical lymph node metastasis ($P = 0.020$ and $P < 0.009$, respectively), tumor depth of invasion ($P = 0.001$ and $P < 0.001$, respectively), and microvessel density indicated by CD34 staining ($P = 0.001$ and $P < 0.001$, respectively). Nodal metastasis ($P = 0.022$), T stage ($P = 0.002$), and positive VEGF expression ($P = 0.044$) were statistically significant prognostic factors for disease-specific survival. CONCLUSION: Vascular endothelial growth factor and Notch1 expression are significantly related to cervical lymph node metastasis and depth of invasion in tongue cancer patients.

Kamata, I., Y. Ishikawa, et al. (2009). "Significance of lymphatic invasion and cancer invasion-related proteins on lymph node metastasis in gastric cancer." J Gastroenterol Hepatol **24**(9): 1527-33.

BACKGROUND AND AIMS: Cancer invasion and metastasis are critical events for patient prognosis; however, the most important step in the whole process of lymph node (LN) metastasis in gastric cancer remains obscure. In this study, the significance of cancer cell behaviors, such as cell detachment, stromal invasion and lymphatic invasion on regional LN metastasis in gastric cancer was investigated by comprehensive immunohistochemistry. METHODS: A total of 210 cases with gastric cancer were selected. These consisted of 105 cases with regional LN metastasis (LN[+] group) and 105 cases without LN metastasis (LN[-] group). Both groups exhibited the same depth of invasion. Cancer tissues were subjected to immunohistochemistry with antibodies against

claudin-3, claudin-4, beta-catenin, matrix metalloproteinase (MMP)-1, and MMP-2, as well as endothelial markers of lymphatic vessel endothelial hyaluronan receptor-1 and von Willebrand factor for the objective discrimination between lymphatics and blood vessels. The expression of each protein as well as the histopathological parameters were compared between LN(+) and LN(-) groups. RESULTS: Along with lymphatic invasion by cancer cells and gross tumor size, MMP-1 expression in cancer cells at the invasive front of the primary tumor was a significant, independent predictor of LN metastasis. The expression of claudins and beta-catenin was associated with the histopathological type of cancer, but not with LN status. CONCLUSION: Among the cancer invasion-related proteins examined, MMP-1 plays a vital role in LN metastasis of gastric cancer. Tumor size, lymphatic invasion and MMP-1 expression level at the invasive front were the predictive factors of LN metastasis of gastric cancer.

Kanao, H., T. Enomoto, et al. (2005). "Overexpression of LAMP3/TSC403/DC-LAMP promotes metastasis in uterine cervical cancer." Cancer Res **65**(19): 8640-5.

LAMP3 (DC-LAMP, TSC403, CD208) was originally isolated as a gene specifically expressed in lung tissues. LAMP3 is located on a chromosome 3q segment that is frequently amplified in some human cancers, including uterine cervical cancer. Because two other members of the LAMP family of lysosomal membrane glycoproteins, LAMP1 and LAMP2, were previously implicated in potentially modulating the interaction of vascular endothelial and cancer cells, we hypothesized that LAMP3 might also play an important part in metastasis. To clarify the metastatic potential of LAMP3 in cervical cancers, we transfected a LAMP3 expression vector into a human uterine cervical cancer cell line, TCS. In an in vitro invasion assay, the migration of LAMP3-overexpressing TCS cells was significantly higher than in control TCS cells. In an in vivo metastasis assay, distant metastasis was detected in 9 of 11 LAMP3-overexpressing TCS cell-injected mice and in only 1 of 11 control mice. Histologic study showed that LAMP3-overexpressing cells readily invaded into the lymph-vascular space. In clinical samples, quantitative real-time reverse transcription-PCR (RT-PCR) analyses showed that LAMP3 mRNA was significantly up-regulated in 47 of 47 (100%) cervical cancers and in 2 of 15 (13%) cervical intraepithelial neoplasias, compared with a low level of LAMP3 mRNA expressed in normal uterine cervixes. Interestingly, high LAMP3 expression was significantly correlated with the overall survival of patients with stage I/II cervical cancers. These findings indicate that LAMP3 overexpression is associated with an enhanced metastatic potential and may be a prognostic factor for cervical cancer.

Kang, H., G. Watkins, et al. (2005). "The elevated level of CXCR4 is correlated with nodal metastasis of human breast cancer." Breast **14**(5): 360-7.

CXCR4, the receptor for stromal cell-derived factor-1(SDF-1), belongs to the chemokine receptor family and has been shown to play an important role in regulating the directional migration of breast cancer cells to sites of metastasis. In the present study, we evaluated the expression of CXCR4 and its association with pathological features and clinical outcome in human breast cancer. Expression of CXCR4 in eight breast cancer cell lines and breast cancer tissues was investigated using conventional PCR. Levels of CXCR4 transcript and protein were examined in human breast cancer tissues (n=120) and corresponding normal tissues (n=32) using real-time quantitative PCR and immunohistochemistry, respectively. The level of CXCR4 expression was analyzed against tumour types, grade, nodal status, recurrence, metastasis, and survival over a median 120 month follow-up period. The expression of CXCR4 was detected in all breast cancer cell lines examined, as well as in breast cancer tissues and breast normal tissues. Breast cancer tissues highly expressed CXCR4 compared with corresponding normal tissues (P=0.029). The level of CXCR4 expression showed a significant difference between node-positive group and node-negative group (19+/-13 vs. 49.7+/-9, respectively, P=0.03). The level of CXCR4 expression was marginal, yet statistically insignificant, higher in tumours from patients with metastatic disease compared with those who remained disease free. No correlation was seen between levels of CXCR4 and the overall survival, although at higher levels of CXCR4 linked to shorter disease free survival (113.0 vs. 136.7 months in patients with low CXCR4, P=0.14, Cox proportional test). The level of CXCR4 expression is significantly correlated with lymph node metastasis. The elevated levels of CXCR4 suggest that the patient has high possibility of lymph node metastasis. CXCR4 may be a useful prognostic indicator and a potential therapeutic target in cancer therapies in patients with breast cancer.

Kang, J. S., S. Y. Bae, et al. (2009). "Interleukin-18 increases metastasis and immune escape of stomach cancer via the downregulation of CD70 and maintenance of CD44." Carcinogenesis **30**(12): 1987-96.

Cancer cells metastasize to the other site after escaping from the immune system and CD70, CD44 and vascular endothelial growth factor (VEGF) play

important roles in this process. It is recently reported that interleukin (IL)-18 is closely related with the pathogenesis of skin tumor. Therefore, we investigated the role of endogenous IL-18 from stomach cancer on the immune escape mechanism and metastasis via the regulation of CD70, CD44 and VEGF expression. IL-18 and IL-18R expressions were not only investigated on tumor tissues (n = 10), and sera (n = 20) from stomach cancer patients, but also on human stomach cancer cell lines. IL-18 and IL-18R expressions were found on stomach cancer cell lines and tumor tissues. In addition, IL-18 levels were elevated in sera from cancer patients ($P < 0.05$), compared with sera from normal individuals. Changes in CD70, CD44 and VEGF expression by flow cytometry, immunoblotting and enzyme-linked immunosorbent assay and immune susceptibility by (51)Cr-release assay were investigated, after silencing or neutralization of endogenous IL-18. CD70 expression was increased and it increases immune susceptibility of cancer cells. In contrast, CD44 and VEGF expression was decreased and it suppresses neovascularization and the metastasis of stomach cancer. After inoculation of IL-18 small interfering RNA (siRNA)-transfected stomach cancer cells into Balb/C (nu/nu) mice, regression of tumor mass was determined by measuring of tumor size. And the number and location of metastatic lesions were investigated by hematoxylin and eosin staining. The regression of tumor mass and the suppression of metastasis were observed in the mice, which are injected with IL-18 siRNA-transfected cell lines. Our data suggest that endogenous IL-18 might facilitate stomach cancer cell immune escape by suppressing CD70 and increasing metastatic ability by upregulating CD44 and VEGF.

Katsman, A., K. Umezawa, et al. (2009). "Chemosensitization and immunosensitization of resistant cancer cells to apoptosis and inhibition of metastasis by the specific NF-kappaB inhibitor DHMEQ." Curr Pharm Des **15**(7): 792-808.

Tumors develop resistance to cytotoxic apoptotic stimuli induced by various chemotherapeutic drugs and immunotherapies. Therefore, there is a need to overcome chemo- and immuno-resistance of tumors through the development of small molecules, as sensitizing agents, aimed at targeting gene products that regulate the apoptotic pathways and allow therapeutics to be effective. The constitutively activated NF-kappaB (nuclear factor kappa B) signaling pathway is involved in cell survival, inflammation and metastasis and is invariably constitutively activated in most cancers. Consequently, NF-kappaB is intimately involved in the regulation of resistance to cytotoxic drugs. A novel NF-kappaB inhibitor, DHMEQ (dehydroxymethylepoxyquinomicin), inhibits the translocation of NF-kappaB into the nucleus as well as inhibits DNA binding of NF-kappaB components and was shown to be a potent chemo- and immuno-sensitizing agent and in combination with cytotoxic therapeutics resulted in significant reversal of resistance and tumor cell death. This review will present various lines of evidence supporting the therapeutic efficacy of DHMEQ when used in combination with conventional/new cytotoxic drugs in the treatment of resistant tumor cells as well as in the prevention of metastasis.

Katsuta, M., M. Miyashita, et al. (2005). "Correlation of hypoxia inducible factor-1alpha with lymphatic metastasis via vascular endothelial growth factor-C in human esophageal cancer." Exp Mol Pathol **78**(2): 123-30.

Hypoxia inducible factor (HIF)-1alpha is a transcription factor that regulates the transcription of genes associated with cell proliferation and vascular development. In various cancer tissues, HIF-1alpha is associated with clinicopathological factors, such as the tumor size, histological grade, and lymph node status. Although HIF-1alpha plays a critical role in tumor growth by inducing vascular endothelial growth factor (VEGF), it is unclarified whether HIF-1alpha affects lymphatic metastasis. The purpose of this study is to clarify the correlation of HIF-1alpha protein expression with lymph node metastasis in esophageal squamous cell carcinoma (ESCC). The expressions of HIF-1alpha and VEGF-C, which is one of the main lymphangiogenic factors, were examined in five ESCC cell lines and 48 surgical specimens of ESCC. HIF-1alpha and VEGF-C mRNAs were expressed in all the five ESCC cell lines as determined by RT-PCR analysis. Immunohistochemically, 34 of the 48 patients (70.8%) were positive for HIF-1alpha and 29 patients (60.4%) were positive for VEGF-C. Clinicopathologically, HIF-1alpha expression correlated with lymphatic invasion and VEGF-C expression ($P = 0.003$ and $P = 0.01$, respectively). Furthermore, HIF-1alpha expression tended to correlate with lymph node metastasis ($P = 0.09$). These findings suggest that HIF-1alpha plays a role in lymphatic invasion and lymph node metastasis through the induction of VEGF-C in ESCC.

Kazama, S., T. Watanabe, et al. (2006). "Tumour budding at the deepest invasive margin correlates with lymph node metastasis in submucosal colorectal cancer detected by anticytokeratin antibody CAM5.2." Br J Cancer **94**(2): 293-8.

In the past few years, tumour budding at the invasive margin has been reported as a new risk factor

for lymph node metastasis in advanced colorectal cancers, but it is sometimes difficult to detect tumour budding in submucosal colorectal cancer by haematoxylin and eosin staining. We immunohistochemically examined tumour budding at the deepest invasive margin of 56 surgically resected submucosal colorectal carcinomas using anticytokeratin antibody CAM5.2, furthermore checked by AE1/AE3, and determined the relation between tumour budding and clinicopathological factors. Moreover, we used the monoclonal antibody D2-40 for immunohistochemistry to detect lymphatic involvement. Tumour budding was detected in 42 cases (75.0%), and the budding-positive group showed a significantly higher rate of lymph node metastasis (including isolated tumour cells) (16/42 vs 0/14; P=0.004) than the budding-negative group. The sensitivity and negative predictive value of tumour budding alone for lymph node metastasis were superior to those of lymphatic invasion alone. Furthermore, the specificity and positive predictive value of the combination of either lymphatic invasion or tumour budding were superior to those of lymphatic invasion alone. Tumour budding detected immunohistochemically by using CAM5.2 is a newly found risk factor for lymph node metastasis and may help to avoid oversurgery in the future.

Kobayashi, D., M. Yamada, et al. (2002). "Overexpression of early growth response-1 as a metastasis-regulatory factor in gastric cancer." Anticancer Res **22**(6C): 3963-70.

BACKGROUND: To investigate the potential role of a nuclear transcription factor, early growth response-1 (Egr-1), in formation and progression of gastric cancer, we compared its expression in gastric cancers with that in non-cancerous tissues. MATERIALS AND METHODS: Egr-1 mRNA expression was measured using TaqMan RT-PCR. The corresponding protein expression was examined immunohistochemically. RESULTS: Egr-1 mRNA expression was significantly higher in gastric cancer tissues than in normal mucosa ($p < 0.0005$). These differences were also reflected by protein product expression. Moreover, Egr-1 mRNA expression was higher in cases with metastasis to lymph nodes or remote organs. In cultured gastric cancer cells known to have a high metastatic potential, expression of this mRNA was higher than that of parental cells. CONCLUSION: It was suggested that Egr-1 has a significant role in carcinogenesis and in cancer progression, especially metastasis. Measurement of this mRNA should be useful for evaluation of the metastatic potential of gastric cancer.

Kobayashi, H., M. Suzuki, et al. (2004). "Genetic down-regulation of phosphoinositide 3-kinase by bikunin correlates with suppression of invasion and metastasis in human ovarian cancer HRA cells." J Biol Chem **279**(8): 6371-9.

Using a cDNA microarray analysis, we previously found that exposure of a highly invasive ovarian cancer cell line HRA with bikunin, a Kunitz-type protease inhibitor, or bikunin gene overexpression markedly reduced phosphoinositide kinase (PI3K) p85 gene expression, demonstrating that PI3K may be a candidate bikunin target gene. To clarify how reduced levels of PI3K may confer repressed invasiveness, we transfected HRA cells with PI3K p85 antisense-oligodeoxynucleotide (AS-ODN) and compared the properties of the transfected cells with those of parental cells and sense (S)-ODN cells. We have also demonstrated previously that transforming growth factor-beta1 (TGF-beta1) stimulates urokinase-type plasminogen activator (uPA)-dependent invasion and metastasis of HRA cells. Here, we show that 1) TGF-beta1 induced a rapid increase of the PI3K activity that was accompanied by increased expression (5-fold) of the uPA mRNA; 2) pharmacological inhibition of PI3K or AS-PI3K ODN transfection inhibited TGF-beta1-stimulated Akt phosphorylation; 3) both PI3K pharmacological inhibitors and forced expression of AS-PI3K ODN reduced TGF-beta1-stimulated uPA mRNA and protein expression by approximately 70% compared with controls; 4) concentrations of PI3K inhibitors, sufficient to inhibit uPA up-regulation, inhibited TGF-beta1-dependent HRA cell invasion; 5) the AS-PI3K ODN cells had a decreased ability to invade the extracellular matrix layer as compared with controls; and 6) when the AS-PI3K ODN cells were injected intraperitoneally into nude mice, the mice developed smaller intraperitoneal tumors and showed longer survival. We conclude that PI3K plays an essential role in promoting uPA-mediated invasive phenotype in HRA cells. Our data identify a novel role for PI3K as a bikunin target gene on uPA up-regulation and invasion.

Kodama, J., Hasengaowa, et al. (2007). "Association of CXCR4 and CCR7 chemokine receptor expression and lymph node metastasis in human cervical cancer." Ann Oncol **18**(1): 70-6.

BACKGROUND: The chemokine receptors CXCR4 and CCR7 have been suggested to play an important role in cancer invasion and metastasis. The expression of these receptors in human cervical cancer, however, has seldom been characterized. PATIENTS AND METHODS: We investigated the expression of CXCR4 and CCR7 in cervical cancer specimens and determined the association between

their expression and the clinicopathological features observed, including patient outcome. RESULTS: CXCR4 expression was significantly higher in elderly patients (P=0.025); it was also significantly increased in patients with cancers displaying large tumor size (P=0.010), deep stromal invasion (P=0.0004), lymph-vascular space involvement (P=0.0002), or lymph node metastasis (P<0.0001). CCR7 expression was significantly higher in cases of squamous cell carcinomas (P=0.010) and in patients with cancers showing large tumor size (P<0.0001), deep stromal invasion (P<0.0001), vaginal invasion (P=0.047), lymph-vascular space involvement (P=0.012), or lymph node metastasis (P<0.0001). Logistic regression analysis revealed that deep stromal invasion (P=0.017) and CXCR4 (P=0.016) and CCR7 (P=0.022) expression were independent factors that influenced pelvic lymph node metastasis. The disease-free survival and overall survival (OS) rates of patients exhibiting both CXCR4 and CCR7 expression were significantly reduced (P<0.0001). In addition, the expression of both CXCR4 and CCR7 was an independent prognostic factor for OS (95% confidence interval=1.03-17.86; P=0.046). CONCLUSIONS: CXCR4 and CCR7 expression may be associated with lymph node metastasis; moreover, the expression of these receptors can serve as an indicator of poor prognosis in patients with cervical cancer.

Koide, N., H. Watanabe, et al. (2000). "Four resections for hepatic metastasis from gastric cancer: histochemical analysis of cell proliferation, apoptosis, and angiogenesis." J Gastroenterol **35**(2): 150-4.

In a patient with gastric cancer (GC) associated with one synchronous and three metachronous hepatic metastases (HM), who underwent four hepatectomies, we carried out histochemical investigations regarding cell proliferation, apoptosis, and angiogenesis in the GC and HM. Tissue samples were taken from the primary GC and four HM. Ki-67 immunostaining was performed to evaluate cell proliferation and determine the labeling index (Ki-67 LI; ie, the percentage of cancer cells with nuclei stained for Ki-67). Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end labeling (TUNEL) was performed to evaluate apoptosis and determine the apoptotic index (ie, the percentage of TUNEL-positive cells), and immunostaining for factor VIII-related antigen was performed to evaluate angiogenesis and measure microvessel density (MVD). The Ki-67 LI was 43.2% in the primary GC and 39.9% in the synchronous HM, and the LI increased with the number of resections of metachronous HM. The apoptotic index was 3.36% in the primary GC, and 5.30% in the synchronous HM, and the index decreased after further resections of the metachronous HM. The MVD was 35 in the primary GC, and 22 in the synchronous HM, and it increased with the number of resections of metachronous HM. The primary GC in this patient may have strongly influenced the growth of HM through effects on cell proliferation, apoptosis, and angiogenesis.

Kondo, Y., S. Arii, et al. (2000). "Enhancement of angiogenesis, tumor growth, and metastasis by transfection of vascular endothelial growth factor into LoVo human colon cancer cell line." Clin Cancer Res **6**(2): 622-30.

The expression of vascular endothelial growth factor (VEGF), a highly potent angiogenic molecule, is thought to be correlated with the development of colon cancer; however, direct evidence for a role of VEGF in metastasis is lacking. This study was designed to more directly establish the role of VEGF in the growth and metastasis of human colon cancer using a genetically engineered cancer cell line. A stable VEGF gene-transfected human colon cancer cell line, LoVo, was made by genetic manipulation using eukaryotic expression constructs designed to express the complete VEGF121 cDNA in the sense orientation. Transfected clones were screened for VEGF121 mRNA expression by Northern blot analysis and for VEGF121 protein expression by Western blot analysis. Consequently, we obtained S17 cells that expressed a high level of both VEGF mRNA and VEGF protein. A vector-transfected clone (V7 cell) was used as a control. The experiment with the dorsal air sac method revealed that S17 cells elicited a stronger directional out-growth of capillaries than V7 cells. S17 cells formed faster-growing tumors than did V7 cells when xenografted s.c. into nude mice, although there was no significant difference in their in vitro proliferation. Tumors derived from S17 cells had more vascularity, as assessed by counting capillary vessels after staining with factor VIII, than did tumors derived from V7 cells ($P < 0.05$). With regard to the metastatic potential, S17 cells exhibited a higher capacity for both hepatic metastasis after the splenic portal inoculation and peritoneal dissemination after i.p. injection than did V7 cells. However, S17 cells showed no apparent metastasis, despite their rapid growth after orthotopic implantation. In conclusion, the present study showed clearly that VEGF plays an important role in cancer growth due to stimulation of angiogenesis by accelerating cell growth after reaching the target organs.

Kozlow, W. and T. A. Guise (2005). "Breast cancer metastasis to bone: mechanisms of osteolysis and

implications for therapy." J Mammary Gland Biol Neoplasia **10**(2): 169-80.

The most common skeletal complication of breast cancer is osteolytic bone metastasis. Bone metastases are present in 80% of patients with advanced disease and cause significant morbidity. They are most often osteolytic, but can be osteoblastic or mixed. Tumor cells, osteoblasts, osteoclasts and bone matrix are the four components of a vicious cycle necessary for the initiation and development of bone metastases. Tumor cell gene expression is modified by interaction with bone-derived factors. For example, parathyroid hormone related protein (PTHrP), a tumor cell factor, is upregulated by bone-derived transforming growth factor beta (TGFbeta). Tumor cell factors, in turn, act upon bone cells to cause dysregulated bone destruction and formation. PTHrP increases osteoblast expression of RANK (receptor activator of NFkappaB) ligand which, in turn, activates osteoclasts. PTHrP-independent osteolytic factors, such as interleukin [IL]-11 and IL-8, also contribute to the vicious cycle. Other tumor-bone interactions, such as stimulation of tumor-homing through the CXCR4 chemokine receptor by its bone-derived ligand stromal-derived factor-1 (SDF-1), may be responsible for the site-specific predilection of breast cancer for bone. These factors and their roles in fueling the vicious cycle may identify novel targets for therapies to prevent metastasis.

Kucia, M., R. Reca, et al. (2005). "Trafficking of normal stem cells and metastasis of cancer stem cells involve similar mechanisms: pivotal role of the SDF-1-CXCR4 axis." Stem Cells **23**(7): 879-94.

The alpha-chemokine stromal-derived factor (SDF)-1 and the G-protein-coupled seven-span transmembrane receptor CXCR4 axis regulates the trafficking of various cell types. In this review, we present the concept that the SDF-1-CXCR4 axis is a master regulator of trafficking of both normal and cancer stem cells. Supporting this is growing evidence that SDF-1 plays a pivotal role in the regulation of trafficking of normal hematopoietic stem cells (HSCs) and their homing/retention in bone marrow. Moreover, functional CXCR4 is also expressed on nonhematopoietic tissue-committed stem/progenitor cells (TCSCs); hence, the SDF-1-CXCR4 axis emerges as a pivotal regulator of trafficking of various types of stem cells in the body. Furthermore, because most if not all malignancies originate in the stem/progenitor cell compartment, cancer stem cells also express CXCR4 on their surface and, as a result, the SDF-1-CXCR4 axis is also involved in directing their trafficking/metastasis to organs that highly express SDF-1 (e.g., lymph nodes, lungs, liver, and bones). Hence, we postulate that the metastasis of cancer stem cells and trafficking of normal stem cells involve similar mechanisms, and we discuss here the common molecular mechanisms involved in these processes. Finally, the responsiveness of CXCR4+ normal and malignant stem cells to an SDF-1 gradient may be regulated positively/primed by several small molecules related to inflammation which enhance incorporation of CXCR4 into membrane lipid rafts, or may be inhibited/blocked by small CXCR4 antagonist peptides. Consequently, strategies aimed at modulating the SDF-1-CXCR4 axis could have important clinical applications both in regenerative medicine to deliver normal stem cells to the tissues/organs and in clinical hematology/oncology to inhibit metastasis of cancer stem cells.

Kudo-Saito, C., H. Shirako, et al. (2009). "Cancer metastasis is accelerated through immunosuppression during Snail-induced EMT of cancer cells." Cancer Cell **15**(3): 195-206.

Epithelial-mesenchymal transition (EMT) is a key step toward cancer metastasis, and Snail is a major transcription factor governing EMT. Here, we demonstrate that Snail-induced EMT accelerates cancer metastasis through not only enhanced invasion but also induction of immunosuppression. Murine and human melanoma cells with typical EMT features after snail transduction induced regulatory T cells and impaired dendritic cells in vitro and in vivo partly through TSP1 production. Although Snail(+) melanoma did not respond to immunotherapy, intratumoral injection with snail-specific siRNA or anti-TSP1 monoclonal antibody significantly inhibited tumor growth and metastasis following increase of tumor-specific tumor-infiltrating lymphocytes and systemic immune responses. These results suggest that inhibition of Snail-induced EMT could simultaneously suppress both tumor metastasis and immunosuppression in cancer patients.

Kurahara, H., S. Takao, et al. (2004). "Impact of vascular endothelial growth factor-C and -D expression in human pancreatic cancer: its relationship to lymph node metastasis." Clin Cancer Res **10**(24): 8413-20.

PURPOSE: The aim of this study was to evaluate the expression of vascular endothelial growth factor (VEGF)-C and -D in pancreatic cancer and to reveal its relation to lymph node metastasis. EXPERIMENTAL DESIGN: Formalin-fixed, paraffin-embedded blocks were obtained from 58 patients with pancreatic head cancer. All of the patients underwent a curative resection. The total number of resected lymph nodes was 1,058. The expressions of VEGF-C and -D were evaluated by

immunohistochemical staining. To evaluate the relation to lymph node metastasis, the expressions of VEGF-C and -D between the marginal and central portions in the tumor were compared. When >25% of the tumor cells showed distinct staining, the portion was judged as high expression. RESULTS: The two groups with high expression of VEGF-C (P = 0.015) and VEGF-D (P = 0.020) in the marginal portion had a significantly higher incidence of lymph node metastasis compared with the groups with low expression, respectively. Furthermore, the group with high expression of both VEGF-C and -D in the marginal portion had a higher incidence of lymph node metastasis compared with the group with low expression (P = 0.007). The 5-year survival rate of patients with high expression of both VEGF-C and -D in the marginal portion was significantly lower than that of patients with low expression of both VEGF-C and -D (P = 0.017). CONCLUSIONS: VEGF-C and -D expression in tumor cells in the marginal portion of the tumor significantly associated with lymphatic metastasis and prognosis in patients with pancreatic head cancer.

Liao, D., C. Corle, et al. (2007). "Hypoxia-inducible factor-1alpha is a key regulator of metastasis in a transgenic model of cancer initiation and progression." Cancer Res **67**(2): 563-72.

Adaptation to hypoxia is a critical step in tumor progression and is, in part, regulated by the transcription factor hypoxia-inducible factor-1alpha (HIF-1alpha). Xenograft models have been extensively used to characterize the role of HIF-1alpha in experimental cancers. Although these models provide an understanding of tumor growth at terminal stages of malignancy, they do not address tumor initiation or metastatic progression. To elucidate these roles, HIF-1alpha was conditionally deleted in the mammary epithelium of a transgenic mouse model for metastatic breast cancer. Conditional deletion of HIF-1alpha in the mammary epithelium resulted in delayed tumor onset and retarded tumor growth; this was correlated with decreased tumor cell proliferation. Tumors with conditional deletion of HIF-1alpha were also less vascular during early tumor progression. Perhaps most surprisingly, deletion of HIF-1alpha in the mammary epithelium resulted in decreased pulmonary metastasis. These results show that whereas HIF-1alpha is not required for the initiation of breast tumor growth or tumor cell metastasis, the transcriptional activity of HIF-1alpha is a significant positive regulator of tumor progression and metastatic potential.

Liao, J., A. Schneider, et al. (2006). "Extracellular calcium as a candidate mediator of prostate cancer skeletal metastasis." Cancer Res **66**(18): 9065-73.

Prostate cancer almost exclusively metastasizes to skeletal sites, indicating that the bone provides a favorable microenvironment for its localization and progression. A natural yet understudied factor in bone that could facilitate tumor localization is elevated extracellular calcium ([Ca2+]o). The present study found that elevated [Ca2+]o (2.5 mmol/L) enhanced proliferation of skeletal metastatic prostate cell lines (PC-3 and C4-2B), but not the nonskeletal metastatic, epithelial-derived prostate cell line LNCaP. The proliferative effect of elevated [Ca2+]o was associated with higher expression of the calcium-sensing receptor (CaSR), a heterotrimeric G-protein-coupled receptor that is the predominant cell-surface sensor for [Ca2+]o. Knockdown of the CaSR via RNA interference reduced cell proliferation in vitro and metastatic progression in vivo. CaSR signaling in PC-3 cells was evaluated by measuring the elevated [Ca2+]o-dependent inhibition of cyclic AMP accumulation, induced by either prostaglandin E2 or forskolin. Elevated [Ca2+]o stabilized expression of cyclin D1, a protein required for cell cycle transition. Furthermore, elevated [Ca2+]o triggered activation of the Akt signaling pathway and enhanced PC-3 cell attachment. Both pertussis toxin (a G-protein inhibitor) and LY294002 (an inhibitor of Akt signaling) reduced cell attachment. These data suggest that elevated [Ca2+]o following increased bone remodeling could facilitate metastatic localization of prostate cancer via the CaSR and the Akt signaling pathway. Taken together, [Ca2+]o is a candidate mediator of prostate cancer bone metastasis.

Lin, B. R., C. C. Chang, et al. (2005). "Connective tissue growth factor inhibits metastasis and acts as an independent prognostic marker in colorectal cancer." Gastroenterology **128**(1): 9-23.

BACKGROUND & AIMS: Connective tissue growth factor (CTGF) has been shown to be implicated in tumor development and progression. The aim of this study was to investigate the role of CTGF in progression of colorectal cancer (CRC). METHODS: Immunohistochemical staining of specimens from 119 patients with CRC was performed. Liposome-mediated transfection was used to introduce a CTGF expression vector into CRC cell lines. Transfectants were tested in invasive ability and experimental hepatic metastasis in BALB/c mice. Furthermore, a FOPflash/TOPflash reporter assay was performed to investigate CTGF on the beta-catenin/T-cell factor signaling pathway. RESULTS: Patients with stage II and stage III CRC whose tumors

displayed high CTGF expression had a significantly higher overall survival and a disease-free advantage over patients with CRC with low CTGF expression. Alterations in the CTGF level in CRC cell lines modulated their invasive ability with an inverse correlation. In addition, a reduction in the CTGF level of CT26 cells after stable transfection with antisense CTGF resulted in increased liver metastasis in BALB/c mice. The activity of the beta-catenin/T-cell factor signaling pathway and its downstream effector gene matrix metalloproteinase 7 in these CTGF-transfected cells was strongly attenuated. Blockage of matrix metalloproteinase 7 with its neutralizing antibodies inhibited increased invasiveness in antisense CTGF-transfected CT26 cells. CONCLUSIONS: Our results implicate CTGF as a key regulator of CRC invasion and metastasis, and it appears to be a useful and better prognosis factor for patients with stage II and stage III CRC.

Lin, Y., P. J. Buckhaults, et al. (2009). "Association of the actin-binding protein transgelin with lymph node metastasis in human colorectal cancer." Neoplasia **11**(9): 864-73.

Metastatic dissemination of primary tumors is responsible for 90% of colorectal cancer (CRC) deaths. The presence of positive lymph nodes, which separates stage I/II from stage III CRC, is a particularly key factor in patient management. Here, we describe results of a quantitative proteomic survey to identify molecular correlates of node status. Laser capture microdissection and two-dimensional difference gel electrophoresis were used to establish expression profiles for 980 discrete protein features in 24 human CRC specimens. Protein abundances were determined with a median technical coefficient of variation of 10%, which provided an ability to detect small differences between cancer subtypes. Transgelin, a 23-kDa actin-binding protein, emerged as a top-ranked candidate biomarker of node status. The area under the receiver operating characteristic curve for transgelin in predicting node status was 0.868 (P = .002). Significantly increased frequency of moderate- and high-level transgelin expression in node-positive CRC was also seen using semiquantitative immunohistochemistry to analyze 94 independent CRC specimens on tissue microarrays (P = .036). Follow-up studies in CRC cell lines demonstrated roles for transgelin in promoting invasion, survival, and resistance to anoikis. Transgelin localizes to the nucleus of CRC cells, and its sequence and properties suggest that it may participate in regulation of the transcriptional program associated with the epithelial-to-mesenchymal transition.

Liu, J., M. Ikeguchi, et al. (2002). "Re-expression of the cadherin-catenin complex in lymph nodes with metastasis in advanced gastric cancer: the relationship with patient survival." J Exp Clin Cancer Res **21**(1): 65-71.

The cadherin-catenin complex has been recognized as an important factor associated with tumor metastasis. However, the clinical significance of the expression of adhesion molecules in lymph nodes with metastasis remains unclear. The aim of this study was to investigate the clinical significance of the re-expression of the cadherin-catenin complex in metastatic lymph nodes in patients with advanced gastric cancer. Immunohistochemical expression of E-cadherin, alpha- and beta-catenin were analyzed in 96 primary gastric cancers with serosal invasion and in 79 lymph nodes with metastasis. The expression levels of these adhesion molecules in primary tumors and lymph nodes with metastasis were compared. Ninety-four out of 96 primary tumors (98%) showed reduced expression of adhesion molecules. Out of 79 cases with lymph node metastasis, increased expression of one or more adhesion molecules in metastatic foci as compared with primary tumors was detected in 52 cases (66%). Re-expression of adhesion molecules in metastatic lymph nodes was detected in a more advanced stage. The overall 5-year survival rate of the 52 patients who had lymph nodes with metastasis with re-expression of adhesion molecules (8%) was significantly poorer than that of the 27 who had lymph nodes with metastasis without re-expression of adhesion molecules (33%, P = 0.0012). The re-expression of the cadherin-catenin complex in lymph nodes with metastasis may play an important role in the growth of cancer cells in metastatic foci. A comparison of the expression patterns of adhesion molecules between the primary tumor and metastatic lymph nodes may provide new prognostic information for patients with advanced gastric cancer.

Loges, S., H. Clausen, et al. (2007). "Determination of microvessel density by quantitative real-time PCR in esophageal cancer: correlation with histologic methods, angiogenic growth factor expression, and lymph node metastasis." Clin Cancer Res **13**(1): 76-80.

PURPOSE: Angiogenesis and lymphangiogenesis are important steps in tumor growth and dissemination and are of prognostic importance in solid tumors. The determination of microvessel density (MVD) by immunohistology is subject to considerable variability between different laboratories and observers. We compared MVD determination by immunohistology and quantitative real-time PCR and correlated the results with clinical variables. EXPERIMENTAL DESIGN: The

expression of endothelial antigens vascular endothelial cadherin (CD144), P1H12 (CD146), tie-2, and VEGFR-2, and lymphatic endothelial markers VEGFR-3, Prox, and LYVE was assessed by quantitative PCR (qPCR) in primary surgical samples. The expression of angiogenetic growth factors VEGF-A, VEGF-C, VEGF-D, angiopoietin-1, and angiopoietin-2 was quantified by PCR and correlated with MVD and clinical variables. RESULTS: The expression of endothelial antigens vascular endothelial cadherin (CD144), P1H12 (CD146), tie-2, and VEGFR-2 correlated with each other in 54 samples of primary esophageal cancer ($P < 0.0001$ for all comparisons). MVD determined immunohistologically by CD31 staining in a subgroup of 35 patients correlated significantly with the qPCR method. The expression of angiogenetic growth factors VEGF-A, VEGF-C, VEGF-D, angiopoietin-1, and angiopoietin-2 was significantly associated with MVD ($P < 0.0001$ for all comparisons). Analysis of the expression of lymphendothelial markers VEGFR-3, Prox, and LYVE revealed concordant results, indicating that quantification of lymphendothelial cells is possible by qPCR. The presence of lymph node metastasis on surgical specimens was significantly correlated with MVD ($P < 0.003$), VEGFR-2 ($P < 0.048$), and VEGF-C ($P < 0.042$) expression. CONCLUSIONS: These results indicate that quantification of MVD by qPCR in surgical samples of esophageal carcinoma yields similar results with immunohistology. Interestingly, the extent of angiogenesis and lymphangiogenesis was not related in individual tumor samples. Lymph node metastases could be predicted by MVD and VEGF-C expression.

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

Rapamycin is an effective inhibitor of human renal cancer metastasis. BACKGROUND: Human renal cell cancer (RCC) is common and is 10 to 100 times more frequent in patients with end-stage renal disease (ESRD) and candidates for renal transplantation. Treatment of metastatic RCC is largely ineffective and is further undermined by immunosuppressive therapy in transplant recipients. A treatment regimen that prevents transplant rejection while constraining RCC progression would be of high value. METHODS: We developed a human RCC pulmonary metastasis model using human RCC 786-O as the tumor challenge and the severe combined immunodeficient (SCID) beige mouse as the host. We explored the effect of rapamycin, cyclosporine, or rapamycin plus cyclosporine on the development of pulmonary metastases and survival. The effects of the drugs on tumor cell growth, apoptosis, and expression of vascular endothelial growth factor (VEGF-A) and transforming growth factor beta1 (TGF-beta1) were also investigated. RESULTS: Rapamycin reduced, whereas cyclosporine increased, the number of pulmonary metastases. Rapamycin was effective in cyclosporine-treated mice, and rapamycin or rapamycin plus cyclosporine prolonged survival. Rapamycin growth arrested RCC 786-O at the G1 phase and reduced VEGF-A expression. Immunostaining of lung tissues for von Willebrand factor was minimal and circulating levels of VEGF-A and TGF-beta1 were lower in the rapamycin-treated mice compared to untreated or cyclosporine-treated mice. CONCLUSION: Our findings support the idea that rapamycin may be of value for patients with RCC and that its antitumor efficacy is realized by cell cycle arrest and targeted reduction of VEGF-A and TGF-beta1. A regimen of rapamycin and cyclosporine, demonstrated to be effective in reducing acute rejection of renal allografts, may prevent RCC progression as well, and has the potential to prevent mortality due to RCC in patients with ESRD who have received renal allografts.

Luo, J. L., W. Tan, et al. (2007). "Nuclear cytokine-activated IKKalpha controls prostate cancer metastasis by repressing Maspin." Nature **446**(7136): 690-4.

Inflammation enhances tumour promotion through NF-kappaB-dependent mechanisms. NF-kappaB was also proposed to promote metastatogenesis through epithelial-mesenchymal transition. Yet a mechanistic link between inflammation and metastasis is missing. We identified a role for IkappaB kinase alpha (IKKalpha), activated by receptor activator of NF-kappaB (RANK/TNFRSF11A), in mammary epithelial proliferation during pregnancy. Owing to similarities between mammary and prostate epithelia, we examined IKKalpha involvement in prostate cancer and its progression. Here we show that a mutation that prevents IKKalpha activation slows down CaP growth and inhibits metastatogenesis in TRAMP mice, which express SV40 T antigen in the prostate epithelium. Decreased metastasis correlated with elevated expression of the metastasis suppressor Maspin, the ablation of which restored metastatic activity. IKKalpha activation by RANK ligand (RANKL/TNFSF11) inhibits Maspin expression in prostate epithelial cells, whereas repression of Maspin transcription requires nuclear translocation of active IKKalpha. The amount of active nuclear IKKalpha in mouse and human prostate cancer correlates with metastatic progression, reduced Maspin expression and infiltration of prostate tumours with RANKL-expressing inflammatory cells. We propose that tumour-infiltrating RANKL-expressing cells lead to

nuclear IKKalpha activation and inhibition of Maspin transcription, thereby promoting the metastatic phenotype.

Malik, F. A., A. J. Sanders, et al. (2009). "KAI-1/CD82, the molecule and clinical implication in cancer and cancer metastasis." Histol Histopathol **24**(4): 519-30.

CD82, also known as KAI-1, structurally belongs to tetraspanin family while categorised as metastasis suppressor gene on functional grounds. KAI1/CD82 is localized on cell membrane and form interactions with other tetraspanins, integrins and chemokines which are respectively responsible for cell migration, adhesion and signalling. In recent years apart from its significant involvement in the suppression of secondary tumours it has also been observed that KAI1/CD82 plays a vital role in virus binding and its entry inside the cell. Decreased expression of KAI1/CD82 molecule results in aggravating cancer progression. Altered expression levels of KAI1/CD82 molecule in different types of human cancer have been implicated as having prognostic value and linking to the long term survival of the patients. Increased level of KAI1/CD82 also results in the suppression of secondary tumour growth. Increased expression of this molecule results in reduced cell invasion and cell migration due to endocytosis of epidermal growth factor receptors (EGFR). Thus, KAI-1/CD82 is a pivotal molecule in the regulation of cancer cells' behaviour and has important clinical and therapeutic implications in cancer.

Maruyama, Y., M. Ono, et al. (2006). "Tumor growth suppression in pancreatic cancer by a putative metastasis suppressor gene Cap43/NDRG1/Drg-1 through modulation of angiogenesis." Cancer Res **66**(12): 6233-42.

Cap43 has been identified as a nickel- and calcium-induced gene, and is also known as N-myc downstream-regulated gene 1 (NDRG1), Drg-1 and rit42. It is also reported that overexpression of Cap43 suppresses metastasis of some malignancies, but its precise role remains unclear. In this study, we asked how Cap43 could modulate the tumor growth of pancreatic cancer. Stable Cap43 cDNA transfectants of pancreatic cancer cells with Cap43 overexpression showed similar growth rates in culture as their control counterparts with low Cap43 protein level. By contrast, Cap43 overexpression showed a marked decrease in tumor growth rates in vivo. Moreover, a marked reduction in tumor-induced angiogenesis was observed. Gelatinolytic activity by matrix metalloproteinase-9 and invasive ability in Matrigel invasion activity were markedly decreased in pancreatic cancer cell lines with high Cap43 expression. Cellular expression of matrix metalloproteinase-9 and two major angiogenic factors, vascular endothelial growth factor and interleukin-8, were also significantly decreased in cell lines with Cap43 overexpression as compared with their parental counterparts. Immunohistochemical analysis of specimens from 65 patients with pancreatic ductal adenocarcinoma showed a significant association between Cap43 expression and tumor microvascular density ($P = 0.0001$) as well as depth of invasion ($P = 0.0003$), histopathologic grading ($P = 0.0244$), and overall survival rates for patients with pancreatic cancer ($P = 0.0062$). Thus, Cap43 could play a key role in the angiogenic on- or off-switch of tumor stroma in pancreatic ductal adenocarcinoma.

Matsumori, Y., S. Yano, et al. (2006). "ZD6474, an inhibitor of vascular endothelial growth factor receptor tyrosine kinase, inhibits growth of experimental lung metastasis and production of malignant pleural effusions in a non-small cell lung cancer model." Oncol Res **16**(1): 15-26.

ZD6474 is a novel, orally active inhibitor of vascular endothelial growth factor receptor-2 (VEGFR-2) tyrosine kinase, with some additional activity against epidermal growth factor receptor (EGFR) tyrosine kinase. The purpose of this study was to determine the potential of ZD6474 in the control of established experimental lung metastasis and pleural effusions produced by human non-small cell lung cancer (NSCLC) cells. PC14PE6 (adenocarcinoma) and H226 (squamous cell carcinoma) cells express high levels of EGFR and only PC14PE6 cells overexpress VEGF. Neither ZD6474 nor the EGFR tyrosine kinase inhibitor gefitinib inhibit proliferation of PC14PE6 or H226 cells in vitro. Both PC14PE6 and H226 cells inoculated intravenously into nude mice induced multiple lung nodules after 5-7 weeks. In addition, PC14PE6 cells produced bloody pleural effusions. Daily oral treatment with ZD6474 did not reduce the number of lung nodules produced by PC14PE6 or H226 cells, but did reduce the lung weight and the size of lung nodules. ZD6474 also inhibited the production of pleural effusions by PC14PE6 cells. Histological analyses of lung lesions revealed that ZD6474 treatment inhibited activation of VEGFR-2 and reduced tumor vascularization and tumor cell proliferation. Therapeutic effects of ZD6474 were considered likely to be due to inhibition of VEGFR-2 tyrosine kinase because gefitinib was inactive in this model. These results indicate that ZD6474, an inhibitor of VEGFR-2, may be useful in controlling the growth of established lung metastasis and pleural effusions by NSCLC.

Matsusue, R., H. Kubo, et al. (2009). "Hepatic stellate cells promote liver metastasis of colon cancer cells by the action of SDF-1/CXCR4 axis." Ann Surg Oncol **16**(9): 2645-53.

BACKGROUND: It has been determined that the chemokine receptor CXCR4 and its ligand stromal cell-derived factor-1 (SDF-1) regulate several key processes in a wide variety of cancers. However, the function and mechanism of the SDF-1/CXCR4 system in the metastasis of colorectal cancer remain controversial. METHODS: Immunohistochemistry was performed to examine quantitatively the expression of CXCR4 in 40 human samples of colorectal cancer and liver metastasis. The functions of SDF-1 on HCT116 colon cancer cells were investigated in vitro. We subcutaneously inoculated HCT116 cells with hepatic stellate cells (HSCs) expressing SDF-1. The CXCR4 inhibitor AMD3100 was tested in vitro and in vivo. RESULTS: By quantitatively counting the number of cells, it was shown that there are more CXCR4-positive cells at the metastatic site in the liver compared with the primary sites. We demonstrated the effect of SDF-1 on the invasion and antiapoptosis of HCT116 cells in vitro. In mouse experiment of liver metastasis, intraperitoneal administration of AMD3100 blocked the metastatic potential of HCT116 cells. Furthermore, we found that alpha-smooth muscle actin (alpha-SMA)-positive myofibroblasts derived from HSCs, surrounding the liver metastasis foci, secreted SDF-1. The subcutaneous inoculation of HCT116 cells with HSCs promoted the tumor initiation in nude mice, indicating the importance of the direct interaction between these cells in vivo. CONCLUSION: These results suggest that HSCs play important role in liver metastasis of colon cancer cells by the action of SDF-1/CXCR4 axis and provide preclinical evidence that blockade of the axis is a target for antimetastasis therapy.

Mehrotra, J., M. Vali, et al. (2004). "Very high frequency of hypermethylated genes in breast cancer metastasis to the bone, brain, and lung." Clin Cancer Res **10**(9): 3104-9.

PURPOSE: Most often it is not the primary tumor, but metastasis to distant organs that results in the death of breast cancer patients. To characterize molecular alterations in breast cancer metastasis, we investigated the frequency of hypermethylation of five genes (Cyclin D2, RAR-beta, Twist, RASSF1A, and HIN-1) in metastasis to four common sites: lymph node, bone, brain, and lung. EXPERIMENTAL DESIGN: Methylation-specific PCR for the five genes was performed on DNA extracted from archival paraffin-embedded specimens of paired primary breast cancer and its lymph nodes (LN) metastasis (n = 25 each); in independent samples of metastasis to the bone (n = 12), brain (n = 8), and lung (n = 10); and in normal bone, brain, and lung (n = 22). RESULTS: No hypermethylation was detected in the five genes in the normal host tissues. In paired samples, LN metastasis had a trend of higher prevalence of methylation compared with the primary breast carcinoma for all five genes with significance for HIN-1 ($P = 0.04$). Compared with the primary breast carcinomas, all five genes had higher methylation frequencies in the bone, brain, and lung metastasis, with HIN-1 and RAR-beta methylation being significantly higher ($P < 0.01$) in each group. Loss of expression of all five genes correlated, with a few exceptions, to hypermethylation of their promoter sequences in metastatic carcinoma cells microdissected from LNs. CONCLUSION: The frequent presence of hypermethylated genes in locoregional and distant metastasis could render them particularly susceptible to therapy targeted toward gene reactivation combining demethylating agents, histone deacetylase inhibitors, and/or differentiating agents.

Missiaglia, E., E. Blaveri, et al. (2004). "Analysis of gene expression in cancer cell lines identifies candidate markers for pancreatic tumorigenesis and metastasis." Int J Cancer **112**(1): 100-12.

Pancreatic cancer is a highly aggressive type of malignancy and the prognosis for disease presenting typically at a late stage is extremely poor. A comprehensive understanding of its molecular genetics is required in order to develop new approaches to clinical management. To date, serial analysis of gene expression and more recently oligo/cDNA microarray technologies have been employed in order to identify genes involved in pancreatic neoplasia that can be developed as diagnostic markers and drug targets for this dismal disease. This study describes the expression profile obtained from 20 pancreatic cell lines using cDNA microarrays containing 9,932 human gene elements. Numerous genes were identified as being differentially expressed, some of which have been previously implicated in pancreatic adenocarcinoma (S100P, S100A4, prostate stem cell antigen, lipocalin 2, claudins 3 and 4, trefoil factors 1 and 2) as well as several novel genes. The differentially expressed genes identified are involved in a variety of cellular functions, including control of transcription, regulation of the cell cycle, proteolysis, cell adhesion and signaling. Validation of our array results was performed by exploring the SAGEmap database and by immunohistochemistry for a selection of 4 genes that have not previously been studied in pancreatic cancer: anterior gradient 2 homologue (Xenopus

laevis), insulin-like growth factor binding protein 3 and 4 and Forkhead box J1. Immunostaining was performed using pancreas-specific tissue microarrays containing core biopsies from 305 clinical specimens. In addition, using statistical group comparison and hierarchical clustering, a selection of genes was identified that may be linked to the site of metastasis from which these cell lines were isolated.

Mochizuki, H., A. Matsubara, et al. (2004). "Interaction of ligand-receptor system between stromal-cell-derived factor-1 and CXC chemokine receptor 4 in human prostate cancer: a possible predictor of metastasis." Biochem Biophys Res Commun **320**(3): 656-63.

Interaction of ligand-receptor systems between stromal-cell-derived factor-1 (SDF-1) and CXC chemokine receptor 4 (CXCR4) is closely involved in the organ specificity of cancer metastasis. We hypothesized that SDF-1-CXCR4 ligand-receptor system plays an important role in prostate cancer metastasis. To test this hypothesis, expression level of SDF-1 and CXCR4 was analyzed in prostate cancer (PC) cell lines (LNCaP, PC3, and DU145) and normal prostate epithelial cell line (PrEC). We also performed migration assay and MTT assay to investigate the chemotactic effect and growth-promoting effect of SDF-1 on DU145 and PC3 cells, respectively. Furthermore, we performed immunohistochemical analysis of CXCR4 expression in tissues from 35 cases of human prostate cancer. CXCR4 expression was detected in all three prostate cancer cell lines, but not in PrECs. SDF-1 significantly enhanced the migration of PC3 and DU145 cells in a dose-dependent manner, and anti-CXCR4 antibody inhibited this chemotactic effect. However, SDF-1 itself did not significantly stimulate the cell growth rate of prostate cancer cell lines. Positive CXCR4 protein was found in 20 out of 35 clinical PC samples (57.1%). Three patients with lung metastasis showed definitely positive CXCR4 immunostaining. Logistic regression analysis revealed that positive expression of CXCR4 protein was an independent and superior predictor for bone metastasis to Gleason sum ($P < 0.05$). Furthermore, among PC patients with PSA greater than 20 ng/mL, the positive rate of CXCR4 protein was significantly higher in patients with bone metastasis than in those with no bone metastasis ($P = 0.017$). These findings suggest that the interaction between SDF-1 and CXCR4 ligand-receptor system is involved in the process of PC metastasis by the activation of cancer cell migration. This is the first report to investigate the role of interaction of ligand-receptor systems between SDF-1 and CXCR4 in prostate cancer metastasis.

Montel, V., A. Gaultier, et al. (2007). "The low-density lipoprotein receptor-related protein regulates cancer cell survival and metastasis development." Cancer Res **67**(20): 9817-24.

Low-density lipoprotein receptor-related protein-1 (LRP-1) is a multifunctional receptor involved in receptor-mediated endocytosis and cell signaling. In this study, we show that LRP-1 is abundantly expressed in severe combined immunodeficient (SCID) mouse xenografts by various human cancer cell lines that express very low or undetectable levels of LRP-1 when cultured in 21% O2 in vitro (standard cell culture conditions). To test whether LRP-1 expression in vivo may be explained by hypoxia in the xenografts, CL16 cells, which are derived from the MDA-MB-435 cell line, were cultured in 1.0% O2. A substantial increase in LRP-1 expression was observed. To test the activity of LRP-1 in cancer progression in vivo, LRP-1 expression was silenced in CL16 cells with short hairpin RNA. These cells formed tumors in SCID mice, in which LRP-1 expression remained silenced. Although LRP-1 gene silencing did not inhibit CL16 cell dissemination from the primary tumors to the lungs, the pulmonary metastases failed to enlarge, suggesting compromised survival or growth at the implantation site. In cell culture experiments, significantly increased cell death was observed when LRP-1-silenced CL16 cells were exposed to CoCl2, which models changes that occur in hypoxia. Furthermore, LRP-1-silenced cells expressed decreased levels of vascular endothelial growth factor in response to 1.0% O2. These results suggest mechanisms by which LRP-1 may facilitate the development and growth of cancer metastases in vivo.

Moreau, J. E., K. Anderson, et al. (2007). "Tissue-engineered bone serves as a target for metastasis of human breast cancer in a mouse model." Cancer Res **67**(21): 10304-8.

The high frequency and mortality associated with breast cancer metastasis to bone has motivated efforts to elucidate tumor-stroma interactions in the bone microenvironment contributing to invasion and proliferation of metastatic cells. The development of engineered tissues has prompted the integration of engineered bone scaffolds into animal models as potential targets for metastatic spread. Silk scaffolds were coupled with bone morphogenetic protein-2 (BMP-2), seeded with bone marrow stromal cells (BMSC), and maintained in culture for 7 weeks, 4 weeks, and 1 day before s.c. implant in a mouse model of human breast cancer metastasis from the orthotopic site. Following injection of SUM1315 cells into mouse mammary fat pads, tumor burden of implanted tissues was observed only in 1-day scaffolds. Scaffold

development and implantation was then reinitiated to identify the elements of the engineered bone that contribute to metastatic spread. Untreated scaffolds were compared with BMP-2-coupled, BMSC-seeded, or BMP-2/BMSC-combined treatment. Migration of SUM1315 cells was detected in four of four mice bearing scaffolds with BMP-2 treatment and with BMSC treatment, respectively, whereas only one of six mice of the BMP-2/BMSC combination showed evidence of metastatic spread. Histology confirmed active matrix modeling and stromal cell/fibroblast infiltration in scaffolds positive for the presence of metastasis. These results show the first successful integration of engineered tissues in a model system of human breast cancer metastasis. This novel platform now can be used in continued investigation of the bone environment and stem cell contributions to the process of breast cancer metastasis.

Motoyama, S., M. Miura, et al. (2009). "CRP genetic polymorphism is associated with lymph node metastasis in thoracic esophageal squamous cell cancer." Ann Surg Oncol **16**(9): 2479-85.

BACKGROUND: Lymph node involvement is the most important prognostic factor in thoracic esophageal cancer. A more accurate molecular technique for diagnosing lymph node metastasis and a better understanding of the molecular mechanisms governing lymph node metastasis would be highly desirable. The purpose of this study is to examine the association between inflammation-related genetic polymorphisms and lymph node metastasis. METHODS: The study participants were 113 Japanese patients undergoing curative surgery for thoracic esophageal squamous cell cancer. DNA was extracted from blood samples and genetic polymorphisms in C-reactive protein (CRP), tumor necrosis factor (TNF)-alpha and -beta, interferon (IFN)-gamma, transforming growth factor (TGF)-beta, interleukin (IL)-1beta, IL-1 receptor antagonist, IL-2, IL-4, IL-6, IL-6 receptor, IL-10, and IL-12beta were investigated using the polymerase chain reaction-restriction fragment length polymorphism method. We then assessed the association between inflammation-related genes and lymph node metastasis. RESULTS: For CRP 1846C>T polymorphism, the frequency of the 1846T/T genotype was significantly higher in patients with lymph node metastasis (P = 0.0043), and the odds ratio (3.040) derived from logistic regression models indicated that the 1846T/T genotype significantly increases the likelihood of lymph node metastasis. In submucosal cancer, the utility of CRP 1846C>T polymorphism for predicting lymph node involvement was superior to usual methods (computed tomography and ultrasonography), with positive and negative predictive values of 69% and 75%, respectively. CONCLUSIONS: These findings suggest that CRP polymorphism is a potentially effective predictor of lymph node metastasis and may thus be useful for deciding on treatment strategy.

Muguruma, H., S. Yano, et al. (2005). "Reveromycin A inhibits osteolytic bone metastasis of small-cell lung cancer cells, SBC-5, through an antiosteoclastic activity." Clin Cancer Res **11**(24 Pt 1): 8822-8.

PURPOSE: The purpose of this study was to determine therapeutic effect of a novel antibiotic, reveromycin A, against osteolytic bone metastasis of human small cell lung cancer (SBC-5) cells. RESULTS: Reveromycin A induced apoptosis specifically in osteoclasts in vitro. Although reveromycin A did not inhibit SBC-5 cell proliferation, it suppressed the expression of parathyroid hormone-related peptide. Intravenous inoculation of SBC-5 cells in natural killer cell-depleted severe combined immunodeficient mice produced experimental metastases in multiple organs, including the bone. Daily administration of reveromycin A inhibited the bone metastasis, but not visceral metastasis, in a dose-dependent manner. Histologic analyses revealed that although treatment with reveromycin A did not affect the number of proliferating tumor cells, it decreased the number of osteoclasts and increased apoptotic cells in bone lesions. CONCLUSIONS: These findings suggest that reveromycin A may inhibit osteolytic bone metastasis through suppression of osteoclast activity by directly inducing apoptosis and indirectly inhibiting tumor cell-derived parathyroid hormone-related peptide production. Therefore, reveromycin A may be a novel, potent therapeutic agent against osteolytic bone metastasis of lung cancer in humans.

Muller-Tidow, C., S. Diederichs, et al. (2005). "Identification of metastasis-associated receptor tyrosine kinases in non-small cell lung cancer." Cancer Res **65**(5): 1778-82.

Development of distant metastasis after tumor resection is the leading cause of death in early-stage non-small cell lung cancer (NSCLC). Receptor tyrosine kinases (RTK) are involved in tumorigenesis but only few RTKs have been systematically studied in NSCLC. Here, we provide quantitative real-time reverse transcription-PCR expression data of all RTKs (n=56) in primary tumors of 70 patients with early-stage (I-IIIA) NSCLC. Overall, 33 RTKs were expressed in at least 25% of the patients. Several RTKs were significantly expressed higher in tumors that ultimately metastasized. The hazard risk for metastasis development in stage I/II disease was increased at least 3-fold for tumors with high

expression levels of insulin receptor, neurotrophic tyrosine receptor kinase 1, epidermal growth factor receptor, ERBB2, ERBB3, platelet-derived growth factor receptor beta, fibroblast growth factor receptor 1, or leukocyte tyrosine kinase. Relative risks were reduced 3-fold by expression of EPHB6 or DKFZ1. Three members of the epidermal growth factor receptor family were associated with a high risk of metastasis, emphasizing the validity of our data. High ERBB3 expression was significantly associated with decreased survival. Taken together, our genome-wide RTK expression map uncovered the previously unknown value of several RTKs as potential markers for prognosis and metastasis prediction in early-stage NSCLC. The identified RTKs represent promising novel candidates for further functional analyses.

Na, I. K., C. Scheibenbogen, et al. (2008). "Nuclear expression of CXCR4 in tumor cells of non-small cell lung cancer is correlated with lymph node metastasis." Hum Pathol **39**(12): 1751-5.

The stromal-derived factor 1alpha (CXCL12)/chemokine receptor CXCR4 system plays an important role in the metastatic process of a variety of cancers, with CXCR4 frequently expressed by tumor cells homing to CXCL12-rich compartments. The current study evaluated a possible association of CXCR4 expression with lymph node metastasis in primary non-small cell lung cancer. CXCR4 expression levels were evaluated using immunohistology in 46 non-small cell lung cancer specimens of patients without or with lymph node involvement (N0 = 24, N1/N2/N3 = 22). Evaluation of immunostaining was performed semiquantitatively by visual assessment. Statistical analyses with multiple testing adjustments for confirmatory comparisons were performed to assess relevant parameters associated with lymph node metastases. In all samples of non-small cell lung cancer, tumor cells stained positively for cytoplasmic CXCR4. The intensity of the CXCR4 staining varied considerably between specimens: 2 (4%) tumors demonstrated weak cytoplasmic CXCR4, 22 (48%) intermediate, and 22 (48%) strong staining. Membranous staining was absent; however, nuclear staining of CXCR4 was observed in 5 non-small cell lung cancer samples. Statistical analyses of the association between presence of lymph node metastases and CXCR4 expression levels revealed that cytoplasmic CXCR4 expression was not associated with the presence of lymph node metastases. However, nuclear CXCR4 was significantly correlated with increasing lymph node stage (P = .008), linear-to-linear association. The association between aberrant expression of CXCR4 in the nucleus of non-small cell lung cancer and metastasis to lymph nodes points toward a potential tumor metastasis promoting function of nuclear CXCR4.

Nagatsuka, I., N. Yamada, et al. (2002). "Inhibitory effect of a selective cyclooxygenase-2 inhibitor on liver metastasis of colon cancer." Int J Cancer **100**(5): 515-9.

COX-2 overexpression is recognized in various cancers, but the role of COX-2 in the progression of cancer, including the liver metastasis of colon cancer, is not clearly understood. We examined the role of COX-2 in the mechanism of liver metastasis of colon cancer, using a highly metastasizable colon carcinoma cell line, LM-H3. A COX-2 inhibitor, JTE-522, inhibited cell proliferation and invasion of LM-H3 in vitro and clearly reduced the number of metastatic nodules on the surface of nude mouse livers in vivo. We also examined the effects of JTE-522 on the production of growth factors and MMPs through the use of ELISA and gelatin zymography, respectively. JTE-522 downregulated PDGF production by LM-H3 but had no influence on VEGF production. JTE-522 also inhibited MMP-2 secretion by LM-H3. JTE-522 downregulated PGE(2) production, but the associated changes in PGE(2) did not affect PDGF and VEGF production by LM-H3. We conclude that JTE-522 downregulated the cell proliferation and invasive potential of LM-H3 by reducing the production of PDGF and MMP-2 and hypothesize that these inhibitory effects on the production of PDGF and MMP-2 can lead to inhibition of liver metastasis of colon cancer. These data indicate that the COX-2 inhibitor JTE-522 has a high potential for use as a clinical agent for the treatment of liver metastasis of colon cancer.

Nakamura, E. S., K. Koizumi, et al. (2006). "RANKL-induced CCL22/macrophage-derived chemokine produced from osteoclasts potentially promotes the bone metastasis of lung cancer expressing its receptor CCR4." Clin Exp Metastasis **23**(1): 9-18.

Chemokines are now known to play an important role in cancer growth and metastasis. Here we report that differentiating osteoclasts constitutively produce CCL22 (also called macrophage-derived chemokine) and potentially promote bone metastasis of lung cancer expressing its receptor CCR4. We first examined expression of chemokines by differentiating osteoclasts. CCL22 was selectively upregulated in osteoclast-like cells derived from RAW264.7 cells and mouse bone marrow cells upon stimulation with RANKL (receptor activator of nuclear factor-kappaB ligand). In addition, a human lung cancer cell line SBC-5 that efficiently metastasized to bone when intravenously injected into NK cell-depleted SCID mice was found to express CCR4. Stimulation of

SBC-5 cells with CCL22 induced cell migration and also enhanced phosphorylation of protein kinase B/Akt and extracellular signal-regulated kinase (ERK). Furthermore, immunohistochemical analysis of bone metastasis lesions demonstrated close co-localization of tartrate-resistant alkaline phosphatase (TRAP)-positive osteoclasts expressing CCL22 and SBC-5 cells expressing CCR4. Collectively, these results suggest that osteoclasts may promote bone metastasis of cancer cells expressing CCR4 in the bone marrow by producing its ligand CCL22.

Narla, G., A. DiFeo, et al. (2008). "KLF6-SV1 overexpression accelerates human and mouse prostate cancer progression and metastasis." J Clin Invest **118**(8): 2711-21.

Metastatic prostate cancer (PCa) is one of the leading causes of death from cancer in men. The molecular mechanisms underlying the transition from localized tumor to hormone-refractory metastatic PCa remain largely unknown, and their identification is key for predicting prognosis and targeted therapy. Here we demonstrated that increased expression of a splice variant of the Kruppel-like factor 6 (KLF6) tumor suppressor gene, known as KLF6-SV1, in tumors from men after prostatectomy predicted markedly poorer survival and disease recurrence profiles. Analysis of tumor samples revealed that KLF6-SV1 levels were specifically upregulated in hormone-refractory metastatic PCa. In 2 complementary mouse models of metastatic PCa, KLF6-SV1-overexpressing PCa cells were shown by in vivo and ex vivo bioluminescent imaging to metastasize more rapidly and to disseminate to lymph nodes, bone, and brain more often. Interestingly, while KLF6-SV1 overexpression increased metastasis, it did not affect localized tumor growth. KLF6-SV1 inhibition using RNAi induced spontaneous apoptosis in cultured PCa cell lines and suppressed tumor growth in mice. Together, these findings demonstrate that KLF6-SV1 expression levels in PCa tumors at the time of diagnosis can predict the metastatic behavior of the tumor; thus, KLF-SV1 may represent a novel therapeutic target.

Nicolson, G. L., A. Nawa, et al. (2003). "Tumor metastasis-associated human MTA1 gene and its MTA1 protein product: role in epithelial cancer cell invasion, proliferation and nuclear regulation." Clin Exp Metastasis **20**(1): 19-24.

Using differential cDNA library screening techniques based on metastatic and nonmetastatic rat mammary adenocarcinoma cell lines, we previously cloned and sequenced the metastasis-associated gene mta1. Using homology to the rat mta1 gene, we cloned the human MTA1 gene and found it to be over-expressed in a variety of human cell lines (breast, ovarian, lung, gastric and colorectal cancer but not melanoma or sarcoma) and cancerous tissues (breast, esophageal, colorectal, gastric and pancreatic cancer). We found a close similarity between the human MTA1 and rat mta1 genes (88% and 96% identities of the nucleotide and predicted amino acid sequences, respectively). Both genes encode novel proteins that contain a proline rich region (SH3-binding motif), a putative zinc finger motif, a leucine zipper motif and 5 copies of the SPXX motif found in gene regulatory proteins. Using Southern blot analysis the MTA1 gene was highly conserved, and using Northern blot analysis MTA1 transcripts were found in virtually all human cell lines (melanoma, breast, cervix and ovarian carcinoma cells and normal breast epithelial cells). However, the expression level of the MTA1 gene in normal breast epithelial cells was approximately 50% of that found in rapidly growing adenocarcinoma and atypical epithelial cell lines. Experimental inhibition of MTA1 protein expression using antisense phosphorothioate oligonucleotides resulted in inhibition of growth and invasion of human MDA-MB-231 breast cancer cells with relatively high MTA1 expression. Furthermore, the MTA1 protein was localized in the nuclei of cells transfected with a mammalian expression vector containing a full-length MTA1 gene. Although some MTA1 protein was found in the cytoplasm, the vast majority of MTA1 protein was localized in the nucleus. Examination of recombinate MTA1 and related MTA2 proteins suggests that MTA1 protein is a histone deacetylase. It also appears to behave like a GATA-element transcription factor, since transfection of a GATA-element reporter into MTA1-expressing cells resulted in 10-20-fold increase in reporter expression over poorly MTA1-expressing cells. Since it was reported that nucleosome remodeling histone deacetylase complex (NuRD complex) involved in chromatin remodeling contains MTA1 protein and a MTA1-related protein (MTA2), we examined NuRD complexes for the presence of MTA1 protein and found an association of this protein with histone deacetylase. The results suggest that the MTA1 protein may serve multiple functions in cellular signaling, chromosome remodeling and transcription processes that are important in the progression, invasion and growth of metastatic epithelial cells.

Ninomiya, S., M. Inomata, et al. (2009). "Effect of bevacizumab, a humanized monoclonal antibody to vascular endothelial growth factor, on peritoneal metastasis of MNK-45P human gastric cancer in mice." J Surg Res **154**(2): 196-202.

BACKGROUND: The aim of this study was to clarify the effect of bevacizumab on gastric cancer

with peritoneal metastasis in nude mice. MATERIALS AND METHODS: The expression of vascular endothelial growth factor mRNA (VEGF mRNA) in four gastric cancer cell lines, NCI-N87, MKN-45, MKN-45P, and Kato-III, was examined by polymerase chain reaction. We created a model of peritoneal metastasis by injecting mice with the human gastric cancer cell line MKN-45P. Mice were injected intraperitoneally with bevacizumab (0.1 mg/100 microL) on days 5-14, after inoculation (n = 10) or with phosphate-buffered saline (PBS) over the same time period (n = 10). The maximum abdominal circumference, ascites volume, and the total number and weight of peritoneal tumors were measured. To assess the effect of bevacizumab on angiogenesis, immunohistochemical analysis was performed. RESULTS: VEGF mRNA was expressed at a high level in MKN-45P cells as well as MKN-45 and Kato-III. The mean maximum abdominal circumference and ascites volume in the bevacizumab group were significantly less than those in the control group ($P < 0.001$, respectively). The total weight of disseminated tumors in the bevacizumab group was also significantly less than that in the control group ($P < 0.01$). In addition, immunohistochemical analysis of CD31-stained peritoneally disseminated nodules showed that the vessel area in the bevacizumab group was significantly less than that in the control group ($P < 0.001$). CONCLUSIONS: These results show that intraperitoneal administration of bevacizumab inhibits peritoneal metastasis and reduces malignant ascites in tumor-bearing mice.

Nishimori, H., T. Yasoshima, et al. (2000). "A novel experimental mouse model of peritoneal dissemination of human gastric cancer cells: different mechanisms in peritoneal dissemination and hematogenous metastasis." Jpn J Cancer Res **91**(7): 715-22.

We established a new cell line, AZ-P7a, with high peritoneal-metastatic potential in nude mice. AZ-P7a cells were derived from the human gastric carcinoma line AZ-521, which has low capacity for peritoneal dissemination. AZ-P7a cells developed peritoneal metastasis in 11 / 14 (78.6%) mice, whereas the parental AZ-521 cells developed metastasis in 2 / 6 (33.3%) mice. The metastatic foci in the peritoneum showed essentially the same histological appearance as those induced by parental cells. The tumorigenicity and the motile activity of AZ-P7a cells were stronger than those of the parental AZ-521 cells; in contrast, adhesion to the extracellular matrix and the production of vascular endothelial growth factor by AZ-P7a cells were decreased. In fluorescence-activated cell sorter (FACS) analysis, AZ-P7a cells expressed significantly greater levels of integrins alpha2, alpha3, alpha5, alpha6 and alphavbeta5, as compared with AZ-521 cells. However, alpha1, alpha4, alphavbeta3, hCD44H, hCD44v3, hCD44v6 and hCD44v10 were not expressed in either cell line. AZ-P7a cells developed no liver metastasis when administered by the intrasplenic injection method, though the highly liver metastatic cell line AZ-H5c showed the same rate of peritoneal dissemination as that exhibited by AZ-P7a cells after intraabdominal injection. These findings suggested that the mechanism of peritoneal dissemination differed from that of hematogenous metastasis. Moreover, the latter appears to be controlled by more complex mechanisms than the former. Thus, this cell line might be useful for investigating the mechanism of peritoneal dissemination of human gastric cancer.

Nishimori, H., T. Yasoshima, et al. (2002). "A novel nude mouse model of liver metastasis and peritoneal dissemination from the same human pancreatic cancer line." Pancreas **24**(3): 242-50.

INTRODUCTION: Recently, several mice models have been used for investigating cancer metastasis. However, there are no metastatic and peritoneal dominated variants from the same parental cell line. AIM AND METHODOLOGY: To elucidate the mechanisms of metastasis, we established highly liver metastatic and peritoneal disseminated models in nude mice, and then characterized several factors related to metastasis in these cells. We established a series of well-characterized sublines that showed metastatic potentials to different organ sites of nude mice. Two sublines were selected sequentially from the parental pancreatic cancer cell line, HPC-4, resulting in a highly liver metastatic cell line, HPC-4H4, and a highly peritoneal disseminated cell line, HPC-4P4a. Using these three cell lines, we investigated several biologic properties and mRNA levels of differentially expressed genes involved in cancer metastasis. RESULTS: The tumorigenicity, the motile activity, and the adhesive activity of metastatic sublines were higher than those of parental HPC-4 cells. Macroscopic and microscopic findings and the DNA ploidy pattern were the same among the three cell lines. In addition, HPC-4H4 cells expressed clearly higher levels of vascular endothelial growth factor and IL-8 expression than did HPC-4P4a cells. In fluorescence-activated cell sorter analysis of adhesion molecules, the expression of integrin-alpha2 was enhanced in HPC-4 cells, integrin-alphavbeta5 was enhanced in HPC-4H4 cells, and integrin-alpha3 was enhanced in HPC-4P4a cells. Osteopontin, vascular endothelial growth factor, and hepatocyte growth factor were among the genes that were upregulated in HPC-4H4 cells compared with HPC-4P4a cells. HPC-4P4a cells did not metastasize to the

liver by intrasplenic injection. Conversely, HPC-4H4 cells metastasized remarkably to the peritoneum by intraabdominal injection. CONCLUSION: These sublines are the first reported liver metastatic and peritoneal disseminated models derived from the same parental cell lines. The results of our study suggest that the process of hematogenous metastasis is not the same as that of peritoneal dissemination.

Nogi, H., H. Takeyama, et al. (2003). "Detection of MUC1 and keratin 19 mRNAs in the bone marrow by quantitative RT-PCR predicts the risk of distant metastasis in breast cancer patients." Breast Cancer **10**(1): 74-81.

BACKGROUND: Early detection of micrometastasis in bone marrow is critical for the prognosis of breast cancer patients. Quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) has been used to detect cancer cells in bone marrow, but its utility as a prognostic factor still remains obscure. MATERIALS AND METHODS: Bone marrow samples were aspirated from the anterosuperior iliac spine of 34 patients, immediately after their surgical procedures had been completed. Control samples were also obtained from 10 healthy adult volunteers. The total RNA was extracted from the mononuclear cells, and the expression levels ofbeta-actin, MUC1 and keratin 19 mRNAs were studied by quantitative RT-PCR. Each mRNA level was scored according to the expression level. The sum of these expression scores was defined as the composite expression score, which was employed as the basis of the evaluation. RESULTS: The mean follow-up period was 45 months. Nine patients developed distant metastases, and one developed local recurrence. The 4-year disease relapse rates were 75% (RR=19.38; 95% CI: 1.94-193.20), 28% (RR=3.64; 95% CI: 0.43-31.18), and 8.3% for patients with composite expression scores of 5/6, 3/4 and 2, respectively. The difference among the three groups was statistically significant (log-rank test: p=0.0029), and multivariate analysis also found the composite expression score to be an independent prognostic factor. CONCLUSIONS: Breast cancer patients who show a high composite expression score in bone marrow have a significantly higher risk of recurrence.

Ohashi, S., S. Okamura, et al. (2007). "Clinicopathological variables associated with lymph node metastasis in submucosal invasive gastric cancer." Gastric Cancer **10**(4): 241-50.

BACKGROUND: We aimed to elucidate clinicopathological variables associated with lymph node metastasis of submucosal invasive gastric cancer. METHODS: Specimens were surgically resected from 201 patients who had primary submucosal gastric cancer. We studied 39 consecutive patients with lymph node metastasis and 162 patients without lymph node metastasis. We compared the following clinicopathological characteristics of the patients in relation to lymph node metastasis: age, sex, tumor size, histology, extent of submucosal invasion, lymphatic and venous invasion, and ulceration of the tumor. Submucosal invasion was divided subjectively into sm1, sm2, and sm3 (representing invasion of the upper-, middle-, and lower-third of the submucosa, respectively). We also studied the relationship between lymph node metastasis of submucosal gastric cancer and immunohistochemistry for p53, Ki67, vascular endothelial growth factor (VEGF), alpha-fetoprotein, sLe(a), and dendritic cells (DCs). RESULTS: In terms of conventional pathological factors, lymph node metastasis in submucosal gastric cancer was related to tumor size ($P = 0.002$), depth of submucosal invasion ($P = 0.001$), lymphatic invasion ($P < 0.0001$), and venous invasion ($P = 0.012$). Lymph node metastasis in sm1 gastric cancer was significantly related to VEGF expression ($P = 0.047$). Also, lymph node metastasis in sm3 gastric cancer was significantly correlated with DC expression ($P = 0.016$). Multivariate analysis showed that tumor size, tumor invasion depth in the submucosal layer, and lymphatic invasion were independent predictors of nodal metastasis in submucosal gastric cancer. CONCLUSION: Conventional pathological factors, such as tumor size, depth of submucosal invasion, and lymphatic invasion, have a significant influence on lymph node metastasis. VEGF expression and DC expression may be helpful predictors of lymph node metastasis in patients with sm1 and sm3 gastric cancer, respectively.

Ohta, Y., Z. Nozaki, et al. (2001). "The predictive value of vascular endothelial growth factor and nm23 for the diagnosis of occult metastasis in non-small cell lung cancer." Jpn J Cancer Res **92**(3): 361-6.

We assessed the association of vascular endothelial growth factor (VEGF) and nm23 expression with occult micrometastasis in lung cancer. As destination sites for micrometastasis, we scrutinized lymph node (LN) and bone marrow (BM) specimens. For LN, 122 stage I patients who had received curative operations were studied. As regards BM, 203 patients in stage I - IV who underwent operations were registered. Immunohistochemical anti-cytokeratin staining was used to detect microdissemination of cancer cells. The VEGF and the nm23 expression at the primary sites were immunohistochemically studied in 285 cases in total. The percentages of the patients with microdissemination were 28.7% for LN and 42.4% for BM. The outcome for the patients with LN or BM

microdissemination was significantly worse than that for patients without it. The increased VEGF and the decreased nm23 expression within primary tumors were significantly associated with LN and BM microdissemination. The results indicate possible value of using these biological markers to predict the risk of systemic micrometastasis in non-small cell lung cancer.

Ojima, H., S. Sasaki, et al. (2003). "Utility of serosal stamp cytology as an indicator for high-risk peritoneal metastasis in colorectal cancer surgery." Hepatogastroenterology **50**(49): 87-90.

BACKGROUND/AIMS: Although peritoneal lavage cytology is widely performed during surgery for gastric cancer and the results have been reported to provide an accurate prognostic factor, its value has not been well established in colorectal cancer. In this study, we demonstrated the utility of serosal stamp cytology from the viewpoint of cell adhesion molecules. METHODOLOGY: Between 1997 and 1999, peritoneal lavage cytology and serosal stamp cytology were performed in 34 patients with resectable colorectal cancer. Epithelial cadherin (E-cadherin) was examined as an index of the progress degree of peritoneal metastasis. RESULTS: Although peritoneal lavage cytology was positive in one case, serosal stamp cytology was positive in 10 cases. E-cadherin expression was lost in all peritoneal lavage cytology and/or serosal stamp cytology positive patients. CONCLUSIONS: Our data indicate that serosal stamp cytology is more sensitive and simple than peritoneal lavage cytology. Serosal stamp cytology may be useful in identifying patients at high risk for peritoneal recurrence.

Okayama, T., S. Kokura, et al. (2009). "Antitumor effect of pretreatment for colon cancer cells with hyperthermia plus geranylgeranylacetone in experimental metastasis models and a subcutaneous tumor model of colon cancer in mice." Int J Hyperthermia **25**(2): 141-9.

PURPOSE: We examined whether hyperthermia attenuated the metastatic potential of colon cancer through the induction of heat shock protein 70 (Hsp70). MATERIALS AND METHODS: Colon26 cells were separated into four groups: (1) no pretreatment, (2) hyperthermia at 42 degrees C for 1 hour, (3) pretreatment with geranylgeranylacetone (GGA) 10(-6) M for 2 hours, and (4) hyperthermia after GGA treatment. We measured cell viabilities and the contents of Hsp70. We assessed nuclear factor-kappa-B (NF-kappa-B) status with and without tumor necrosis factor-alpha (TNF-alpha) stimulation. For in vivo study, colon26 cells were injected via the tail vein or into a subcutaneous area of mice and the numbers of lung metastatic nodules or the volumes of subcutaneous tumors were assessed. Untreated cells were incubated with PKH26. Experimental metastasis models were then generated and used to assess the fixed cancer cells. RESULTS: Tumor development in the subcutaneous tumor models and cell viabilities were similar among the four groups. However, the GGA plus hyperthermia group had fewer lung metastatic nodules in the experimental lung metastasis model and higher Hsp70 induction than the other cell groups. The GGA plus hyperthermia pretreatment group also showed a lower number of fixed cells in lungs and lower activation of NF-kappa-B by TNF-alpha than the other cell groups. CONCLUSIONS: It is suggested the metastatic potential but not the proliferation potency of cancer cells is inhibited by the transient induction of Hsp70.

Okuyama, N., A. Matsumine, et al. (2008). "Matrix metalloproteinase-1 is a crucial bone metastasis factor in a human breast cancer-derived highly invasive cell line." Oncol Rep **20**(6): 1497-504.

Bone metastasis is one of the most severe cancer complications. To analyze the mechanism of bone metastasis, we established highly invasive cell lines from the human breast cancer cell line MDA-MB-231 using an in vitro sequential selection system. The cell lines, MDA-231-S10 and MDA-231-S5, were more invasive and more motile than the parental cell line. Moreover, MDA-231-S10 metastasized to bone more often when inoculated into the arterial circulation of nude mice. MDA-231-S10-bearing nude mice had a significantly poorer prognosis, and their bony metastatic tumors grew more rapidly than those of the mice bearing the parental cell line (MDA-231-P). Given that a high expression of matrix metalloproteinase (MMP) is reported to be associated with cancer invasiveness, we examined MMP expression. Our results showed that the expression of MMP-3, -5, -7, -9, -13 and -14 was decreased on Multiplex real-time quantitative RT-PCR analysis in the two new cell lines. The zymographic analysis showed no MMP-2 activity and a decreased MMP-9 activity in MDA-231-S10. However, the expression of MMP-1 in MDA-231-S10 was increased. We therefore concluded that MMP-1 plays a crucial role in breast cancer bone metastasis. Furthermore, our MDA-231-derived cell lines are useful analytical models of MMP-1- associated breast cancer bone metastasis.

Osanai, M., H. Chiba, et al. (2002). "Hepatocyte nuclear factor (HNF)-4alpha induces expression of endothelial Fas ligand (FasL) to prevent cancer cell transmigration: a novel defense mechanism of

endothelium against cancer metastasis." Jpn J Cancer Res **93**(5): 532-41.

Endothelial Fas ligand (FasL) contributes to the "immune privilege" of tissues such as testis and eye, in which apoptosis is induced in infiltrating Fas-positive activated T cells and results in the inhibition of leukocyte extravasation. In this study, we examined the role of endothelial FasL in controlling cancer cell transmigration using rat lung endothelial (RLE) cell line bearing a doxycycline-inducible hepatocyte nuclear factor (HNF)-4alpha expression system. We showed that a detectable level of FasL was expressed in RLE cells and that this expression was markedly up-regulated and well correlated to the degree of HNF-4alpha expression in a time-dependent manner. When various cancer cells were overlaid on an RLE monolayer sheet, we examined the ability of endothelial FasL to induce massive apoptosis in Fas-expressing cancer cells and found a causal link to inhibition of the transmigration. Finally, we showed that FasL was expressed in capillaries of the rat brain by immunohistochemical staining, suggesting that FasL serves its functions not only in vitro, but also in vivo. These results raise the possibility that HNF-4alpha is involved in regulating cancer cell transmigration by modulating the Fas-FasL system.

Otsuka, S., M. Hanibuchi, et al. (2009). "A bone metastasis model with osteolytic and osteoblastic properties of human lung cancer ACC-LC-319/bone2 in natural killer cell-depleted severe combined immunodeficient mice." Oncol Res **17**(11-12): 581-91.

Lung cancer is commonly associated with multiple-organ metastasis, and bone is a frequent metastatic site for lung cancer. Lung cancer frequently develops osteolytic, and less frequently osteoblastic, metastasis to bone. Osteolytic metastasis models of lung cancer have been reported, but no osteoblastic metastasis model is available for lung cancer. In the present study, we established a reproducible model of human lung cancer with both osteolytic and osteoblastic changes in natural killer cell-depleted severe combined immunodeficient mice. Intravenous inoculation of ACC-LC-319/bone2 cells resulted in the development of metastatic colonies in the lung, liver, and bone of the mice. As assessed sequentially by X-ray photographs, osteolytic bone lesions were observed by day 28, and then osteoblastic lesions were detected by day 35. Histological examination revealed the presence of bony spurs, a hallmark of osteoblastic bone metastasis, where osteoclasts were hardly observed. Treatment with an anti-human vascular endothelial growth factor antibody, bevacizumab, as well as zoledronate, inhibited the number of experimental bone metastases, including osteoblastic changes produced by ACC-LC-319/bone2 cells. These results indicate that our bone metastasis model by ACC-LC319/bone2 might be useful to understand the molecular pathogenesis of osteolytic and osteoblastic metastasis, and to identify molecular targets to control bone metastasis of lung cancer.

Park, H. R., S. K. Min, et al. (2003). "Expression of osteoprotegerin and RANK ligand in breast cancer bone metastasis." J Korean Med Sci **18**(4): 541-6.

Bone destruction is primarily mediated by osteoclastic bone resorption, and cancer cells stimulate the formation and activation of osteoclasts next to metastatic foci. Accumulating evidences indicate that receptor activator of NF-kB ligand (RANKL) is the ultimate extracellular mediator that stimulates osteoclast differentiation into mature osteoclasts. In contrast, osteoprotegerin (OPG) inhibits osteoclast development. In order to elucidate a mechanism for cancer-induced osteoclastogenesis, cells from a human breast cancer line, MDA-MB-231, were directly co-cultured with ST2, MC3T3-E1, or with primary mouse calvarial cells. Osteoclast-like cells and tartarate resistant acid phosphatase (TRAP) activities were then quantitated. We examined these cell lines and samples from breast cancer by RT-PCR for the expressions of OPG and RANKL mRNA. Compared to controls, co-culture of MDA-MB-231 cells with stromal or osteoblastic cells induced an increase in number of osteoclasts and TRAP activities. MDA-MB-231 cells alone or breast cancer samples did not express RANKL mRNA. However, co-culture of these cancer cells with stromal or osteoblastic cells induced RANKL mRNA expression and decreased OPG mRNA expression. These experiments demonstrate that direct interactions between breast cancer and stromal or osteoblastic cells induce osteoclastogenesis in vitro through modulating RANKL expression.

Park, S., A. J. Holmes-Tisch, et al. (2009). "Discordance of molecular biomarkers associated with epidermal growth factor receptor pathway between primary tumors and lymph node metastasis in non-small cell lung cancer." J Thorac Oncol **4**(7): 809-15.

INTRODUCTION: For the identification of the patients who most likely benefit from epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors in non-small cell lung cancer (NSCLC), molecular assays are considered to be of paramount importance. Given the heterogeneity of NSCLC at the molecular level, this study was conducted to determine the discrepancy in EGFR mutations between primary tumors and the corresponding lymph node metastasis. PATIENTS AND METHODS: Surgically resected 101 paired primary NSCLC and

metastatic lymph nodes were evaluated for the EGFR mutations by direct DNA sequencing and heteroduplex analysis. RESULTS: EGFR mutation was detected in 29.7% (30 of 101) of the primary tumors and in 27.7% of lymph node metastases (28 of 101) by either direct sequencing or heteroduplex analysis, respectively. By direct sequencing, 12 cases (11.9%) showed discordance in EGFR mutations between primary tumors and metastasis. In 11 cases, EGFR mutations were detected only in the primary tumor, whereas 1 case only in lymph node metastases. By heteroduplex analysis, 17 cases (16.8%) were discordant. Ten cases were primary tumor positive and lymph node negative, whereas seven cases were lymph node positive and primary tumor negative. CONCLUSIONS: A considerable proportion of NSCLC showed discrepancy in EGFR mutations between primary tumors and metastatic lymph nodes, suggesting tumor heterogeneity at the molecular level during the process of metastasis.

Preet, A., R. K. Ganju, et al. (2008). "Delta9-Tetrahydrocannabinol inhibits epithelial growth factor-induced lung cancer cell migration in vitro as well as its growth and metastasis in vivo." Oncogene **27**(3): 339-46.

Delta(9)-Tetrahydrocannabinol (THC) is the primary cannabinoid of marijuana and has been shown to either potentiate or inhibit tumor growth, depending on the type of cancer and its pathogenesis. Little is known about the activity of cannabinoids like THC on epidermal growth factor receptor-overexpressing lung cancers, which are often highly aggressive and resistant to chemotherapy. In this study, we characterized the effects of THC on the EGF-induced growth and metastasis of human non-small cell lung cancer using the cell lines A549 and SW-1573 as in vitro models. We found that these cells express the cannabinoid receptors CB(1) and CB(2), known targets for THC action, and that THC inhibited EGF-induced growth, chemotaxis and chemoinvasion. Moreover, signaling studies indicated that THC may act by inhibiting the EGF-induced phosphorylation of ERK1/2, JNK1/2 and AKT. THC also induced the phosphorylation of focal adhesion kinase at tyrosine 397. Additionally, in in vivo studies in severe combined immunodeficient mice, there was significant inhibition of the subcutaneous tumor growth and lung metastasis of A549 cells in THC-treated animals as compared to vehicle-treated controls. Tumor samples from THC-treated animals revealed antiproliferative and antiangiogenic effects of THC. Our study suggests that cannabinoids like THC should be explored as novel therapeutic molecules in controlling the growth and metastasis of certain lung cancers.

Psaila, B., R. N. Kaplan, et al. (2006). "Priming the 'soil' for breast cancer metastasis: the pre-metastatic niche." Breast Dis **26**: 65-74.

The long prevailing model of metastasis recognizes the importance of both "seed" and "soil" for metastatic progression [1]. Much attention has focused on understanding the molecular and genetic factors that confer an intrinsic metastatic advantage to certain tumor cells. Meanwhile, changes occurring within distant tissues, creating a "soil" conducive for tumor invasion, have been largely neglected. Bone marrow-derived hematopoietic progenitor cells (HPCs) recently emerged as key players in initiating these early changes, creating a receptive microenvironment at designated sites for distant tumor growth and establishing the "Pre-Metastatic Niche" [2]. This insight into the earliest stages in the metastatic cascade revises our concept of the metastatic "microenvironment" to include physiological cells recruited from the bone marrow. Moreover, the concept of pre-metastatic tissues as 'niches' similar to physiological stem cell niches establishes a paradigm in which disseminated tumor cells may reside within a highly defined microcosm, both supportive and regulatory, and which may confer specific functions on indwelling cells. Understanding the cellular and molecular cross-talk between "seed" and "soil" may further our understanding of the factors that govern both site-specific patterning in metastasis and the phenomenon of tumor dormancy. This may lead to therapeutic strategies to detect and prevent metastasis at its earliest inception.

Qian, J., A. Dong, et al. (2007). "Suppression of type 1 Insulin-like growth factor receptor expression by small interfering RNA inhibits A549 human lung cancer cell invasion in vitro and metastasis in xenograft nude mice." Acta Biochim Biophys Sin (Shanghai) **39**(2): 137-47.

Cancer invasion and metastasis, involving a variety of pathological processes and cytophysiological changes, contribute to the high mortality of lung cancer. The type 1 insulin-like growth factor receptor (IGF-1R), associated with cancer progression and invasion, is a potential anti-invasion and anti-metastasis target in lung cancer. To inhibit the invasive properties of lung cancer cells, we successfully down-regulated IGF-1R gene expression in A549 human lung cancer cells by small interfering RNA (siRNA) technology, and evaluated its effects on invasion-related gene expression, tumor cell in vitro invasion, and metastasis in xenograft nude mice. A549 cells transfected with a plasmid expressing hairpin siRNA for IGF-1R showed a significantly decreased IGF-1R expression at the mRNA level as well as the

protein level. In biological assays, transfected A549 cells showed a significant reduction of cell-matrix adhesion, migration and invasion. Consistent with these results, we found that down-regulation of IGR-1R concomitantly accompanied by a large reduction in invasion-related gene expressions, including MMP-2, MMP-9, u-PA, and IGF-1R specific downstream p-Akt. Direct tail vein injections of plasmid expressing hairpin siRNA for IGF-1R significantly inhibited the formation of lung metastases in nude mice. Our results showed the therapeutic potential of siRNA as a method for gene therapy in inhibiting lung cancer invasion and metastasis.

Qin, L., L. Liao, et al. (2008). "The AIB1 oncogene promotes breast cancer metastasis by activation of PEA3-mediated matrix metalloproteinase 2 (MMP2) and MMP9 expression." Mol Cell Biol **28**(19): 5937-50.

Amplified-in-breast cancer 1 (AIB1) is an overexpressed transcriptional coactivator in breast cancer. Although overproduced AIB1 is oncogenic, its role and underlying mechanisms in metastasis remain unclear. Here, mammary tumorigenesis and lung metastasis were investigated in wild-type (WT) and AIB1(-/-) mice harboring the mouse mammary tumor virus-polyomavirus middle T (PyMT) transgene. All WT/PyMT mice developed massive lung metastasis, but AIB1(-/-)/PyMT mice with comparable mammary tumors had significantly less lung metastasis. The recipient mice with transplanted AIB1(-/-)/PyMT tumors also had much less lung metastasis than the recipient mice with transplanted WT/PyMT tumors. WT/PyMT tumor cells expressed mesenchymal markers such as vimentin and N-cadherin, migrated and invaded rapidly, and formed disorganized cellular masses in three-dimensional cultures. In contrast, AIB1(-/-)/PyMT tumor cells maintained epithelial markers such as E-cadherin and ZO-1, migrated and invaded slowly, and still formed polarized acinar structures in three-dimensional cultures. Molecular analyses revealed that AIB1 served as a PEA3 coactivator and formed complexes with PEA3 on matrix metalloproteinase 2 (MMP2) and MMP9 promoters to enhance their expression in both mouse and human breast cancer cells. In 560 human breast tumors, AIB1 expression was found to be positively associated with PEA3, MMP2, and MMP9. These findings suggest a new alternative strategy for controlling the deleterious roles of these MMPs in breast cancer by inhibiting their upstream coregulator AIB1.

Que, H. F., H. F. Chen, et al. (2008). "Effect of runing II on the growth and metastasis of transplanted tumor in mammary cancer-bearing mice and its mechanism." J Tradit Chin Med **28**(4): 293-8.

OBJECTIVE: To study the effect of Runing II (a Chinese herbal preparation for mammary cancer) on the growth and metastasis of transplanted tumor of mammary cancer MA-891-bearing TA2 mice and its mechanism. METHODS: The model of mammary cancer MA-891 cell strain transplanted tumor of TA2 mice with lung metastasis were developed to observe the effect of Runing II on the growth and metastasis of the transplanted tumor. The immunohistochemical method and image analysis were adopted to detect the levels of vascular endothelial growth factor (VEGF), vascular endothelial growth factor receptor (VEGFR), and micro-vessel count (MVC) and micro-vessel area (MVA). RESULTS: In the Runing II group, the tumor weight inhibition rate and the lung metastasis inhibition rate were 37.3% and 65.4% respectively, the tumor growth and lung metastasis were obviously inhibited; And the levels of VEGF and VEGFR, MVC and MVA were significantly decreased as compared with those in the tumor-bearing control group ($P<0.05$). CONCLUSION: The Chinese herbal preparation Running II can inhibit the metastasis of tumor through inhibiting the angiogenesis, and the mechanism is possibly related with down-regulation of VEGF and VEGFR expression.

Rahman, K. M., F. H. Sarkar, et al. (2006). "Therapeutic intervention of experimental breast cancer bone metastasis by indole-3-carbinol in SCID-human mouse model." Mol Cancer Ther **5**(11): 2747-56.

Several lines of experimental evidence have suggested that chemokine receptor CXCR4, a metastasis-promoting molecule, may play important roles in breast cancer bone metastasis. There is emerging evidence linking CXCR4 to matrix metalloproteinases (MMP) as well as their regulator nuclear factor-kappaB (NF-kappaB), a key transcription factor, which is known to activate metastasis-promoting molecules for many types of malignancies, including breast cancer. A recent study also showed that promoter region of CXCR4 has several NF-kappaB-binding sites, suggesting that there may be a cross-talk between CXCR4 and NF-kappaB. We have shown previously that indole-3-carbinol (I3C), a natural compound present in vegetables of the genus Brassica, can inhibit NF-kappaB in breast cancer cells. However, there are no reports in the literature showing any effect of I3C on CXCR4 expression in vitro and in vivo. We therefore examined whether I3C could inhibit bone metastasis of breast cancer by inhibiting CXCR4 and MMP-9 expression mediated via the inhibition of the NF-kappaB signaling pathway. Here, we have modified

the severe combined immunodeficient (SCID)-human mouse model of experimental bone metastasis for use with the MDA-MB-231 breast cancer cell line. In this animal model, we found that I3C significantly inhibited MDA-MB-231 bone tumor growth, and our results were correlated with the down-regulation of NF-kappaB. Moreover, we found that I3C significantly inhibited the expression of multiple genes involved in the control of metastasis and invasion in vitro and in vivo, especially the expression of CXCR4 and MMP-9 along with pro-MMP-9, with concomitant decrease in Bcl-2 and increase in the proapoptotic protein Bax. From these results, we conclude that the CXCR4/NF-kappaB pathway is critical during I3C-induced inhibition of experimental breast cancer bone metastasis. These results also suggest that I3C could be a promising agent for the prevention and/or treatment of breast cancer bone metastasis in the future.

Rha, S. Y., S. H. Noh, et al. (1999). "Modulation of biological phenotypes for tumor growth and metastasis by target-specific biological inhibitors in gastric cancer." Int J Mol Med **4**(2): 203-12.

For tumor progression, a cascade of linked sequential biological events is essential. We tried to test whether biological therapy can modulate specific biological phenotypes and increase the anti-tumor effect when combined with chemotherapy. Five human gastric cancer cell lines (YCC-1, YCC-2, YCC-3, YCC-7, AGS) were used in these studies. Pentosan polysulfate (PPS) as a heparin-binding growth factor inhibitor, Tranexamic acid as a plasmin inhibitor, Lovastatin as an adhesion inhibitor and Adriamycin as a chemotherapeutic agent were selected. The effects of each drug on colony formation and tumor cell proliferation were evaluated by soft agar assay and cell proliferation assay, respectively to test direct anti-tumor effect. The expression of uPA, PAI-1 was determined by ELISA, while MMPs activity was evaluated by zymography. PPS suppressed the colony-forming activity as much as Adriamycin did, but it showed only cytostatic effects in cell proliferation assay. Migration capacity using Boyden chamber assay was more closely correlated with adhesive capacity than uPA or MMP-2 expression. The motility inhibitory effect of Tranexamic acid was observed in the YCC-7 cell line, which expressed all the required biological phenotypes for migration. In AGS, with high cell motility and adhesiveness, the adhesion was inhibited by Lovastatin and most of the inhibitory effect was recovered by Mevalonate. When PPS was combined with Adriamycin on the Adriamycin-resistant, midkine (MK) gene expressing YCC-7 cell line, the growth inhibition rate increased up to 84%, while that for a single treatment of PPS or Adriamycin was 40% and 22%, respectively (p=0.001). When we combined Tranexamic acid and Adriamycin, we observed the synergistic effect in YCC-3 and YCC-7, while no combined effect was found in YCC-1. The combination of Lovastatin and Adriamycin did not show any combined effects in any of the cell lines. In conclusion, a synergistic anti-proliferative effect (chemo-sensitization) with combined chemo-biotherapy was found in cancer cells with specific biological target, MK. The anti-motility effect was the greatest when the gastric cancer cells expressed all the specific biological phenotypes.

Rowland-Goldsmith, M. A., H. Maruyama, et al. (2002). "Soluble type II transforming growth factor-beta receptor attenuates expression of metastasis-associated genes and suppresses pancreatic cancer cell metastasis." Mol Cancer Ther **1**(3): 161-7.

Pancreatic ductal adenocarcinoma (PDAC) is a deadly malignancy that frequently metastasizes and that overexpresses transforming growth factor-beta s (TGF-beta s). To determine whether TGF-beta s can act to enhance the metastatic potential of PDAC, PANC-1 human pancreatic cancer cells were transfected with an expression construct encoding a soluble type II TGF-beta receptor (sT beta RII) that blocks cellular responsiveness to TGF-beta 1. When injected s.c. in athymic mice, PANC-1 clones expressing sT beta RII exhibited decreased tumor growth in comparison with sham-transfected cells and attenuated expression of plasminogen activator inhibitor 1 (PAI-1), a gene associated with tumor growth. When tested in an orthotopic mouse model, these clones formed small intrapancreatic tumors that exhibited a suppressed metastatic capacity and decreased expression of plasminogen activator inhibitor 1 and the metastasis-associated urokinase plasminogen activator. These results indicate that TGF-beta s act in vivo to enhance the expression of genes that promote the growth and metastasis of pancreatic cancer cells and suggest that sT beta RII may ultimately have a therapeutic benefit in PDAC.

Sachdev, D., J. S. Hartell, et al. (2004). "A dominant negative type I insulin-like growth factor receptor inhibits metastasis of human cancer cells." J Biol Chem **279**(6): 5017-24.

We have previously shown that LCC6 wild-type (WT) cells, a metastatic variant of MDA-MB-435 cancer cells originally derived from a breast cancer patient, exhibit enhanced motility in response to IGF-I compared with the parent MDA-MB-435 cells. To further understand the role of the type I insulin-like growth factor (IGF) receptor (IGF1R) in cancer metastasis we inhibited signaling via IGF1R using a

C-terminal-truncated IGF1R. The truncated receptor retains the ligand binding domain but lacks the autophosphorylated tyrosine residues in the carboxyl terminus. Cells stably transfected with this truncated receptor (LCC6-DN cells) overexpressed the truncated IGF1R messenger RNA nearly 50-fold over endogenous receptor. The truncated receptor in the LCC6-DN cells behaved in a dominant negative manner to inhibit endogenous IGF1R activation by IGF-I. Compared with the LCC6-WT cells, LCC6-DN cells failed to phosphorylate the adaptor proteins insulin receptor substrate-1 and -2 in response to IGF-I and did not activate Akt after exposure to IGF-I. Unlike LCC6-WT cells, LCC6-DN cells did not show enhanced motility in response to IGF-I. To assay for metastasis, LCC6-WT and LCC6-DN cells were injected into the mammary fat pads of mice, and the primary xenograft tumors were removed after 21 days. Mice sacrificed 5 weeks later showed multiple lung metastases derived from LCC-WT xenografts, whereas mice harboring LCC6-DN xenografts showed no lung metastases. Our data show that IGF1R can regulate several aspects of the malignant phenotype. In these cells, metastasis but not proliferation requires IGF1R function.

Sakao, Y., H. Miyamoto, et al. (2006). "Prognostic significance of metastasis to the highest mediastinal lymph node in nonsmall cell lung cancer." Ann Thorac Surg **81**(1): 292-7.

BACKGROUND: We have tried to clarify the prognostic significance of metastasis to the highest mediastinal (HM) lymph node in patients with N2 lung cancer who underwent complete dissection of superior mediastinal (including HM) lymph nodes. METHODS: This study analyzed 53 patients with N2 nonsmall cell lung cancer who underwent surgical procedures such as lobectomy plus hilar and mediastinal node dissection (T4, neoadjuvant therapy cases were excluded). For patients whose cancer was in the left lung, we performed surgery through the median sternotomy in order to dissect superior mediastinal nodes. The clinicopathologic records of the patients were examined for prognostic factors such as age, sex, side, histology, tumor location, tumor size, clinical node (cN) number, preoperative serum carcinoembryonic antigen level, number of metastatic stations, and HM lymph node involvement. RESULTS: A univariate analysis showed that tumor size (T1/T2-3), cN factor (cN1-2/cN0), N2 level (multiple/single), and metastasis to the HM node were significant prognostic factors. In the multivariate analysis, metastasis to the HM lymph node remained a significant prognostic factor ($p = 0.026$). The 3-year survival rates were 52% in patients without metastasis to the HM lymph node and 21% in patients with metastasis to the HM lymph node ($p < 0.001$). Furthermore, when HM nodal involvement was absent, the 5-year survival rate was 33% even in patients with multilevel N2 status, 45% in patients with cN1-2 status, and 47% in patients with pT2-3 tumor status. CONCLUSIONS: Highest mediastinal lymph node involvement is prognostic of highly advanced N2 disease resulting in poor outcome. The results also suggest that patients with no involvement of the HM lymph node can experience acceptable postoperative outcomes even if they have multilevel N2 status, positive cN status, or T2-3 tumor status.

Satcher, R. L., Jr., K. Dvorkin, et al. (2004). "Gene expression in cancer cells is influenced by contact with bone cells in a novel coculture system that models bone metastasis." Clin Orthop Relat Res(426): 54-63.

Contact between bone cells and cancer cells (heterotypic cell contact) is thought to play a central role in the initial growth and progression of metastatic cells. Attempts at studying heterotypic contact in vitro and in vivo have been confounded by difficulty in controlling how and when heterotypic contact occurs between unlike cells. A novel model, the micropatterned coculture system, is described that quantifies and controls heterotypic contact between cancer cells and bone cells in vitro. The micropatterned coculture system is biocompatible, and is modified easily to accommodate two or more different populations of cells. Immunofluorescence of cocultures of prostate cancer-3 cells and osteoblasts show the precise control of cell interactions. Ribonucleic acid of sufficient quantity and quality is isolated readily from cells cocultured on the micropatterned coculture system. The expression of the metastasis associated genes urokinase plasminogen activator, insulinlike growth factor binding protein-1 and insulinlike growth factor binding protein-3 are regulated in response to heterotypic contact and soluble factors respectively. A model of bone metastasis based on the micropatterned coculture system technology will streamline the process for testing therapeutic agents, so that more molecules can be identified for animal and clinical testing at less cost and in less time than using conventional methods.

Sato, F., Y. Shimada, et al. (1999). "Expression of vascular endothelial growth factor, matrix metalloproteinase-9 and E-cadherin in the process of lymph node metastasis in oesophageal cancer." Br J Cancer **80**(9): 1366-72.

Lymph node metastasis is a strong independent prognostic factor for oesophageal cancer. The expression of matrix metalloproteinases (MMPs)

and reduction of E-cadherin correlate with lymph node metastasis of oesophageal cancer. We previously reported that the expression of vascular endothelial growth factor (VEGF) is associated with lymph node metastasis. This study was designed to determine whether VEGF, MMP-9 and E-cadherin expression is stable or changes in the process of lymph node metastasis of oesophageal cancer. Using immunohistochemistry, we detected VEGF, MMP-9 and E-cadherin expression in paraffin-embedded specimens of oesophageal squamous cell carcinoma. We classified 134 primary tumours and 174 nodal metastases using two different criteria: the absence [Group N(-)] or presence [Group N(+)] of nodal metastasis, and the stage of metastasis--Early Stage (cancer cells < 50% of lymph node) or Late Stage (> or = 50%)--and compared the expression among two groups and among two stages. The expression rates of Group N(-), Group N(+), Early Stage and Late Stage are as follows: VEGF (49%, 74%, 60%, 33%), MMP-9 (76%, 65%, 95%, 69%) and E-cadherin (49%, 24%, 55%, 38%). VEGF expression was down-regulated in Late Stage lymph node metastasis, while MMP-9 expression was elevated in Early Stage metastasis. E-cadherin expression is restored somewhat in Early Stage metastasis, but suppressed again in Late Stage metastasis. These data suggest that the expression of VEGF, MMP-9 and E-cadherin each change in the process of lymph node metastasis in oesophageal cancer, and that the patterns of change are different.

Sawada, T., K. Kimura, et al. (2006). "TGF-beta1 down-regulates ICAM-1 expression and enhances liver metastasis of pancreatic cancer." Adv Med Sci **51**: 60-5.

PURPOSE: In order to study the regulation of adhesion-molecule expression by cytokines, we have investigated the effect of transforming growth factor-beta1. (TGF-beta1) on the expression of intercellular adhesion molecule-1 (ICAM-1) in human pancreatic cancer cell lines. MATERIAL AND METHODS: By using three pancreatic cancer cell lines, SW1990, CAPAN-2 and PANC-1, the effect of TGF-beta1 on expression of ICAM-1, cancer cell immunogenicity and liver metastasis were investigated. RESULTS: Cell surface ICAM-1 expression by ELISA on three cell lines were all reduced significantly by following incubation with various concentrations of TGF-beta1 and down-regulation of ICAM-1 expression was also observed at the mRNA level. Corresponding to the down expression of ICAM-1, the adhesion of peripheral blood mononuclear lymphocytes (PBMLs) to cancer cells and cancer cell cytotoxicity during co-culture with PBMLs were remarkably decreased by treatment with TGF-beta1. Furthermore, enhanced liver metastatic potential by in vivo splenic injection was observed in CAPAN-2 cells pretreated with TGF-beta1. CONCLUSIONS: Since decreased expression of ICAM-1 has been known to contribute to cancer cell escape from immunologic recognition and cytotoxicity by effector cells, the present results indicate that unknown function of TGF-beta1 in the tumor progression and metastasis of pancreatic cancer.

Shibata, M. A., J. Morimoto, et al. (2008). "Combination therapy with short interfering RNA vectors against VEGF-C and VEGF-A suppresses lymph node and lung metastasis in a mouse immunocompetent mammary cancer model." Cancer Gene Ther **15**(12): 776-86.

Cancer metastasis contributes significantly to cancer mortality and is facilitated by lymphangiogenesis and angiogenesis. Vascular endothelial growth factor-C (VEGF-C) and VEGF-A are involved in lymphangiogenesis and angiogenesis. To inhibit metastasis, combination therapy with vector-based small interfering RNA (siRNA) against VEGF-C and/or VEGF-A was conducted on murine metastatic mammary cancer. Syngeneic, inoculated, metastatic mammary cancers received direct intratumoral injection of plasmid siRNA vector targeting VEGF-C (psiRNA-VEGF-C), VEGF-A (psiRNA-VEGF-A), both VEGF-C and VEGF-A (both psiRNA-VEGF-C and psiRNA-VEGF-A vectors injected, referred to as the psiRNA-VEGF-C+A group) or a scrambled sequence (psiRNA-SCR) as control, once a week for 8 weeks. Gene electrotransfer was performed on the tumors after each injection. Tumor volume was significantly lower in the psiRNA-VEGF-A and the psiRNA-VEGF-C+A groups throughout the study. Lymph node metastasis was significantly less frequent in all therapeutic groups, whereas the multiplicity of lung metastases was significantly lower in the psiRNA-VEGF-C+A group only. All siRNA therapeutic groups showed a significant reduction in the number of dilated lymphatic vessels containing intraluminal cancer cells and microvessel density. Our data suggest that specific silencing of the VEGF-C or VEGF-A gene alone can inhibit lymph node metastasis. However, combination siRNA therapy targeting both VEGF-C and VEGF-A inhibits both lymph node and lung metastasis, rendering this combined therapy more beneficial than either alone. The observed anti-metastatic activity of siRNA-expressing vectors targeting VEGF-C or VEGF-A may be of high clinical significance in the treatment of metastatic breast cancer.

Shimizu, K., H. Kubo, et al. (2004). "Suppression of VEGFR-3 signaling inhibits lymph node metastasis in gastric cancer." Cancer Sci **95**(4): 328-33.

In gastric cancer, lymph node metastasis is one of the major prognostic factors and forms the basis for surgical removal of local lymph nodes. Recently, several studies have demonstrated that overexpression of lymphangiogenic growth factor VEGF-C or VEGF-D induces tumor lymphangiogenesis and promotes lymphatic metastasis in mouse tumor models. We examined whether these processes could be inhibited in naturally metastatic tumors by blocking of their cognate receptor VEGFR-3 signaling pathway. Using a mouse orthotopic gastric cancer model which has a high frequency of lymph node metastasis, we estimated lymphatic vessels in gastric cancers by immunostaining for VEGFR-3 and other specific lymphatic markers, LYVE-1 and prox-1. Then we systemically administered anti-VEGFR-3 blocking antibodies. This treatment resulted in the inhibition of regional lymph node metastasis and reduction of lymphatic vessel density in the primary tumors. In addition, increased density of LYVE-1-positive lymphatic vessels of primary tumors was closely correlated with lymph node metastasis in human samples of gastric cancer. Antilymphangiogenesis by inhibiting VEGFR-3 signaling could provide a potential strategy for the prevention of lymph node metastasis in gastric cancer.

Shimo, T., S. Kubota, et al. (2006). "Pathogenic role of connective tissue growth factor (CTGF/CCN2) in osteolytic metastasis of breast cancer." J Bone Miner Res **21**(7): 1045-59.

The role of CTGF/CCN2 in osteolytic metastasis by breast cancer cells and its mechanism of action were studied. Osteolytic metastasis accompanied by CCN2 and PTHrP overproduction was efficiently inhibited by an anti-CCN2 antibody. Furthermore, we found that CCN2 was induced by PTHrP through PKA-, PKC-, and ERK-mediated pathways therein. INTRODUCTION: Connective tissue growth factor (CTGF/CCN2) is a mediator of local angiogenesis induced by breast cancer, but its role in osteolytic metastasis has not been evaluated. PTH-related peptide (PTHrP) is another critical factor in the development of the osteolytic metastasis. Using both in vivo and in vitro approaches, we studied whether/how neutralization of CCN2 prevented bone metastasis and how PTHrP signaling is related. MATERIALS AND METHODS: A mouse model of bone metastasis by human breast cancer cell line MDA231 was treated with a CCN2-neutralizing antibody, and osteolytic bone metastases were assessed on radiographs and immunohistochemistry. Ccn2 gene expression and transcription were examined by Northern blot and luciferase analysis. Immunoblot analysis and kinase inhibitors were used to identify the signaling pathways implicated. Anti-angiogenic/osteoclastogenic effects of ccn2 downregulation were also evaluated. RESULTS: Treatment of mice with a CCN2-neutralizing antibody greatly decreased osteolytic bone metastasis, microvasculature, and osteoclasts involved. The antibody also suppressed the growth of subcutaneous tumor in vivo and proliferation and migration of human umbilical vein endothelial cells (HUVECs) in vitro. Downregulation of ccn2 also repressed osteoclastogenesis. CCN2 expression was specifically observed in cancer cells producing PTHrP and type I PTH/PTHrP receptor (PTH1R) invaded the bone marrow, and PTHrP strongly upregulated ccn2 in MDA231 cells in vitro. Activation of protein kinase C (PKC) and protein kinase A (PKA) was necessary and sufficient for the stimulation of ccn2 by PTHrP. Indeed, inhibition of the extracellular signal-regulated kinase (ERK1/2), PKC, or PKA by specific inhibitors counteracted the stimulation of ccn2 expression. Incubation of MDA231 cells with PTHrP induced the activation of ERK1/2. Consistent with these findings, inhibition of PKC prevented PTHrP-induced ERK1/2 activation, whereas 12-O-tetradecanoylphorbol13-acetate (TPA), a stimulator of PKC, upregulated it. CONCLUSIONS: CCN2 was critically involved in osteolytic metastasis and was induced by PKA- and PKC-dependent activation of ERK1/2 signaling by PTHrP. Thus, CCN2 may be a new molecular target for anti-osteolytic therapy to shut off the PTHrP-CCN2 signaling pathway.

Shinohara, T., T. Miki, et al. (2001). "Nuclear factor-kappaB-dependent expression of metastasis suppressor KAI1/CD82 gene in lung cancer cell lines expressing mutant p53." Cancer Res **61**(2): 673-8.

KAI1/CD82 has been shown to be a metastasis suppressor for several human cancers, and a recent study revealed that wild-type tumor suppressor p53 can directly activate KAI1/CD82 gene expression. However, the response of KAI1/CD82 expression in cancer cells to exogenous stimulants has not been investigated. The present study examined whether tumor necrosis factor (TNF), which mediates many of the cellular responses associated with inflammatory reactions or cancer progression, can affect the KAI1/CD82 expression in lung cancer cells and, if so, whether nuclear factor (NF)-kappaB, a key molecule in TNF-mediated gene expression, is involved in the mechanism of KAI1/CD82 induction. Our results demonstrated that expression of KAI1/CD82 in PC-14 cells expressing mutant p53 could be augmented by TNF-alpha, and that transfer of the gene for a specific inhibitor of NF-kappaB, IkappaB alphaSR (mutant IkappaB alpha; NF-kappaB super-repressor), into PC-14 cells could inhibit this

augmentation. The amount of NF-kappaB in the nucleus of PC-14/IkappaB alphaSR cells correlated well with KAI1/CD82 mRNA and protein expression. In addition, IkappaB alphaSR gene transfer inhibited the spontaneous expression of KAI1/CD82 protein in KAI1/CD82-high-expressing RERF-LC-OK cells, which contain a mutant-type p53. These observations indicate that NF-kappaB activation may play a role in the regulation of KAI1/CD82 expression in lung cancer cells independently of wild-type p53, and suggest that KAI1/CD82 expression may be regulated by interaction with the host microenvironment.

Shintani, S., T. Ishikawa, et al. (2004). "Growth-regulated oncogene-1 expression is associated with angiogenesis and lymph node metastasis in human oral cancer." Oncology **66**(4): 316-22.

Growth-regulated oncogene-1 (GRO-1) is an autocrine growth factor in melanoma and is a member of the CXC family of chemokines which promote chemotaxis of granulocytes and endothelia through binding to CXC receptor 2. A previous article noted that GRO-1 was upregulated in oral cancer using a genome-wide microarray approach. We have examined the expression of GRO-1 in 9 oral squamous cell carcinoma (OSCC) cell lines and 94 OSCC specimens. Using real-time quantitative polymerase chain reaction analyses, GRO-1 expressions were varied in OSCC cell lines. Of the 94 OSCC specimens, 37 (39.4%) showed GRO-1 cytoplasmic immunostaining, and microvessel density revealed a correlation between GRO-1 expression and tumor angiogenesis. GRO-1 expression was also associated with leukocyte infiltration, and lymph node metastasis. These findings suggest a possible relationship between the expression level of GRO-1 and tumor progression.

Shintani, S., C. Li, et al. (2003). "Gefitinib ('Iressa'), an epidermal growth factor receptor tyrosine kinase inhibitor, mediates the inhibition of lymph node metastasis in oral cancer cells." Cancer Lett **201**(2): 149-55.

High expression of epidermal growth factor receptor (EGFR) is frequently observed in many solid tumor types including oral squamous cell carcinomas (OSCC). This study investigated whether treatment with gefitinib ('Iressa'), an EGFR-tyrosine kinase inhibitor, would inhibit the metastatic spread in OSCC cells. This was evaluated using orthotopic xenografts of highly metastatic OSCC. Metastasis was observed in six of 13 gefitinib treated animals (46.2%), compared with all of 12 control animals (100%). After exposure to gefitinib, OSCC cells showed a marked reduction in cell adhesion ability to fibronectin and in the expression of integrin alpha3, alphav, beta1, beta4, beta5 and beta6.

Shintani, Y., S. Higashiyama, et al. (2004). "Overexpression of ADAM9 in non-small cell lung cancer correlates with brain metastasis." Cancer Res **64**(12): 4190-6.

The "a disintegrin and metalloprotease" (ADAM) family contributes to regulation of the cell-cell and cell-matrix interactions that are critical determinants of malignancy. To determine the relationship between metastasis and ADAM proteins, we compared the mRNA levels of ADAM9, -10, -12, -15, and -17 in sublines of an EBC-1 lung cancer cell line that were highly metastatic to either brain or bone. ADAM9 mRNA levels were significantly higher in highly brain-metastatic sublines than in the parent or highly bone-metastatic sublines. To elucidate the role of ADAM9 in brain metastasis, we stably transfected A549 and EBC-1 cells with a full-length ADAM9 expression vector. Compared with mock-transfectants, ADAM9 overexpression resulted in increased invasive capacity in response to nerve growth factor, increased adhesion to brain tissue, and increased expression of integrin alpha 3 and beta 1 subunits. Administration of the anti-beta 1 monoclonal antibody attenuated this increase in invasive and adhesive activity. Intravenous administration of ADAM9-overexpressing A549 cells to mice resulted in micrometastatic foci in the brain and multiple metastatic colonies in the lungs. In contrast, administration of parent and mock-transfected A549 cells to mice resulted in lung tumors without brain metastasis. These results suggest that ADAM9 overexpression enhances cell adhesion and invasion of non-small cell lung cancer cells via modulation of other adhesion molecules and changes in sensitivity to growth factors, thereby promoting metastatic capacity to the brain.

Singh, D., D. D. Joshi, et al. (2000). "Increased expression of preprotachykinin-I and neurokinin receptors in human breast cancer cells: implications for bone marrow metastasis." Proc Natl Acad Sci U S A **97**(1): 388-93.

Neuropeptides are implicated in many tumors, breast cancer (BC) included. Preprotachykinin-I (PPT-I) encodes multiple neuropeptides with pleiotropic functions such as neurotransmission, immune/hematopoietic modulation, angiogenesis, and mitogenesis. PPT-I is constitutively expressed in some tumors. In this study, we investigated a role for PPT-I and its receptors, neurokinin-1 (NK-1) and NK-2, in BC by using quantitative reverse transcription-PCR, ELISA, and in situ hybridization. Compared with normal mammary epithelial cells (n = 2) and benign breast biopsies (n =

21), BC cell lines (n = 7) and malignant breast biopsies (n = 25) showed increased expression of PPT-I and NK-1. NK-2 levels were high in normal and malignant cells. Specific NK-1 and NK-2 antagonists inhibited BC cell proliferation, suggesting autocrine and/or intercrine stimulation of BC cells by PPT-I peptides. NK-2 showed no effect on the proliferation of normal cells but mediated the proliferation of BC cells. Cytosolic extracts from malignant BC cells enhanced PPT-I translation whereas extracts from normal mammary epithelial cells caused no change. These enhancing effects may be protein-specific because a similar increase was observed for IL-6 translation and no effect was observed for IL-1alpha and stem cell factor. The data suggest that PPT-I peptides and their receptors may be important in BC development. Considering that PPT-I peptides are hematopoietic modulators, these results could be extended to understand early integration of BC cells in the bone marrow, a preferred site of metastasis. Molecular signaling transduced by PPT-I peptides and the mechanism that enhances translation of PPT-I mRNA could lead to innovative strategies for BC treatments and metastasis.

Singh, L. S., M. Berk, et al. (2007). "Ovarian cancer G protein-coupled receptor 1, a new metastasis suppressor gene in prostate cancer." J Natl Cancer Inst **99**(17): 1313-27.

BACKGROUND: Metastasis is a process by which tumors spread from primary organs to other sites in the body and is the major cause of death for cancer patients. The ovarian cancer G protein-coupled receptor 1 (OGR1) gene has been shown to be expressed at lower levels in metastatic compared with primary prostate cancer tissues. METHODS: We used an orthotopic mouse metastasis model, in which we injected PC3 metastatic human prostate cancer cells stably transfected with empty vector (vector-PC3) or OGR1-expressing vector (OGR1-PC3) into the prostate lobes of athymic or NOD/SCID mice (n = 3-8 mice per group). Migration of PC3 cells transiently transfected with vector control or with OGR1- or GPR4 (a G protein-coupled receptor with the highest homology to OGR1)-expressing vectors was measured in vitro by Boyden chamber assays. G protein alpha-inhibitory subunit 1 (G alpha(i1)) expression after treatment with pertussis toxin (PTX) was measured using immunoblotting analysis. The inhibitory factor present in the conditioned medium was extracted using organic solvents and analyzed by mass spectrometry. RESULTS: In vivo, all 26 mice carrying tumors that were derived from vector-PC3 cells developed prostate cancer metastases (mean = 100%, 95% confidence interval [CI] = 83.97% to 100%) but few (4 of 32) mice carrying tumors derived from OGR1-expressing PC3 cells (mean = 12.50%, 95% CI = 4.08% to 29.93%) developed metastases. However, exogenous OGR1 overexpression had no effect on primary prostate tumor growth in vivo. In vitro, expression of OGR1, but not GPR4, inhibited cell migration (mean percentage of cells migrated, 30.2% versus 100%, difference = 69.8%, 95% CI = 63.0% to 75.9%; P<.001) via increased expression of G alpha(i1) and the secretion of a chloroform/methanol-extractable heat-insensitive factor into the conditioned medium through a PTX-sensitive pathway. CONCLUSION: OGR1 is a novel metastasis suppressor gene for prostate cancer. OGR1's constitutive activity via G alpha(i) contributes to its inhibitory effect on cell migration in vitro.

Stathopoulos, G. T., T. P. Sherrill, et al. (2008). "Use of bioluminescent imaging to investigate the role of nuclear factor-kappaBeta in experimental non-small cell lung cancer metastasis." Clin Exp Metastasis **25**(1): 43-51.

Nuclear factor (NF)-kappaB is frequently over-expressed in non-small cell lung cancer (NSCLC), but the exact role of this observation remains unclear. In this regard, activation of the transcription factor may govern distinct steps of NSCLC progression, such as carcinogenesis, angiogenesis, and metastasis. In these studies we attempted to dissect the effects of two proteins of the NF-kappaB pathway (p65/RelA and IkappaBetaalpha) on experimental metastasis of murine NSCLC, using a novel approach of bioluminescent detection of NF-kappaB activation in tumor cells. Stable integration of a NF-kappaBeta reporter confirmed high basal activation of the transcription factor in mouse NSCLC cells in vitro and during experimental metastasis to the lungs, like human NSCLC. In the mouse model of NSCLC metastasis, NF-kappaBeta-dependent luciferase expression served as a reliable indicator of tumor cell delivery to the lungs, establishment of metastatic tumors, and lung tumor burden. In vitro transient p65/RelA and IkappaBetaalpha gene transfer to mouse NSCLC cells resulted, respectively, in significant NF-kappaB activation and inhibition, without affecting cell growth. However, p65/RelA overexpression in NSCLC cells drastically reduced in vivo metastasis to the lungs, while overexpression of IkappaBetaalpha had no effect. In conclusion, using bioluminescent detection of NF-kappaB activation in mouse lug adenocarcinoma cells, we found a negative impact of p65/RelA on NSCLC metastasis.

Stathopoulos, G. T., T. P. Sherrill, et al. (2008). "Host nuclear factor-kappaB activation potentiates lung cancer metastasis." Mol Cancer Res **6**(3): 364-71.

Epidemiologic and experimental evidence suggests that a link exists between inflammation and cancer, although this relationship has only recently begun to be elucidated for lung cancer, the most frequently fatal human tumor. Nuclear factor-kappaB (NF-kappaB), a transcription factor that controls innate immune responses in the lungs, has been implicated as an important determinant of cancer cell proliferative and metastatic potential; however, its role in lung tumorigenesis is uncertain. Here, we specifically examine the role of NF-kappaB-induced airway inflammation in lung cancer metastasis using a model of intravenous injection of Lewis lung carcinoma cells into immunocompetent C57Bl/6 mice. Induction of lung inflammation by direct and specific NF-kappaB activation in airway epithelial cells potentiates lung adenocarcinoma metastasis. Moreover, we identify resident lung macrophages as crucial effectors of lung susceptibility to metastatic cancer growth. We conclude that NF-kappaB activity in host tissue is a significant factor in the development of lung metastasis.

Su, J. L., P. C. Yang, et al. (2006). "The VEGF-C/Flt-4 axis promotes invasion and metastasis of cancer cells." Cancer Cell **9**(3): 209-23.

Flt-4, a VEGF receptor, is activated by its specific ligand, VEGF-C. The resultant signaling pathway promotes angiogenesis and/or lymphangiogenesis. This report provides evidence that the VEGF-C/Flt-4 axis enhances cancer cell mobility and invasiveness and contributes to the promotion of cancer cell metastasis. VEGF-C/Flt-4-mediated invasion and metastasis of cancer cells were found to require upregulation of the neural cell adhesion molecule contactin-1 through activation of the Src-p38 MAPK-C/EBP-dependent pathway. Examination of tumor tissues from various types of cancers revealed high levels of Flt-4 and VEGF-C expression that correlated closely with clinical metastasis and patient survival. The VEGF-C/Flt-4 axis, through upregulation of contactin-1, may regulate the invasive capacity in different types of cancer cells.

Suarez-Cuervo, C., M. A. Merrell, et al. (2004). "Breast cancer cells with inhibition of p38alpha have decreased MMP-9 activity and exhibit decreased bone metastasis in mice." Clin Exp Metastasis **21**(6): 525-33.

p38 belongs to a family of mitogen-activated protein kinases, which transfer extracellular signals into intracellular responses. p38 is also frequently detected in clinical breast cancer specimens, but its role as a prognostic factor is not known. Of the various p38 isoforms, p38alpha has been shown to mediate the in vitro invasiveness of breast cancer cells through up-regulation of urokinase plasminogen activator (uPA). We studied the role of p38alpha in breast cancer bone metastases, using dominant negative blockade approach. Human MDA-MB-231 breast cancer clones stably expressing dominant negative p38alpha (p38/AF) exhibited decreased basal MMP-9 activity. TGF-beta1-induced MMP-9 activity was also blunted in these clones, as compared with controls in which TGF-betal up-regulated MMP-9 activity. Consistent with these findings, SB202190, a specific p38 inhibitor, also inhibited TGF-beta1-induced MMP-9 activity in parental cells. The p38/AF clones exhibited also reduced uPA production after growth on vitronectin and decreased cell motility, as compared with controls. VEGF production levels in all the studied clones were similar. The p38/AF clone, which had similar in vitro growth rate as the control pcDNA3 clone, formed significantly less bone metastases in a mouse model, as compared with the control clone. In conclusion, inhibition of the p38alpha pathway results in decreased MMP-9 activity, impaired uPA expression and decreased motility, all of which may contribute to the decreased formation of bone metastasis.

Sulzer, M. A., M. P. Leers, et al. (1998). "Reduced E-cadherin expression is associated with increased lymph node metastasis and unfavorable prognosis in non-small cell lung cancer." Am J Respir Crit Care Med **157**(4 Pt 1): 1319-23.

E-cadherin is a calcium-dependent, epithelial cell adhesion molecule whose reduced expression has been associated with tumor dedifferentiation and increased lymph node metastasis in clinical studies involving several carcinomas. In this study, 111 patients who had previously undergone complete resection and systematic mediastinal lymph node dissection for non-small cell lung cancer (NSCLC) were studied retrospectively. In the primary tumor, as well as in the lymph node metastases, E-cadherin expression was detected by immunohistochemistry using a monoclonal antibody (HECD-1; Takara, Otsu, Japan). There was a significant inverse correlation between E-cadherin expression and lymph node stage (Pearson correlation coefficient -0.52, $p = 0.0001$) as well as tumor differentiation (Pearson correlation coefficient -0.27, $p = 0.005$). Moreover, Kaplan and Meier survival estimates showed a significant correlation between E-cadherin expression and patient survival in log rank testing ($p = 0.006$). In the patient group with the highest proportion of E-cadherin positive tumor cells, 60% of the patients were still estimated to be alive at 36 mo, versus 32% of the patients in the group classified as showing negative E-cadherin expression. Our findings provide clinical evidence that reduced E-cadherin expression is

associated with tumor dedifferentiation, increased lymphogenous metastasis and poor survival. It seems therefore that E-cadherin expression might be an important prognostic factor in NSCLC.

Sumiyoshi, Y., Y. Yamashita, et al. (2000). "Expression of CD44, vascular endothelial growth factor, and proliferating cell nuclear antigen in severe venous invasional colorectal cancer and its relationship to liver metastasis." Surg Today **30**(4): 323-7.

The first step in liver metastasis is venous invasion by cancer cells from the primary tumor. However, even among cases where the histology shows extensive venous invasion by the primary tumor, we sometimes find cases without synchronous liver metastases. As a result, there is a strong possibility that, besides the established causes of colorectal cancer and that of cancer cells invading the veins, some other important causes for liver metastasis must exist. We investigated the expression rates of CD44, proliferating cell nuclear antigen (PCNA), and vascular endothelial growth factor (VEGF) in 28 primary colorectal tumors using immunohistological techniques, and examined an association with liver metastasis. Cases that are strongly positive for CD44 or PCNA have a higher rate of synchronous liver metastases than cases with either no expression or a low expression. We could find no correlation between the VEGF expression and synchronous liver metastasis. In cases with severe venous invasion, VEGF is not correlated with liver metastasis whereas CD44 and PCNA are correlated with liver metastasis. In cases where severe venous invasion is histologically observed, an immunohistochemical analysis for CD44 and PCNA should be done to assess the likelihood of liver metastases.

Sun, Y. X., E. A. Pedersen, et al. (2008). "CD26/dipeptidyl peptidase IV regulates prostate cancer metastasis by degrading SDF-1/CXCL12." Clin Exp Metastasis **25**(7): 765-76.

Stromal derived factor-1 (SDF-1 or CXCL12) expressed by osteoblasts and endothelial cells, and its receptors CXCR4 and CXCR7/RDC1 are key molecular determinants in prostate cancer (PCa) metastasis. What drives PCa cells into the extravascular marrow space(s) once they make contact with the blood vessel endothelium, however remains unclear. Here, we evaluated whether degradation of CXCL12 facilitates PCa cell entry into the marrow cavity by locally lowering CXCL12 levels intravascularly. To explore this possibility, co-cultured conditioned media from PCa cells and endothelial cells were evaluated for their ability to degrade biotinylated CXCL12 (bCXCL12). Co-culture of PCa cells/endothelial cells resulted in greater digestion of CXCL12 than was achieved by either cell type alone, and this activity regulated invasion in vitro. The ability to degrade CXCL12 was not however observed in PCa and osteoblasts co-cultures. Fractionation and inhibitor studies suggested that the activity was CD26/dipeptidyl peptidase IV (DPPIV) and possibly other cysteine/serine proteases. By inhibiting CD26/DPPIV, invasion and metastasis of PCa cell lines were enhanced in in vitro and in vivo metastasis assays. Together, these data suggest that the degradation of CXCL12 by CD26/DPPIV may be involved in the metastatic cascades of PCa, and suggests that inhibition of CD26/DPPIV may be a trigger of PCa metastasis.

Sweeney, C. J., S. Mehrotra, et al. (2005). "The sesquiterpene lactone parthenolide in combination with docetaxel reduces metastasis and improves survival in a xenograft model of breast cancer." Mol Cancer Ther **4**(6): 1004-12.

Parthenolide, a sesquiterpene lactone, shows antitumor activity in vitro, which correlates with its ability to inhibit the DNA binding of the antiapoptotic transcription factor nuclear factor kappaB (NF-kappaB) and activation of the c-Jun NH(2)-terminal kinase. In this study, we investigated the chemosensitizing activity of parthenolide in vitro as well as in MDA-MB-231 cell-derived xenograft metastasis model of breast cancer. HBL-100 and MDA-MB-231 cells were used to measure the antitumor and chemosensitizing activity of parthenolide in vitro. Parthenolide was effective either alone or in combination with docetaxel in reducing colony formation, inducing apoptosis and reducing the expression of prometastatic genes IL-8 and the antiapoptotic gene GADD45beta1 in vitro. In an adjuvant setting, animals treated with parthenolide and docetaxel combination showed significantly enhanced survival compared with untreated animals or animals treated with either drug. The enhanced survival in the combination arm was associated with reduced lung metastases. In addition, nuclear NF-kappaB levels were lower in residual tumors and lung metastasis of animals treated with parthenolide, docetaxel, or both. In the established orthotopic model, there was a trend toward slower growth in the parthenolide-treated animals but no statistically significant findings were seen. These results for the first time reveal the significant in vivo chemosensitizing properties of parthenolide in the metastatic breast cancer setting and support the contention that metastases are very reliant on activation of NF-kappaB.

Tahir, S. A., G. Yang, et al. (2001). "Secreted caveolin-1 stimulates cell survival/clonal growth and

contributes to metastasis in androgen-insensitive prostate cancer." Cancer Res **61**(10): 3882-5.

Caveolin-1 is an integral protein of caveolae, known to play important roles in signal transduction and lipid transport. We demonstrate that caveolin-1 expression is significantly increased in primary and metastatic human prostate cancer after androgen ablation therapy. We also show that caveolin-1 is secreted by androgen-insensitive prostate cancer cells, and that this secretion is regulated by steroid hormones. Significantly, caveolin-1 was detected in the MDL(3) fraction of serum specimens from patients with advanced prostate cancer and to a lesser extent in normal subjects. Conditioned media from high passage caveolin-1 secreting, androgen-insensitive, LNCaP cells stimulated increased viability and clonal growth of low passage, caveolin-1-negative, androgen-sensitive, LNCaP cells in vitro, and this effect was blocked by treating the media with caveolin-1 antibody. i.p. injections of caveolin-1 antibody suppressed the orthotopic growth and spontaneous metastasis of highly metastatic, androgen-insensitive caveolin-1-secreting mouse prostate cancer. Overall, our results establish caveolin-1 as an autocrine/paracrine factor that is associated with androgen-insensitive prostate cancer. We demonstrate the potential for caveolin-1 as a therapeutic target for this important malignancy.

Takanami, I. (2006). "Lymphatic microvessel density using D2-40 is associated with nodal metastasis in non-small cell lung cancer." Oncol Rep **15**(2): 437-42.

The monoclonal antibody D2-40 is a new selective marker for lymphatic endothelium. The lymphatic microvessel density (LMVD) using D2-40 has not yet been evaluated in non-small cell lung cancer (NSCLC). The aim of this study was to evaluate LMVD using D2-40 in NSCLC. We investigated LMVD in 77 patients with NSCLC who underwent curative tumor resection. We also determined the relation between LMVD and clinicopathologic factors, VEGF-C and Ang-2 and microvessel density (MVD) using factor VIII-related antigen. The median number of D2-40-positive vessels in the highest LMVD was 25 (range, 5-71). LMVD was significantly associated with tumor status, lymph node metastasis, stage, lymphatic invasion, VEGF-C protein and MVD ($p=0.0149$ for tumor status; $p<0.0001$ for nodal status; $p<0.0001$ for stage; $p=0.0153$ for lymphatic invasion; $p=0.0030$ for VEGF-C, and $p=0.0029$ for MVD). Furthermore, LMVD using D2-40 expression was shown to be an independent predictor of lymph node metastasis by multivariate analysis ($p=0.0070$). These data indicate that a high LMVD by D2-40 may be an indicator of lymph node metastasis in NSCLC.

Takayama, T., K. Miyanishi, et al. (2006). "Colorectal cancer: genetics of development and metastasis." J Gastroenterol **41**(3): 185-92.

It has been well documented that there are two major pathways in colorectal carcinogenesis. One is the chromosomal instability pathway (adenoma-carcinoma sequence), which is characterized by allelic losses on chromosome 5q (APC), 17p (p53), and 18q (DCC/SMAD4), and the other is a pathway that involves microsatellite instability. Recent progress in molecular biology, however, has shown that colorectal carcinogenesis is not necessarily clearly divided into these two pathways, but is in fact more complicated. Other routes, including the transforming growth factor-beta/SMAD pathway, the serrated pathway, and the epigenetic pathway, have been reported. Cross talk among these pathways has also been reported. In the invasion and metastasis steps of colorectal cancers, many more genes have now been identified as being involved in proteolysis, adhesion, angiogenesis, and cell growth. Recently accumulated evidence indicates that colorectal cancer is a genetically heterogeneous and complicated disease.

Takizawa, H., K. Kondo, et al. (2006). "The balance of VEGF-C and VEGFR-3 mRNA is a predictor of lymph node metastasis in non-small cell lung cancer." Br J Cancer **95**(1): 75-9.

A positive association between vascular endothelial growth factor-C (VEGF-C) expression and lymph node metastasis has been reported in several cancers. However, the relationship of VEGF-C and lymph node metastasis in some cancers, including non-small cell lung cancer (NSCLC), is controversial. We evaluated the VEGF-C and vascular endothelial growth factor receptor-3 (VEGFR-3) expression in NSCLC samples from patients who had undergone surgery between 1998 and 2002 using real-time quantitative RT-PCR and immunohistochemical staining. We failed to find a positive association between VEGF-C and VEGFR-3 mRNA expression and lymph node metastasis in NSCLC. An immunohistological study demonstrated that VEGF-C was expressed not only in cancer cells, but also in macrophages in NSCLC, and that VEGFR-3 was expressed in cancer cells, macrophages, type II pneumocytes and lymph vessels. The VEGF-C/VEGFR-3 ratio of the node-positive group was significantly higher than that of the node-negative group. Immunohistochemical staining showed that VEGFR-3 was mainly expressed in cancer cells. The immunoreactivity of VEGF-C and VEGFR-3 was roughly correlated to the mRNA levels of VEGF-C and VEGFR-3 in real-time PCR. VEGF-C mRNA alone has no positive association with lymph node

metastasis in NSCLC. The VEGF-C/VEGFR-3 ratio was positively associated with lymph node metastasis in NSCLC. This suggests that VEGF-C promotes lymph node metastasis while being influenced by the strength of the VEGF-C autocrine loop, and the VEGF-C/VEGFR-3 ratio can be a useful predictor of lymph node metastasis in NSCLC.

Tamura, M., M. Oda, et al. (2004). "The combination assay with circulating vascular endothelial growth factor (VEGF)-C, matrix metalloproteinase-9, and VEGF for diagnosing lymph node metastasis in patients with non-small cell lung cancer." Ann Surg Oncol **11**(10): 928-33.

BACKGROUND: The aim of the present study was to evaluate the diagnostic utility of levels of circulating vascular endothelial growth factor (VEGF)-C, matrix metalloproteinase-9 (MMP-9), and VEGF and to verify that the combination assay of these circulating factors is a clinically useful indicator to predict the presence of lymph node metastasis in non-small cell lung cancer (NSCLC). In the ROC curve analysis, VEGF-C (0.761) had the biggest areas under the ROC curve, followed by MMP-9 (0.723) and VEGF (0.694). Combination assay of three markers had higher sensitivity and specificity for prediction than single-marker assays (AUC = 0.837). CONCLUSIONS: This study has confirmed that combination assay of three markers to determine VEGF-C, MMP-9, and VEGF expression in circulation detects lymph node metastasis in NSCLC with higher accuracy than single-marker assays.

Tamura, M., M. Oda, et al. (2004). "Chest CT and serum vascular endothelial growth factor-C level to diagnose lymph node metastasis in patients with primary non-small cell lung cancer." Chest **126**(2): 342-6.

STUDY OBJECTIVE: Accurate tumor staging is essential for choosing the appropriate treatment strategy for lung cancer. CT of the chest is the most commonly used noninvasive staging method of the lymph node metastasis, but it is far from satisfying. We evaluated whether circulating vascular endothelial growth factor (VEGF)-C could give additional information for diagnosing lymph node metastasis in patients with lung cancer. PATIENTS AND METHOD: Serum samples were obtained from 116 patients with primary non-small cell lung cancer (NSCLC). All patients underwent preoperative CT of the thorax. Clinical T and N stages were compared to the final T and N stages obtained from pathologic findings. Serum VEGF-C concentration was assayed by commercially available sandwich enzyme-linked immunosorbent assay. We evaluated the utility of serum VEGF-C level as a marker for nodal metastasis comparing the utility of CT. RESULTS: Preoperative and final T categories completely agreed in 82.8%. Regarding nodal metastasis, the accuracy of CT was 68.1%. Patients with lymph node metastasis showed higher serum VEGF-C concentrations than those without lymph node metastasis ($p = 0.0007$). Serum VEGF-C reached the highest sensitivity and specificity in diagnosing lymph node metastasis when a cut-off value of 1,850.6 pg/mL was applied (sensitivity, 70.0%; specificity, 77.3%). Serum VEGF-C visually correlated with CT scan in the detection of lymph node metastasis (sensitivity, 74.0%; specificity, 80.3%; positive predictive value, 74.0%; negative predictive value, 80.3%; accuracy, 77.6%). When the cases were limited to adenocarcinoma, better results could be obtained. CONCLUSIONS: Serum VEGF-C is a reliable marker for lymph node metastasis in NSCLC. Serum VEGF-C evaluation and CT examination are complementary to each other for accurate lymph node staging in NSCLC.

Tanaka, S., S. C. Pero, et al. (2006). "Specific peptide ligand for Grb7 signal transduction protein and pancreatic cancer metastasis." J Natl Cancer Inst **98**(7): 491-8.

BACKGROUND: Pancreatic cancer is one of the most aggressive malignancies, with high rates of invasion and metastasis and with generally poor prognosis. We previously found that metastasis was strongly associated with the expression of growth factor receptor-bound protein 7 (Grb7), which contains a Src homology 2 (SH2) domain. In this study, we evaluated Grb7 protein as a molecular target of therapy for metastatic pancreatic cancer. METHODS: Grb7 protein expression was measured by immunohistochemistry in 36 human pancreatic cancer specimens and adjacent normal pancreatic tissue. We synthesized a nonphosphorylated peptide inhibitor that binds specifically to the SH2 domain of Grb7. Intracellular signaling was assessed by immunoprecipitation and immunoblot assays in cultured human pancreatic cancer cells. Cell migration was measured with a modified Boyden chamber method. Peritoneal metastasis of the pancreatic cancer cells was measured with a mouse model. All statistical tests were two-sided. RESULTS: We found that 22 (61%) of 36 pancreatic cancer specimens had higher levels of Grb7 protein than their corresponding normal pancreatic tissue specimens. The Grb7 peptide inhibitor appears to be a promising molecularly targeted therapeutic agent against metastatic pancreatic cancer.

Tanaka, Y., H. Kobayashi, et al. (2004). "Genetic downregulation of pregnancy-associated plasma protein-A (PAPP-A) by bikunin reduces IGF-I-

dependent Akt and ERK1/2 activation and subsequently reduces ovarian cancer cell growth, invasion and metastasis." Int J Cancer **109**(3): 336-47.

A Kunitz-type protease inhibitor, bikunin, downregulates expression of uPA and its receptor uPAR at the mRNA and protein levels in several types of tumor cells. Our recent work showed that, using a cDNA microarray analysis, pregnancy-associated plasma protein-A (PAPP-A) is a candidate bikunin target gene. To clarify how reduced levels of PAPP-A may confer repressed invasiveness, we transfected human ovarian cancer cell line HRA with antisense (AS)-PAPP-A cDNA and compared the properties of the transfected cells to those of parental HRA cells. Here, we show that regulation of uPA mRNA and protein by IGF-I depends on the PI3K and MAPK signaling pathways and phosphorylation of Akt and ERK1/2 is required for IGF-I-mediated cell invasion; that IGFBP-4 protease in HRA cells is identified as PAPP-A; that reduced PAPP-A expression is associated with the upregulation of IGFBP-4 expression; that higher intact IGFBP-4 levels were associated with low invasive potential and growth rate in AS-PAPP-A cells in response to IGF-I; that IGF-I stimulates Akt and ERK1/2 activation of both the control and antisense cells, but the relative potency and efficacy of IGF-I were lower in the antisense cells compared to the control; and that genetic downregulation of PAPP-A reduces the proliferation, invasion and metastasis of HRA cells. In conclusion, our data identify a novel role for PAPP-A as a bikunin target gene. IGF-I-induced IGFBP-4 proteolysis by PAPP-A may enhance cell growth and invasion through IGF-I-dependent Akt and ERK1/2 activation and subsequently upregulation of uPA.

Taylor, A. P. and D. M. Goldenberg (2007). "Role of placenta growth factor in malignancy and evidence that an antagonistic PlGF/Flt-1 peptide inhibits the growth and metastasis of human breast cancer xenografts." Mol Cancer Ther **6**(2): 524-31.

The angiogenic growth factor placenta growth factor (PlGF) is implicated in several pathologic processes, including the growth and spread of cancer. We found by immunohistochemistry that 36% to 60% and 65% of primary breast cancers express PlGF and its receptor Flt-1, respectively. These findings suggest that PlGF may be active in tumor growth and metastasis beyond its role in angiogenesis. It was found that exogenously added PlGF (2 nmol/L), in contrast to vascular endothelial growth factor (2 nmol/L), significantly stimulated in vitro motility and invasion of the human breast tumor lines MCF-7 and MDA-MB-231. A PlGF-2/Flt-1-inhibiting peptide, binding peptide 1 (BP1), that binds Flt-1 at or near the heparin-binding site was identified and synthesized. Both PlGF-stimulated motility and invasion were prevented by treatment with BP1 ($P < 0.05$), as well as by anti-PlGF antibody. Treatment of mice bearing s.c. MDA-MB-231 with BP1 (200 mug i.p., twice per week) decreased the number of spontaneous metastatic lung nodules by 94% ($P < 0.02$), whereas therapy of animals with orthotopic mammary fat pad tumors decreased pulmonary metastases by 82% ($P < 0.02$). These results indicate, for the first time, that PlGF stimulates the metastatic phenotype in these breast cancer cells, whereas therapy with a PlGF-2/Flt-1 heparin-blocking peptide reduces the growth and metastasis of human breast cancer xenografts.

Tokunaga, T., Y. Abe, et al. (2002). "Ribozyme mediated cleavage of cell-associated isoform of vascular endothelial growth factor inhibits liver metastasis of a pancreatic cancer cell line." Int J Oncol **21**(5): 1027-32.

Stromal angiogenesis is an important factor for progression of malignant neoplasms. We used hammerhead ribozymes against vascular endothelial growth factor (VEGF) gene transcripts to down-regulate cell-associated VEGF189 isoform function in the pancreatic cancer cell line MIA PaCa2. MIA PaCa2 transfected with anti-VEGF189 ribozyme did not show any alteration of growth rate under tissue culture. When the transformants were subcutaneously transplanted, tumour volume of the ribozyme-transfected MIA PaCa2 xenografts was significantly smaller ($P<0.01$). No metastasis of MIA PaCa2 transfected with anti-VEGF189 was apparent, while disabled ribozyme-transfected MIA PaCa2 showed significant liver metastasis ($P<0.05$). These results suggested that VEGF189 plays an important role in growth and metastatic potential through alteration of angiogenic balance in cancer.

Tokunaga, T., M. Nakamura, et al. (1999). "Thrombospondin 2 expression is correlated with inhibition of angiogenesis and metastasis of colon cancer." Br J Cancer **79**(2): 354-9.

Two subtypes of thrombospondin (TSP-1 and TSP-2) have inhibitory roles in angiogenesis in vitro, although the biological significance of these TSP isoforms has not been determined in vivo. Vascularity was estimated by CD34 staining, and TSP-2(-)/VEGF-189(+) colon cancers showed significantly increased vessel counts and density in the stroma ($P < 0.0001$). TSP-2(-)/VEGF-189(+) colon cancer patients also showed significantly poorer prognosis compared with those with TSP-2(+)/VEGF-189(-) ($P = 0.0014$). These results suggest that colon cancer metastasis is critically determined by angiogenesis resulting from

the balance between the angioinhibitory factor TSP-2 and angiogenic factor VEGF-189.

Tsuruga, T., S. Nakagawa, et al. (2007). "Loss of Hugl-1 expression associates with lymph node metastasis in endometrial cancer." Oncol Res **16**(9): 431-5.

Mutation of neoplastic tumor suppressor genes, scribble, discs large, and lethal giant larvae (lgl), causes disruption of cell polarity and overproliferation of Drosophila epithelial cells and neuroblasts. Reduced expression of human homologue of lgl, Hugl-1, has been reported to be involved in development and progression of human colon cancer and malignant melanoma. To explore the association between Hugl-1 expression and clinical character in endometrial cancer, we examined the expression of Hugl-1 in primary endometrial cancer tissues. The expression of Hugl-1 mRNA in 86 primary endometrial cancer tissues was examined using semiquantitative reverse transcription polymerase chain reaction (RT-PCR). All samples were categorized into two groups: Hugl-1 positive and Hugl-1 negative. Clinical data of each group were analyzed by Fisher's exact probability test and survival rates of each group were compared by Kaplan-Meier method and Log-rank test. Loss of Hugl-1 expression had correlation with the higher incidence of lymph node metastasis, but not to the patient's age at onset, distant metastasis, clinical stage, lymph or venous vessel invasion, or histopathological grade of differentiation. The Hugl-1-positive group had poorer prognosis compared with the Hugl-1-negative group. These results indicate that loss of Hugl-1 expression in endometrial cancer may contribute to lymph node metastasis and it can be a factor of poor prognosis.

Tsutsumi, S., T. Yanagawa, et al. (2004). "Autocrine motility factor signaling enhances pancreatic cancer metastasis." Clin Cancer Res **10**(22): 7775-84.

PURPOSE: Autocrine motility factor (AMF)/phosphoglucose isomerase (PGI) is a ubiquitous cytosolic enzyme that plays a key role in glycolysis. AMF/PGI is also a multifunctional protein that acts in the extracellular milieu as a potent mitogen/cytokine. Increased expression of AMF/PGI and its receptor has been found in a wide spectrum of malignancies and is associated with cancer progression and metastasis. Recent studies indicated that AMF is induced by hypoxia and enhances the random motility of pancreatic cancer cells. In the present study, the role and regulation of AMF in the growth and metastasis of pancreatic cancer cells were determined. EXPERIMENTAL DESIGN: In this study, we assessed whether overexpression of AMF in human pancreatic cancer cells enhances the liver metastasis using an orthotopic mouse tumor model. We also investigated the intracellular signal transduction pathways of AMF in human pancreatic cancer cell lines. RESULTS: Overexpression of AMF stimulated in vitro invasion of MIA PaCa-2 cells. In vivo, after orthotopic implantation into the pancreas of nude mice, parental and empty vector-transfected MIA PaCa-2 cells produced locally relatively small tumors with no evidence of liver metastasis, whereas AMF-transfected MIA PaCa-2 cells produced the large tumors and liver metastases. In addition, over-expression of AMF leads to down-regulation of E-cadherin expression associated with the up-regulation of the zinc-finger transcription factor SNAIL expression. CONCLUSIONS: The data submitted here show that AMF expression significantly contributes to the aggressive phenotype of human pancreatic cancer and thus may provide a novel prognostic and therapeutic target.

Uchima, Y., T. Sawada, et al. (2003). "Identification of a trypsinogen activity stimulating factor produced by pancreatic cancer cells: its role in tumor invasion and metastasis." Int J Mol Med **12**(6): 871-8.

Trypsinogen/trypsin is one of the major serine proteases and is produced by pancreatic acinar cells. Tumor-associated trypsinogen (TAT) has been reported to be produced by several cancer cell lines. The biological roles and activation mechanisms of both TAT and pancreatic acinar trypsinogen (PAT) have not been elucidated in the context of cancer extension, in particular at the stage of invasion and metastasis. In this study, we investigate the roles played by PAT and TAT in pancreatic cancer invasion. In addition, we determined their mechanisms of activation and identified a trypsinogen activity-stimulating factor (TASF) produced by pancreatic cancer cells. TAT expression and high TAT activity were associated with high invasive and liver metastatic potential in SW1990 and CAPAN-2 cells. Moreover, a trypsinogen activating effect and activity prolonging effect was observed in a mixture of these supernatants with trypsinogen. These cells revealed significantly enhanced invasiveness upon invasion assay and in the presence of PAT. TAT and PAT were activated by TASF, active u-PA, produced by pancreatic cancer cells. Activated TAT and PAT can degrade not only ECM proteins but they can also activate other latent proteases. This ECM-protease-network may form a vicious cycle, thereby promoting tumor cell invasion.

Versteeg, H. H., C. A. Spek, et al. (2004). "Tissue factor and cancer metastasis: the role of intracellular and extracellular signaling pathways." Mol Med **10**(1-6): 6-11.

Tissue factor (TF) initiates the coagulation cascade but also plays a role in cancer and metastasis. This transmembrane protein is frequently upregulated on tumor cells and cells that show metastatic behavior. Furthermore, it is a significant risk factor for hepatic metastasis in patients suffering from colon cancer. Recently, it has been shown that TF, together with its natural ligand factor VIIa, induces intracellular changes, such as signal transduction cascades, gene transcription, and protein synthesis. Moreover, TF:factor VIIa interaction leads to survival of cells that have been stimulated to undergo apoptosis. Together with TF-dependent processes such as angiogenesis, these intracellular phenomena form a plausible explanation for the influence of TF on metastasis. In this review, we will discuss these phenomena in more detail and hypothesize on their role in TF-driven metastasis.

Von Marschall, Z., A. Scholz, et al. (2005). "Vascular endothelial growth factor-D induces lymphangiogenesis and lymphatic metastasis in models of ductal pancreatic cancer." Int J Oncol **27**(3): 669-79.

The presence of lymphatic metastases is a strong indicator for poor prognosis in patients with ductal pancreatic cancer. In order to better understand the mechanisms controlling lymphatic growth and lymph node metastasis in human ductal pancreatic cancer, we analyzed the expression pattern of the vascular endothelial growth factor-D (VEGF-D), its receptor VEGF-receptor-3 (VEGFR-3) and the lymphatic endothelium-specific hyaluronan receptor LYVE-1 in a panel of 19 primary human ductal pancreatic tumors and 10 normal pancreas specimens. We further addressed the biological function of VEGF-D for induction of lymphatic metastasis in a nude mouse xenograft model using two human ductal pancreatic cancer cell lines with overexpression of VEGF-D. Compared to normal human pancreas, pancreatic cancer tissue showed overexpression of VEGF-D and VEGFR-3 in conjunction with a high lymphatic vascularization as determined by immunohistochemistry and in situ hybridization. Tumors derived from VEGF-D-overexpressing cells had a higher microvessel density compared to their mock-controls, as determined based on CD31 immunohistochemistry. Importantly, these tumors also revealed a significant induction of intra- and peritumoral lymphatics, as judged from immunohistochemical detection of LYVE-1 expression. This was associated with a significant increase in lymphatic vessel invasion by tumor cells and an increased rate of lymphatic metastases, as indicated by pan-cytokeratin reactive cells in lymph nodes. Our results suggest that VEGF-D plays a pivotal role in stimulating lymphangiogenesis and lymphatic metastasis in human ductal pancreatic cancer, and therefore represents a novel therapeutic target for this devastating disease.

Wang, J., Y. Cai, et al. (2006). "Increased expression of the metastasis-associated gene Ehm2 in prostate cancer." Prostate **66**(15): 1641-52.

BACKGROUND: Alterations of fibroblast growth factors and their receptors contribute to prostate cancer progression by enhancing cell survival, motility, and proliferation. The expression of the FGFR-4 Arg(388) variant is correlated with the occurrence of pelvic lymph node metastasis and biochemical (PSA) recurrence in men undergoing radical prostatectomy. Ehm2 is an androgen-regulated gene that has been associated with metastasis in other systems, so we sought to determine if it is expressed in prostate cancer and if the FGFR-4 Arg(388) variant can increase its expression. METHODS: Expression of Ehm2 was examined by quantitative RT-PCR and Western blotting in prostate cell lines and by quantitative RT-PCR, in situ hybridization, and immunohistochemistry in prostate tissues. The effect of Ehm2 expression on collagen IV adhesion was tested by transient overexpression and RNA interference. RESULTS: Ehm2 expression is upregulated in prostate cancer cell lines and prostate cancer tissues. Expression of the FGFR-4 Arg(388) variant results in increased expression of Ehm2. Increased expression of Ehm2 leads to decreased adhesion to collagen IV, which has been associated with metastasis in cancers. Analysis of tissue microarrays revealed that increased Ehm2 expression is associated with biochemical recurrence after radical prostatectomy, which is indicative of more aggressive disease. CONCLUSIONS: Ehm2 is overexpressed in prostate cancer and may enhance disease progression and metastasis.

Wang, W. B., S. Boing, et al. (2002). "Identification of metastasis-associated genes in early stage non-small cell lung cancer by subtractive hybridization." Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao (Shanghai) **34**(3): 273-8.

Non-small cell lung cancer (NSCLC) is a leading cause of death and a substantial fraction of patients with surgically resected disease ultimately dies due to distant metastasis. To identify gene expression differences in early stage adenocarcinoma that either did or did not metastasize within a 5-year period, we employed a subtractive hybridization strategy of pooled RNA from primary adenocarcinomas (stage I) of the lung. Individual clones (n=225) of the subtracted cDNA library were sequenced. Further analyses of mRNA expression

levels in a cohort of 70 NSCLC patients (stage I to IIIA) showed that the metastasis association of the identified genes was stage and histology specific. Cox regression analyses identified two genes (EIF4A1, MALA1) to be independent prognostic parameters for patients' survival in stage I and II disease. These findings could help to identify early-stage NSCLC patients at high risk for the development of distant metastasis.

Wegiel, B., A. Bjartell, et al. (2008). "Multiple cellular mechanisms related to cyclin A1 in prostate cancer invasion and metastasis." J Natl Cancer Inst **100**(14): 1022-36.

BACKGROUND: Cyclin A1 is a cell cycle regulator that has been implicated in the progression of prostate cancer. Its role in invasion and metastasis of this disease has not been characterized. PC3 cells that overexpressed cyclin A1 showed increased invasiveness, and inhibition of cyclin A1 expression via shRNA expression reduced invasiveness of these cells. Eight of 10 mice (80%) bearing PC3 cells overexpressing cyclin A1 had infiltration of tumor cells in lymph node, liver, and lung, but all 10 mice bearing tumors expressing control vector were free of liver and lung metastases and only one mouse from this group had lymph node metastasis (P values from Fisher exact tests < .001). Cyclin A1, in concert with AR, bound to and increased expression from the VEGF and MMP2 promoters. CONCLUSIONS: Cyclin A1 contributes to prostate cancer invasion by modulating the expression of MMPs and VEGF and by interacting with AR.

Wen, J., K. Matsumoto, et al. (2004). "Hepatic gene expression of NK4, an HGF-antagonist/angiogenesis inhibitor, suppresses liver metastasis and invasive growth of colon cancer in mice." Cancer Gene Ther **11**(6): 419-30.

Hepatocyte growth factor (HGF) is involved in malignant behavior of cancer cells by enhancing invasion and metastasis. We earlier found that NK4, a four-kringle fragment of HGF, functions as both an HGF antagonist and an angiogenesis inhibitor. We have now carried out studies to determine if hydrodynamics-based delivery and expression of the NK4 gene would inhibit liver metastasis and invasive growth of colon carcinoma cells in mice. When the naked plasmid for NK4 was introduced into mice by hydrodynamics-based gene delivery, a high level of expression of NK4 was predominant in the liver. After intrasplenic inoculation of MC-38 murine colon carcinoma cells, the cells formed numerous metastatic nodules in the liver and showed invasive growth behavior. On the other hand, when mice were given the NK4 plasmid, hepatic gene expression of NK4 inhibited the liver metastasis and subsequent growth associated with a decrease in microvessel density. Likewise, intrahepatic invasion of cancer cells was inhibited by NK4 gene expression, and this anti-invasive effect was associated with in situ inhibition of c-Met receptor tyrosine phosphorylation. Moreover, NK4 gene expression prolonged survival of these mice. Taken together with the knowledge that the majority of deaths from colon cancer are due to liver metastasis, the potential therapeutic use of hepatic gene expression of NK4 for metastatic colon cancer treatment can be given consideration.

Wen, J., K. Matsumoto, et al. (2007). "Inhibition of colon cancer growth and metastasis by NK4 gene repetitive delivery in mice." Biochem Biophys Res Commun **358**(1): 117-23.

NK4, originally prepared as a competitive antagonist for hepatocyte growth factor (HGF), is a bifunctional molecule that acts as an HGF-antagonist and angiogenesis inhibitor. When the expression plasmid for NK4 gene was administered into mice by hydrodynamics-based delivery, the repetitive increase in the plasma NK4 protein level was achieved by repetitive administration of NK4 gene. Mice were subcutaneously implanted with colon cancer cells and weekly given with the NK4 plasmid. The repetitive delivery and expression of NK4 gene inhibited angiogenesis and invasiveness of colon cancer cells in subcutaneous tumor tissue and this was associated with suppression of primary tumor growth. By fifty days after tumor implantation, cancer cells naturally metastasized to the liver, whereas NK4 gene expression potently inhibited liver metastasis. Inhibition of the HGF-Met receptor pathway and tumor angiogenesis by NK4 gene expression has potential therapeutic value toward inhibition of invasion, growth, and metastasis of colon cancer.

Whang, P. G., E. M. Schwarz, et al. (2005). "The effects of RANK blockade and osteoclast depletion in a model of pure osteoblastic prostate cancer metastasis in bone." J Orthop Res **23**(6): 1475-83.

Adenocarcinoma of the prostate exhibits a clear propensity for bone and is associated with the formation of osteoblastic metastases. It has previously been suggested that osteoclast activity may be necessary for the development of these osteoblastic metastases based on data from lytic and mixed lytic-blastic tumors. Here we investigate the effects of complete in vivo osteoclast depletion via the blockade of receptor activator of NF:kappaB (RANK) on the establishment and progression of purely osteoblastic (LAPC-9 cells) bone lesions induced by human prostate cancer cells using a SCID mouse intratibial injection model. The subcutaneous administration of

the RANK antagonist (15 mg/kg) RANK:Fc did not prevent the formation of purely osteoblastic lesions, indicating that osteoclasts may not be essential to the initial development of osteoblastic metastases. However, RANK:Fc protein appeared to inhibit the progression of established osteoblastic lesions, suggesting that osteoclasts may be involved in the subsequent growth of these tumors once they are already present. In contrast, RANK:Fc treatment effectively blocked the establishment and progression of purely osteolytic lesions formed by PC-3 cells, which served as a positive control. These results indicate that in vivo RANK blockade may not be effective for the prevention of osteoblastic metastasis but may potentially represent a novel therapy that limits the growth of established metastatic CaP lesions in bone.

Wiesner, C., S. M. Nabha, et al. (2008). "C-kit and its ligand stem cell factor: potential contribution to prostate cancer bone metastasis." Neoplasia **10**(9): 996-1003.

The tyrosine kinase receptor c-kit and its ligand stem cell factor (SCF) have not been explored in prostate cancer (PC) bone metastasis. Herein, we found that three human PC cell lines and bone marrow stromal cells express a membrane-bound SCF isoform and release a soluble SCF. Bone marrow stromal cells revealed strong expression of c-kit, whereas PC cells showed very low levels of the receptor or did not express it all. Using an experimental model of PC bone metastasis, we found that intraosseous bone tumors formed by otherwise c-kit-negative PC3 cells strongly expressed c-kit, as demonstrated using immunohistochemical and Western blot analyses. Subcutaneous PC3 tumors were, however, c-kit-negative. Both bone and subcutaneous PC3 tumors were positive for SCF. Immunohistochemical analysis of human specimens revealed that the expression frequency of c-kit in epithelial cells was of 5% in benign prostatic hyperplasia, 14% in primary PC, and 40% in PC bone metastases, suggesting an overall trend of increased c-kit expression in clinical PC progression. Stem cell factor expression frequency was more than 80% in all the cases. Our data suggest that the bone microenvironment up-regulates c-kit expression on PC cells, favoring their intraosseous expansion.

Wu, D., H. E. Zhau, et al. (2007). "cAMP-responsive element-binding protein regulates vascular endothelial growth factor expression: implication in human prostate cancer bone metastasis." Oncogene **26**(35): 5070-7.

Aberrant expression of vascular endothelial growth factor (VEGF) is associated with human prostate cancer (PCa) metastasis and poor clinical outcome. We found that both phosphorylation of cyclic AMP-responsive element-binding protein (CREB) and VEGF levels were significantly elevated in patient bone metastatic PCa specimens. A PCa ARCaP progression model demonstrating epithelial-to-mesenchymal transition exhibited increased CREB phosphorylation and VEGF expression as ARCaP cells became progressively more mesenchymal and bone-metastatic. Activation of CREB induced, whereas inhibition of CREB blocked, VEGF expression in ARCaP cells. CREB may regulate VEGF transcription via a hypoxia-inducible factor-dependent mechanism in normoxic conditions. Activation of CREB signaling is involved in the coordinated regulation of VEGF and may pre-dispose to PCa bone metastasis.

Xue, C., F. Liang, et al. (2006). "ErbB3-dependent motility and intravasation in breast cancer metastasis." Cancer Res **66**(3): 1418-26.

A better understanding of how epidermal growth factor receptor family members (ErbBs) contribute to metastasis is important for evaluating ErbB-directed therapies. Activation of ErbB3/ErbB2 heterodimers can affect both proliferation and motility. We find that increasing ErbB3-dependent signaling in orthotopic injection models of breast cancer can enhance intravasation and lung metastasis with no effect on primary tumor growth or microvessel density. Enhanced metastatic ability due to increased expression of ErbB2 or ErbB3 correlated with stronger chemotaxis and invasion responses to heregulin beta1. Suppression of ErbB3 expression reduced both intravasation and metastasis. A human breast cancer tumor tissue microarray showed a significant association between ErbB3 and ErbB2 expression and metastasis independent of tumor size. These results indicate that ErbB3-dependent signaling through ErbB3/ErbB2 heterodimers can contribute to metastasis through enhancing tumor cell invasion and intravasation in vivo and that ErbB-directed therapies may be useful for the inhibition of invasion independent of effects on tumor growth.

Yamamoto, M., H. Kikuchi, et al. (2008). "TSU68 prevents liver metastasis of colon cancer xenografts by modulating the premetastatic niche." Cancer Res **68**(23): 9754-62.

The aim of this study was to investigate the inhibitory effect of TSU68 [(Z)-5-[(1,2-dihydro-2-oxo-3H-indol-3-ylidene)methyl]-2,4-dimethyl-1H-pyrrole-3-p ropanoic acid; SU6668], an inhibitor of vascular endothelial growth factor receptor 2, platelet-derived growth factor receptor beta, and fibroblast growth factor receptor 1 (FGFR1), on colon cancer

liver metastasis, and to test the hypothesis that TSU68 modulates the microenvironment in the liver before the formation of metastasis. First, we implanted the highly metastatic human colon cancer TK-4 orthotopically into the cecal walls of nude mice, followed by twice-daily administration of TSU68 (400 mg/kg/d) or vehicle. Five weeks of treatment with TSU68 significantly inhibited liver metastasis compared with the control group (P<0.001). Next, we analyzed the gene expression profile in premetastatic liver using microarrays. Microarray and quantitative reverse transcription-PCR analysis showed that mRNA levels for the chemokine CXCL1 were significantly increased in tumor-bearing mice compared with non-tumor-bearing mice. Moreover, CXCL1 expression was significantly decreased by TSU68 treatment. CXCR2 expression was detected predominantly on tumor cells in orthotopic tumors compared with ectopic tumors. The number of migrating neutrophils in premetastatic liver was significantly decreased in the TSU68-treated group (P<0.001). The amount of interleukin-12 (IL-12) p40 in the portal vein was significantly decreased by TSU68 (P=0.02). Blockade of both CXCR2 and IL-12 p40 with a neutralizing antibody significantly inhibited liver metastasis. These results suggest that the CXCL1/CXCR2 axis is important in cancer metastasis and that TSU68 may modulate the premetastatic niche in the target organ through suppression of the inflammatory response, which might be an alternative mechanism used by antiangiogenic agents.

Yamashita, H., J. Kitayama, et al. (2007). "Tissue factor expression is a clinical indicator of lymphatic metastasis and poor prognosis in gastric cancer with intestinal phenotype." J Surg Oncol **95**(4): 324-31.

BACKGROUND AND OBJECTIVES: Tissue factor (TF), which normally safeguards vascular integrity by inducing hemostasis upon injury, has received widespread attention in the pathogenesis of cancer progression and metastasis. Aberrantly expressed TF in cancer cells has been reported to be associated with advanced stages of malignancy in various cancers. METHODS: The expression of TF and microvessel density (MVD) were immunohistochemically evaluated in 207 gastric cancers, and their relationship with clinicopathological features was examined. RESULTS: TF was preferentially expressed (41.8%) in intestinal-type cancer at a significantly higher rate than that in diffuse-type cancer (12.1%, P<0.0001). The expression of TF was associated with advanced stage of disease and showed a positive correlation with a higher rate of lymphatic and venous invasion and lymphatic metastasis in intestinal-type, but not in diffuse-type carcinoma. Moreover, TF expression was associated with high MVD in the tumor and a worse outcome only in intestinal-type carcinoma. CONCLUSIONS: TF may be critically involved in tumor progression in intestinal-type, but not in diffuse-type, gastric carcinoma. The difference in clinical features between these two histological types might be partially dependent on TF expression profile.

Yamauchi, T., M. Watanabe, et al. (2003). "The potential for a selective cyclooxygenase-2 inhibitor in the prevention of liver metastasis in human colorectal cancer." Anticancer Res **23**(1A): 245-9.

In a previous report we noted that cyclooxygenase-2 (COX-2) expression in clinical colorectal cancer is closely related to liver metastasis and survival. The aim of the present study was to clarify the role of COX-2 in liver metastasis and to examine the potential for a selective COX-2 inhibitor as a novel therapeutic agent in the treatment of colorectal cancer. MATERIALS AND METHODS: COX-2 expression of 6 kinds of human colon cancer cell lines, with various potentials for liver metastasis, were assessed by Western blot and reverse transcriptase polymerase chain reaction (RT-PCR). In human tumor xenografts/severe combined immune-deficient (SCID) mouse, we examined the effects of a selective COX-2 inhibitor (JTE-522) on tumor growth or liver metastasis of HT-29, a highly-metastatic cell line, or on COLO205, a non-metastatic cell line. The effect of JTE-522 on vascular endothelial growth factor (VEGF) expression and the activity of matrix metalloproteinases (MMPs) in HT-29 and COLO205 were assessed by enzyme-linked immunosorbent assay (ELISA) and gelatin zymography, respectively. RESULTS: COX-2 was expressed in all metastatic cell lines but not in the non-metastatic lines. JTE-522 prevented the liver metastasis of HT-29, but not the subcutaneous growth of HT-29 and COLO205 in SCID mice. In vitro, JTE-522 suppressed VEGF expression, but did not affect MMP production in HT-29; an inhibitory effect was not found in COLO205. CONCLUSION: A selective COX-2 inhibitor of JTE-522, was found to prevent liver metastases of colon cancer by suppressing VEGF expression, and therefore, COX-2 possibly plays an important role in liver metastasis of human colon cancer via the regulation of VEGF expression.

Yano, S., H. Nokihara, et al. (2003). "Multifunctional interleukin-1beta promotes metastasis of human lung cancer cells in SCID mice via enhanced expression of adhesion-, invasion- and angiogenesis-related molecules." Cancer Sci **94**(3): 244-52.

We examined whether interleukin-1 (IL-1), a multifunctional proinflammatory cytokine, progresses

or regresses metastasis of lung cancer. Exogenous IL-1beta enhanced expression of various cytokines (IL-6, IL-8, and vascular endothelial growth factor (VEGF)) and intracellular adhesion molecule-1 (ICAM-1) by A549, PC14, RERF-LC-AI, and SBC-3 cells expressing IL-1 receptors. A549 cells transduced with human IL-1beta-gene with the growth-hormone signaling-peptide sequence (A549/IL-1beta) secreted a large amount of IL-1beta protein. Overexpression of IL-1beta resulted in augmentation of expression of the cytokines, ICAM-1, and matrix metalloproteinase-2 (MMP-2). A549/IL-1beta cells intravenously inoculated into severe combined immunodeficiency (SCID) mice distributed to the lung more efficiently and developed lung metastasis much more rapidly than did control A549 cells. Treatment of SCID mice with anti-IL-1beta antibody inhibited formation of lung metastasis by A549/IL-1beta cells. Moreover, A549/IL-1beta cells inoculated in the subcutis grew more rapidly, without necrosis, than did control A549 cells, which produced smaller tumors with central necrosis, suggesting involvement of angiogenesis in addition to enhanced binding in the high metastatic potential of A549/IL-1beta cells. Histological analyses showed that more host-cell infiltration, fewer apoptotic cells, more vascularization, and higher MMP activity were observed in tumors derived from A549/IL-1beta cells, compared with tumors derived from control A549 cells. These findings suggest that IL-1beta facilitates metastasis of lung cancer via promoting multiple events, including adhesion, invasion and angiogenesis.

Yasuoka, H., M. Tsujimoto, et al. (2008). "Cytoplasmic CXCR4 expression in breast cancer: induction by nitric oxide and correlation with lymph node metastasis and poor prognosis." BMC Cancer **8**: 340.

BACKGROUND: Lymph nodes constitute the first site of metastasis for most malignancies, and the extent of lymph node involvement is a major criterion for evaluating patient prognosis. The CXC chemokine receptor 4 (CXCR4) has been shown to play an important role in lymph node metastasis. Nitric oxide (NO) may also contribute to induction of metastatic ability in human cancers. METHODS: CXCR4 expression was analyzed in primary human breast carcinoma with long-term follow-up. The relationship between nitrotyrosine levels (a biomarker for peroxynitrate formation from NO in vivo) and lymph node status, CXCR4 immunoreactivity, and other established clinico-pathological parameters, as well as prognosis, was analyzed. Nitrite/nitrate levels and CXCR4 expressions were assessed in MDA-MB-231 and SK-BR-3 breast cancer cell lines after induction and/or inhibition of NO synthesis. RESULTS: CXCR4 staining was predominantly cytoplasmic; this was observed in 50%(56/113) of the tumors. Cytoplasmic CXCR4 expression was significantly correlated with nitrotyrosine levels and lymph node metastasis. Kaplan-Meier survival curves showed that cytoplasmic CXCR4 expression was associated with reduced disease-free and overall survival. In multivariate analysis, cytoplasmic CXCR4 expression emerged as a significant independent predictor for overall and disease-free survival. Cytoplasmic expression of functional CXCR4 in MDA-MB-231 and SK-BR-3 cells was increased by treatment with the NO donor DETA NONOate. This increase was abolished by L-NAME, an inhibitor of NOS. CONCLUSION: Our data showed a role for NO in stimulating cytoplasmic CXCR4 expression in vitro. Formation of the biomarker nitrotyrosine was also correlated with CXCR4 expression and lymph node metastasis in vivo. In addition, cytoplasmic CXCR4 expression may serve as a significant prognostic factor for long-term survival in breast cancer.

Yeh, K. Y., J. W. Chang, et al. (2003). "Ovarian metastasis originating from bronchioloalveolar carcinoma: a rare presentation of lung cancer." Jpn J Clin Oncol **33**(8): 404-7.

Ovarian metastasis originating from bronchioloalveolar carcinoma (BAC) has not been reported previously. We report a 63-year-old Chinese woman who was diagnosed as BAC with pleural metastasis in 1997. Four years later, she complained of vaginal bleeding, and a pelvic mass was discovered by an abdominal computerized tomography scan. Tumor debulking and total hysterectomy with bilateral salpingo-oopherectomy were performed. Pathology disclosed well-differentiated adenocarcinoma, with abundant clear cytoplasm, in the ovaries. Furthermore, immunohistochemical staining revealed that the tumor cells from the ovary and pleura were reactive to thyroid transcription factor 1 (TTF-1) and cytokeratin-7 (CK-7) but were negative for cytokeratin-20 (CK-20). The results of immunohistochemical staining, clinical course, and pathological features were compatible with the diagnosis of BAC with ovarian metastasis. In conclusion, to investigate the primary site of a metastatic ovarian cancer, clinicians should not forget the lungs since the incidence of lung cancer in females is increasing. Moreover, a monoclonal antibody panel for TTF-1, CK-7, and CK-20 may facilitate discrimination between primary and metastasized ovarian adenocarcinomas and/or identifying tumors of pulmonary origin.

Yi, B., P. J. Williams, et al. (2002). "Tumor-derived platelet-derived growth factor-BB plays a critical role

in osteosclerotic bone metastasis in an animal model of human breast cancer." Cancer Res **62**(3): 917-23.

Breast cancer produces a variety of growth factors to promote its behavior at primary and secondary sites in autocrine/paracrine manners. However, the role of these growth factors in the colonization of cancer cells in bone, which is one of the most common metastatic sites, is poorly understood. To study this, we established an in vivo model in which the MCF-7 human breast cancer cells caused predominant osteosclerotic bone metastases 20-25 weeks after inoculation into the left cardiac ventricle in female nude mice. To make this model more time efficient, we overexpressed the oncogene Neu, which is associated with aggressive behavior in human breast cancers, in MCF-7 cells (MCF-7/Neu). MCF-7/Neu cells grew without estrogen and developed osteosclerotic bone metastases in 10-12 weeks in animals. Of note, MCF-7/Neu-bearing mice showed substantial plasma levels of human platelet-derived growth factor-BB (hPDGF-BB; 855 +/- 347 pg/ml; mean +/- SE, n = 5), indicating hPDGF-BB production by inoculated MCF-7/Neu cells. MCF-7/Neu cells in culture also produced large amounts of hPDGF-BB. Conditioned medium harvested from MCF-7/Neu cells stimulated osteoblastic bone formation in organ cultures of neonatal mouse calvariae, and a neutralizing antibody to hPDGF-BB blocked the osteoblastic bone formation. Stable transfection of the hPDGF-B AS in MCF-7/Neu cells reduced hPDGF-BB production in culture. Mice bearing these MCF-7/Neu cells with antisense showed reduced bone metastases with decreased plasma hPDGF-BB levels (54 +/- 20 and 35 +/- 21 in two different antisense and 696 +/- 312 pg/ml in empty vector; mean +/- SE; n = 5). Introduction of hPDGF-B cDNA in the MDA-MB-231 human breast cancer cells, which consistently formed osteolytic bone metastases, induced osteosclerotic lesions in the osteolytic bone metastases. In conclusion, we show that MCF-7 cells cause osteosclerotic bone metastases and that Neu enhances this capacity of MCF-7 cells. Our data suggest that MCF-7/Neu-derived hPDGF-BB plays a causative role in the development of osteosclerotic bone metastases in this model.

Yokoi, H., M. Nakata, et al. (2001). "Intraglomerular metastasis from pancreatic cancer." Am J Kidney Dis **37**(6): 1299-303.

Few case reports have shown the presence of metastatic tumor cells in renal glomeruli. We report one case with intraglomerular metastasis proved at renal biopsy. A 60-year-old man suffered from weight loss and fever of unknown origin. Urinalysis revealed proteinuria with cellular and granular casts. Because vasculitis was suspected, renal biopsy was performed. Presence of tumor cells occupying the glomerular capillary lumina was shown by means of light microscopy and electron microscopy. Laboratory findings revealed elevated leukocyte count (28.9 x 10(3)/mm(3)), serum granulocyte colony-stimulating factor (G-CSF) (77 pg/mL), and serum CA 19-9 (21,885 U/mL). The patient soon developed disseminated intravascular coagulation and died. Autopsy findings revealed pancreatic cancer showing positive staining for G-CSF and CA 19-9. Tumor cells in the glomerular capillary lumina showed positive staining for CA 19-9 and proliferating cell nuclear antigen (PCNA). These results suggest that the pancreatic tumor cells producing G-CSF were entrapped in the glomerular capillary lumina where they proliferated. This may have been the first step in renal metastasis.

Yonemura, Y., Y. Endo, et al. (1999). "Role of vascular endothelial growth factor C expression in the development of lymph node metastasis in gastric cancer." Clin Cancer Res **5**(7): 1823-9.

Neogenesis of lymphatic vessel and lymphatic invasion is frequently found in the stroma of cancers, but the mechanisms of this phenomenon remain unclear. Vascular endothelial growth factor C (VEGF-C) is known to be the only growth factor for the lymphatic vascular system, and its receptor has been identified as Flt4. To clarify the mechanism of lymphatic invasion in cancer, we studied the expression of VEGF-C and flt4 genes in gastric cancer tissues. VEGF-C mRNA was mainly expressed in primary tumors (15 of 32; 47%), but the frequency of VEGF-C mRNA expression was low in normal mucosa (4 of 32; 13%). In primary tumors, there was a significant relationship between VEGF-C and flt4 mRNA expression. In contrast, Flt4 was mainly expressed on the lymphatic endothelial cells but not in cancer cells. A strong correlation was found between VEGF-C expression and lymph node status, lymphatic invasion, venous invasion, and tumor infiltrating patterns. Cancer cells in the lymphatic vessels frequently showed intracytoplasmic VEGF-C immunoreactivity. Furthermore, there was a close correlation between VEGF-C tissue status and the grade of lymph node metastasis. Patients with high expression of VEGF-C protein had a significantly poorer prognosis than did those in low VEGF-C expression group. By the Cox regression model, depth of wall invasion, lymph node metastasis, and VEGF-C tissue status emerged as independent prognostic parameters, and the VEGF-C tissue status was ranked third as an independent risk factor for death. These results strongly suggest that cancer cells producing VEGF-C may induce the proliferation and dilation of lymphatic vessels, resulting in the development of

invasion of cancer cells into the lymphatic vessel and lymph node metastasis.

Yoshikawa, R., H. Yanagi, et al. (2006). "ECA39 is a novel distant metastasis-related biomarker in colorectal cancer." World J Gastroenterol **12**(36): 5884-9.

AIM: To investigate the possible role of polysaccharide-K (PSK) -related markers in predicting distant metastasis and in the clinical outcome of colorectal cancer (CRC). METHODS: Firstly, we used protein microarrays to analyze the in vitro expression profiles of potential PSK-related markers in the human colorectal adenocarcinoma cell line SW480, which carries a mutant p53 gene. Then, we investigated the clinical implications of these markers in the prognosis of CRC patients. RESULTS: ECA39, a direct target of c-Myc, was identified as a candidate protein affected by the anti-metastatic effects of PSK. Immunohistochemistry revealed that ECA39 was expressed at significantly higher levels in tumor tissues with distant metastases compared to those without (P<0.00001). Positive ECA39 expression was shown to be highly reliable for the prediction of distant metastases (sensitivity: 86.7%, specificity: 90%, positive predictive value: 86.7%, negative predictive value: 90%). A significantly higher cumulative 5-yr disease free survival rate was observed in the ECA39-negative patient group (77.3%) compared with the ECA39-positive patient group (25.8%) (P<0.05). CONCLUSION: Our results suggest that ECA39 is a dominant predictive factor for distant metastasis in patients with advanced CRC and that its suppression by PSK might represent a useful application of immunotherapy as part of a program of integrated medicine.

Yoshitake, N., H. Fukui, et al. (2008). "Expression of SDF-1 alpha and nuclear CXCR4 predicts lymph node metastasis in colorectal cancer." Br J Cancer **98**(10): 1682-9.

Although stromal cell-derived factor (SDF)-1 alpha and its receptor CXCR4 are experimentally suggested to be involved in tumorigenicity, the clinicopathological significance of their expression in human disease is not fully understood. We examined SDF-1 alpha and CXCR4 expression in colorectal cancers (CRCs) and their related lymph nodes (LNs), and investigated its relationship to clinicopathological features. Specimens of 60 primary CRCs and 27 related LNs were examined immunohistochemically for not only positivity but also immunostaining patterns for SDF-1 alpha and CXCR4. The relationships between clinicopathological features and SDF-1 alpha or CXCR4 expression were then analysed. Stromal cell-derived factor-1 alpha and CXCR4 expression were significantly associated with LN metastasis, tumour stage, and survival of CRC patients. Twenty-nine of 47 CXCR4-positive CRCs (61.7%) showed clear CXCR4 immunoreactivity in the nucleus and a weak signal in the cytoplasm (nuclear type), whereas others showed no nuclear immunoreactivity but a diffuse signal in the cytoplasm and at the plasma membrane (cytomembrane type). Colorectal cancer patients with nuclear CXCR4 expression showed significantly more frequent LN metastasis than did those with cytomembrane expression. Colorectal cancer patients with nuclear CXCR4 expression in the primary lesion frequently had cytomembrane CXCR4-positive tumours in their LNs. In conclusion, expression of SDF-1 alpha and nuclear CXCR4 predicts LN metastasis in CRCs.

Zhang, G., B. He, et al. (2003). "Growth factor signaling induces metastasis genes in transformed cells: molecular connection between Akt kinase and osteopontin in breast cancer." Mol Cell Biol **23**(18): 6507-19.

Malignant tumors are characterized by excessive growth, immortalization, and metastatic spread, whereas benign tumors do not express gene products that mediate invasion. The molecular basis for this difference is incompletely understood. We have screened signal transduction molecules associated with the epidermal growth factor (EGF) receptor and have identified constitutive phosphorylation, indicative of activation, of Akt kinase in MT2994 breast cancer cells. In contrast, cells of the benign breast epithelial cell lines Comma-D and FSK-7 are immortalized through pathways that are independent of the EGF-phosphatidylinositol 3-kinase-Akt kinase cascade, but this is not associated with invasiveness. Transfection of constitutively active Akt kinase causes accelerated cell division and osteopontin expression. Conversely, dominant-negative Akt kinase slows cell cycle progression and suppresses osteopontin expression. The manipulation of osteopontin expression in this setting by transfection of the gene or its antisense does not affect the growth rate of the cells but alters cell motility and anchorage independence. Therefore, Akt kinase activates two distinct genetic programs: the program of growth and survival, which is independent of osteopontin expression, and the program of invasiveness and anchorage independence, which is mediated by osteopontin. These studies define Akt kinase as a molecular bridge between cell cycle progression and dissemination.

Zhang, H., L. C. Stephens, et al. (2006). "Metastasis tumor antigen family proteins during breast cancer progression and metastasis in a reliable mouse model

for human breast cancer." Clin Cancer Res **12**(5): 1479-86.

PURPOSE: Chromatin remodeling pathways are critical in the regulation of cancer-related genes and are currently being explored as potential targets for therapeutic intervention. The metastasis tumor antigen (MTA) family of proteins, MTA1, MTA2, and MTA3, are components of chromatin remodeling pathways with potential roles in breast cancer. Although all three MTA family proteins have been shown to be associated with metastatic progression of breast cancers, the expression characteristic of MTA1-3 proteins in a multistep breast cancer progression model remains unknown. Structural and functional studies have suggested that they are heterogeneous in the Mi-2/NuRD complex, exhibit tissue-specific patterns of expression, and impart unique properties to estrogen receptor-alpha (ERalpha) action. This led us to hypothesize that each member of the MTA family possesses a unique role and interacts with different pathways in the stepwise process of breast cancer development and progression. EXPERIMENTAL DESIGN: MTA family proteins were examined by immunohistochemistry in breast cancer processes ranging from normal duct, to premalignant lesions, to invasive carcinoma, and to metastasized tumors in PyV-mT transgenic mice, which represents a reliable model for multistage tumorigenesis of human breast cancer. We also determined the association of MTA proteins with the status of cell proliferation, ER, E-cadherin and cytoplasmic beta-catenin, and cancer-related coactivators, AIB1 and PELP1. RESULTS: The expression of all three MTA proteins was altered in primary breast tumors. Each MTA protein had a unique expression pattern during the primary breast tumor progression. Altered expression of MTA1 was observed in both premalignant lesion and malignant carcinoma, but an elevated nuclear expression was observed in ER-negative carcinomas. MTA3 was exclusively expressed in a subset of cells of ER-positive premalignant lesions but not in carcinomas. MTA2 expression seems to be unrelated to ER status. Loss of MTA3 expression and more nuclear localization of MTA1 occurred with loss of E-cadherin and decreased cytoplasmic beta-catenin, two molecules essential for epithelial cell adhesion and important tumor cell invasion. At the late stage of tumor formation, MTA1 is usually expressed in the center of tumors. Coincidentally, the distribution of MTA1-positive cells at this stage was complementary to that of AIB1 and PELP1, which were localized to the tumor periphery with relatively active cell proliferation, scattered ER-positive cells and a limited differentiation. In metastasized lung tumors, the expression pattern of MTA-protein expression was distinct from that in primary counterparts. CONCLUSIONS: The findings presented here support the notion that each member of the MTA family might potentially play a stepwise role in a cell type-specific manner during breast cancer progression to metastasis. On the basis of the noted temporal expression patterns of MTA proteins with ER status, cell adhesion-essential regulators (E-cadherin and cytoplasmic beta-catenin), and coactivators, we propose that MTA protein-related chromatin remodeling pathways interact with steroid receptors, growth factor receptors, and other transcriptional signaling pathways to orchestrate the governing of events in breast cancer progression and metastasis.

Zhang, H., S. Yano, et al. (2003). "A novel bisphosphonate minodronate (YM529) specifically inhibits osteolytic bone metastasis produced by human small-cell lung cancer cells in NK-cell depleted SCID mice." Clin Exp Metastasis **20**(2): 153-9.

In the present study, we examined the effects of a newly developed bisphosphonate, minodronate (YM529), on osteolytic bone metastasis caused by lung cancer. Human small-cell lung cancer (SBC-5) cells, injected intravenously into natural killer cell-depleted SCID mice, produced radiologically detectable bone metastasis by day 18 and macroscopically visible visceral metastases (lung, liver, kidney, systemic lymph node) by day 35. Prophylactic treatment with YM529 on day 1 significantly inhibited the formation of osteolytic bone metastasis evaluated on X-ray photographs in a dose-dependent manner. In addition, treatment with YM529 after establishment of bone metastasis (on day 21) also inhibited bone metastasis, although the treatment was more effective when started earlier. Single administration was as effective as repeated treatment, suggesting a sustained inhibitory effect of YM529 on bone metastasis. YM529 reduced the number of osteoclasts in the bone metastatic lesions in vivo, but had no effect on the proliferation or cytokine production of SBC-5 cells in vitro. These results suggest that YM529 is a potent inhibitor of bone metastasis of human lung cancer, probably by suppressing osteoclastic bone resorption. In contrast, treatment with YM529 had no effect on visceral metastasis, even if started on day 1, and did not prolong the survival of the mice. Therefore, development of a combined modality is necessary for prolonging the survival of small-cell lung cancer patients with multiple-organ metastasis.

Zhao, S., K. Venkatasubbarao, et al. (2008). "Inhibition of STAT3 Tyr705 phosphorylation by Smad4 suppresses transforming growth factor beta-mediated invasion and metastasis in pancreatic cancer cells." Cancer Res **68**(11): 4221-8.

The role of Smad4 in transforming growth factor beta (TGFbeta)-mediated epithelial-mesenchymal transition (EMT), invasion, and metastasis was investigated using isogenically matched pancreatic cancer cell lines that differed only in expression of Smad4. Cells expressing Smad4 showed an enhanced TGFbeta-mediated EMT as determined by increased expression of vimentin and decreased expression of beta-catenin and E-cadherin. TGFbeta-mediated invasion was suppressed in Smad4-intact cells as determined by in vitro assays, and these cells showed a reduced metastasis in an orthotopic model of pancreatic cancer. Interestingly, TGFbeta inhibited STAT3(Tyr705) phosphorylation in Smad4-intact cells. The decrease in STAT3(Tyr705) phosphorylation was linked to a TGFbeta/Smad4-dependent and enhanced activation of extracellular signal-regulated kinases, which caused an increase in serine phosphorylation of STAT3(Ser727). Down-regulating signal transducer and activator of transcription 3 (STAT3) expression by short hairpin RNA in Smad4-deficient cells prevented TGFbeta-induced invasion. Conversely, expressing a constitutively activated form of STAT3 (STAT3-C) in Smad4-intact cells enhanced invasion. This study indicates the requirement of STAT3 activity for TGFbeta-induced invasion in pancreatic cancer cells and implicates Smad4-dependent signaling in regulating STAT3 activity. These findings further suggest that loss of Smad4, leading to aberrant activation of STAT3, contributes to the switch of TGFbeta from a tumor-suppressive to a tumor-promoting pathway in pancreatic cancer.

Zheng, J. (2008). "Is SATB1 a master regulator in breast cancer growth and metastasis?" Womens Health (Lond Engl) **4**(4): 329-32.

Evaluation of: Han HJ, Russo J, Kohwi Y et al.: SATB1 reprogrammes gene expression to promote breast tumor growth and metastasis. Nature 452(7184), 187-193 (2008). Metastasis is the most common cause of death in cancer patients. However, the genetic mechanisms involved in the master control genes of metastasis remain unclear. In this study, the authors found that special AT-rich sequence-binding protein 1 (SATB1) expression contributed to breast cancer growth and metastasis. SATB1 expression is detected in aggressive breast cancer cells rather than nonaggressive breast cancer cells. Moreover, by introducing the SATB1 gene into nonmetastatic breast cancer cells, invasive tumors can be induced in mice; whereas, silencing of SATB1 in metastatic cells not only abolishes metastasis and tumor growth in mice, but also returns cells to their normal appearance. These effects are related as SATB1 upregulates metastasis-associated genes while downregulating tumor-suppressor genes through epigenetic modification. The research suggests that SATB1 is a master regulator in the metastasis of breast cancer and, therefore, can be considered as an independent prognostic factor and a potential therapeutic target for breast cancer.

Zheng, R., S. Yano, et al. (2005). "CD9 overexpression suppressed the liver metastasis and malignant ascites via inhibition of proliferation and motility of small-cell lung cancer cells in NK cell-depleted SCID mice." Oncol Res **15**(7-8): 365-72.

CD9, a transmembrane protein known as motility-related protein-1, plays a pivotal role in regulating cell adhesion, motility, and proliferation, and has been regarded as an important metastasis-inhibitory factor of various human cancers. However, little information has been obtained regarding the highly metastatic human small-cell lung cancer (SCLC). In the present study, an SCLC cell line (OS3-R5), lacking CD9 expression, was transfected with human CD9 gene to assess the role of CD9 on the metastatic potential of SCLC. CD9 gene transfection into OS3-R5 cells resulted in cell proliferation and motility in vitro. Parental and mock-transfected OS3-R5 cells developed liver metastasis and malignant ascites when they were intravenously inoculated into NK cell-depleted SCID mice. CD9 gene transfection into OS3-R5 cells caused suppression of the liver metastasis and malignant ascites. Immunohistochemical analysis revealed that the number of proliferating tumor cells was significantly fewer in liver lesions produced by CD9 gene-transfected OS3-R5 cells than those produced by parental or mock control OS3-R5 cells. In addition, no detectable levels of CD9 were expressed in metastatic tumor cells in mice bearing CD9 gene-transfected OS3-R5 cells, as well as those in mice bearing parental or mock control OS3-R5 cells. These results suggest that the restored expression of CD9 in SCLC cells may reduce the metastatic spread of SCLC cells via the inhibition of cell proliferation and motility.

References

1. Baker, D. L., Y. Fujiwara, et al. (2006). "Carba analogs of cyclic phosphatidic acid are selective inhibitors of autotaxin and cancer cell invasion and metastasis." J Biol Chem **281**(32): 22786-93.
2. Bandyopadhyay, A., Y. Zhu, et al. (1999). "A soluble transforming growth factor beta type III receptor suppresses tumorigenicity and metastasis of human breast cancer MDA-MB-231 cells." Cancer Res **59**(19): 5041-6.
3. Bandyopadhyay, S., S. K. Pai, et al. (2004). "Role of the putative tumor metastasis suppressor gene Drg-1 in breast cancer progression." Oncogene **23**(33): 5675-81.
4. Bandyopadhyay, S., Y. Wang, et al. (2006). "The tumor metastasis suppressor gene Drg-1 down-regulates the expression of activating transcription factor 3 in prostate cancer." Cancer Res **66**(24): 11983-90.

5. Bulut, I., M. Meral, et al. (2009). "Analysis of HLA class I and II alleles regarding to lymph node and distant metastasis in patients with non-small cell lung cancer." Lung Cancer **66**(2): 231-6.
6. Burton, J. B., S. J. Priceman, et al. (2008). "Suppression of prostate cancer nodal and systemic metastasis by blockade of the lymphangiogenic axis." Cancer Res **68**(19): 7828-37.
7. Collectively, these results suggest that osteoclasts may promote bone metastasis of cancer cells expressing CCR4 in the bone marrow by producing its ligand CCL22.
8. Chen, H. W., J. Y. Lee, et al. (2008). "Curcumin inhibits lung cancer cell invasion and metastasis through the tumor suppressor HLJ1." Cancer Res **68**(18): 7428-38.
9. Chen, J. H., H. H. Lin, et al. (2008). "Gaseous nitrogen oxide promotes human lung cancer cell line A549 migration, invasion, and metastasis via iNOS-mediated MMP-2 production." Toxicol Sci **106**(2): 364-75.
10. Chen, J., P. M. Stavro, et al. (2002). "Dietary flaxseed inhibits human breast cancer growth and metastasis and downregulates expression of insulin-like growth factor and epidermal growth factor receptor." Nutr Cancer **43**(2): 187-92.
11. Chen, Y. J., W. M. Chang, et al. (2009). "A small-molecule metastasis inhibitor, norcantharidin, downregulates matrix metalloproteinase-9 expression by inhibiting Sp1 transcriptional activity in colorectal cancer cells." Chem Biol Interact **181**(3): 440-6.
12. Chien, C. W., S. C. Lin, et al. (2008). "Regulation of CD151 by hypoxia controls cell adhesion and metastasis in colorectal cancer." Clin Cancer Res **14**(24): 8043-51.
13. Chien, C. Y., C. Y. Su, et al. (2006). "High expressions of CD105 and VEGF in early oral cancer predict potential cervical metastasis." J Surg Oncol **94**(5): 413-7.
14. Choo, M. K., H. Sakurai, et al. (2006). "TAK1-mediated stress signaling pathways are essential for TNF-alpha-promoted pulmonary metastasis of murine colon cancer cells." Int J Cancer **118**(11): 2758-64.
15. Choo, M. K., H. Sakurai, et al. (2008). "A ginseng saponin metabolite suppresses tumor necrosis factor-alpha-promoted metastasis by suppressing nuclear factor-kappaB signaling in murine colon cancer cells." Oncol Rep **19**(3): 595-600.
16. Cicek, M. and M. J. Oursler (2006). "Breast cancer bone metastasis and current small therapeutics." Cancer Metastasis Rev **25**(4): 635-44.
17. Cicek, M., R. Fukuyama, et al. (2005). "Breast cancer metastasis suppressor 1 inhibits gene expression by targeting nuclear factor-kappaB activity." Cancer Res **65**(9): 3586-95.
18. Dabrosin, C., J. Chen, et al. (2002). "Flaxseed inhibits metastasis and decreases extracellular vascular endothelial growth factor in human breast cancer xenografts." Cancer Lett **185**(1): 31-7.
19. Dai, L., L. Gu, et al. (2009). "TWEAK promotes ovarian cancer cell metastasis via NF-kappaB pathway activation and VEGF expression." Cancer Lett **283**(2): 159-67.
20. Das Roy, L., L. B. Pathangey, et al. (2009). "Breast-cancer-associated metastasis is significantly increased in a model of autoimmune arthritis." Breast Cancer Res **11**(4): R56.
21. Dasgupta, S., M. Bhattacharya-Chatterjee, et al. (2006). "Tumor metastasis in an orthotopic murine model of head and neck cancer: possible role of TGF-beta 1 secreted by the tumor cells." J Cell Biochem **97**(5): 1036-51.
22. DiMeo, T. A., K. Anderson, et al. (2009). "A novel lung metastasis signature links Wnt signaling with cancer cell self-renewal and epithelial-mesenchymal transition in basal-like breast cancer." Cancer Res **69**(13): 5364-73.
23. Freudenberg, J. A., Q. Wang, et al. (2009). "The role of HER2 in early breast cancer metastasis and the origins of resistance to HER2-targeted therapies." Exp Mol Pathol **87**(1): 1-11.
24. Fu, H., Z. Hu, et al. (2009). "TGF-beta promotes invasion and metastasis of gastric cancer cells by increasing fascin1 expression via ERK and JNK signal pathways." Acta Biochim Biophys Sin (Shanghai) **41**(8): 648-56.
25. Fukunaga, S., K. Maeda, et al. (2006). "Association between expression of vascular endothelial growth factor C, chemokine receptor CXCR4 and lymph node metastasis in colorectal cancer." Oncology **71**(3-4): 204-11.
26. Guo, Y., A. P. Mazar, et al. (2002). "An antiangiogenic urokinase-derived peptide combined with tamoxifen decreases tumor growth and metastasis in a syngeneic model of breast cancer." Cancer Res **62**(16): 4678-84.
27. Halder, S. K., G. Rachakonda, et al. (2008). "Smad7 induces hepatic metastasis in colorectal cancer." Br J Cancer **99**(6): 957-65.
28. Havens, A. M., Y. Jung, et al. (2006). "The role of sialomucin CD164 (MGC-24v or endolyn) in prostate cancer metastasis." BMC Cancer **6**: 195.
29. Hayashi, S., I. Yokoyama, et al. (1999). "Inhibitory effect on the establishment of hepatic metastasis by transduction of the tissue plasminogen activator gene to murine colon cancer." Cancer Gene Ther **6**(4): 380-4.
30. Hazan, R. B., G. R. Phillips, et al. (2000). "Exogenous expression of N-cadherin in breast cancer cells induces cell migration, invasion, and metastasis." J Cell Biol **148**(4): 779-90.
31. He, B. P., J. J. Wang, et al. (2006). "Differential reactions of microglia to brain metastasis of lung cancer." Mol Med **12**(7-8): 161-70.
32. Helbig, G., K. W. Christopherson, 2nd, et al. (2003). "NF-kappaB promotes breast cancer cell migration and metastasis by inducing the expression of the chemokine receptor CXCR4." J Biol Chem **278**(24): 21631-8.
33. Hinton, C. V., S. Avraham, et al. (2008). "Contributions of integrin-linked kinase to breast cancer metastasis and tumourigenesis." J Cell Mol Med **12**(5A): 1517-26.
34. Holmes, C. E., J. E. Levis, et al. (2009). "Activated platelets enhance ovarian cancer cell invasion in a cellular model of metastasis." Clin Exp Metastasis **26**(7): 653-61.
35. Horiguchi, T., S. Tachikawa, et al. (2000). "Usefulness of serum carboxy-terminal telopeptide of type I collagen (ICTP) as a marker of bone metastasis from lung cancer." Jpn J Clin Oncol **30**(4): 174-9.
36. Hsu, P. I., H. L. Hsieh, et al. (2009). "Loss of RUNX3 expression correlates with differentiation, nodal metastasis, and poor prognosis of gastric cancer." Ann Surg Oncol **16**(6): 1686-94.
37. Huang, W. C., D. Wu, et al. (2006). "beta2-microglobulin is a signaling and growth-promoting factor for human prostate cancer bone metastasis." Cancer Res **66**(18): 9108-16.
38. Ichiki, K., N. Mitani, et al. (2000). "Regulation of activator protein-1 activity in the mediastinal lymph node metastasis of lung cancer." Clin Exp Metastasis **18**(7): 539-45.
39. Ide, H., K. Hatake, et al. (2008). "Serum level of macrophage colony-stimulating factor is increased in prostate cancer patients with bone metastasis." Hum Cell **21**(1): 1-6.
40. Ikeguchi, M., T. Taniguchi, et al. (2000). "Reduced E-cadherin expression and enlargement of cancer nuclei strongly correlate with hematogenic metastasis in colorectal adenocarcinoma." Scand J Gastroenterol **35**(8): 839-46.
41. Ilan, N., M. Elkin, et al. (2006). "Regulation, function and clinical significance of heparanase in cancer metastasis and angiogenesis." Int J Biochem Cell Biol **38**(12): 2018-39.
42. Inoue, M., S. Matsumoto, et al. (2008). "Intraperitoneal administration of a small interfering RNA targeting nuclear factor-kappa B with paclitaxel successfully prolongs the survival of xenograft model mice with peritoneal metastasis of gastric cancer." Int J Cancer **123**(11): 2696-701.
43. Inoue, M., T. Sawada, et al. (2005). "Plasminogen activator inhibitor-1 (PAI-1) gene transfection inhibits the liver

metastasis of pancreatic cancer by preventing angiogenesis." Oncol Rep **14**(6): 1445-51.
44. Iwasaki, T., K. Yamashita, et al. (2002). "Interleukin-18 inhibits osteolytic bone metastasis by human lung cancer cells possibly through suppression of osteoclastic bone-resorption in nude mice." J Immunother **25 Suppl 1**: S52-60.
45. Iwasaki, T., M. Mukai, et al. (2002). "Ipriflavone inhibits osteolytic bone metastasis of human breast cancer cells in a nude mouse model." Int J Cancer **100**(4): 381-7.
46. Jackson, P., M. O. Grimm, et al. (2002). "Relationship between expression of KAI1 metastasis suppressor gene, mRNA levels and p53 in human bladder and prostate cancer cell lines." Urol Oncol **7**(3): 99-104.
47. Jallal, H., M. L. Valentino, et al. (2007). "A Src/Abl kinase inhibitor, SKI-606, blocks breast cancer invasion, growth, and metastasis in vitro and in vivo." Cancer Res **67**(4): 1580-8.
48. Janowska-Wieczorek, A., M. Wysoczynski, et al. (2005). "Microvesicles derived from activated platelets induce metastasis and angiogenesis in lung cancer." Int J Cancer **113**(5): 752-60.
49. Joo, Y. H., C. K. Jung, et al. (2009). "Relationship between vascular endothelial growth factor and Notch1 expression and lymphatic metastasis in tongue cancer." Otolaryngol Head Neck Surg **140**(4): 512-8.
50. Kamata, I., Y. Ishikawa, et al. (2009). "Significance of lymphatic invasion and cancer invasion-related proteins on lymph node metastasis in gastric cancer." J Gastroenterol Hepatol **24**(9): 1527-33.
51. Kanao, H., T. Enomoto, et al. (2005). "Overexpression of LAMP3/TSC403/DC-LAMP promotes metastasis in uterine cervical cancer." Cancer Res **65**(19): 8640-5.
52. Kang, H., G. Watkins, et al. (2005). "The elevated level of CXCR4 is correlated with nodal metastasis of human breast cancer." Breast **14**(5): 360-7.
53. Kang, J. S., S. Y. Bae, et al. (2009). "Interleukin-18 increases metastasis and immune escape of stomach cancer via the downregulation of CD70 and maintenance of CD44." Carcinogenesis **30**(12): 1987-96.
54. Katsman, A., K. Umezawa, et al. (2009). "Chemosensitization and immunosensitization of resistant cancer cells to apoptosis and inhibition of metastasis by the specific NF-kappaB inhibitor DHMEQ." Curr Pharm Des **15**(7): 792-808.
55. Katsuta, M., M. Miyashita, et al. (2005). "Correlation of hypoxia inducible factor-1alpha with lymphatic metastasis via vascular endothelial growth factor-C in human esophageal cancer." Exp Mol Pathol **78**(2): 123-30.
56. Kazama, S., T. Watanabe, et al. (2006). "Tumour budding at the deepest invasive margin correlates with lymph node metastasis in submucosal colorectal cancer detected by anticytokeratin antibody CAM5.2." Br J Cancer **94**(2): 293-8.
57. Kobayashi, D., M. Yamada, et al. (2002). "Overexpression of early growth response-1 as a metastasis-regulatory factor in gastric cancer." Anticancer Res **22**(6C): 3963-70.
58. Kobayashi, H., M. Suzuki, et al. (2004). "Genetic down-regulation of phosphoinositide 3-kinase by bikunin correlates with suppression of invasion and metastasis in human ovarian cancer HRA cells." J Biol Chem **279**(8): 6371-9.
59. Kodama, J., Hasengaowa, et al. (2007). "Association of CXCR4 and CCR7 chemokine receptor expression and lymph node metastasis in human cervical cancer." Ann Oncol **18**(1): 70-6.
60. Koide, N., H. Watanabe, et al. (2000). "Four resections for hepatic metastasis from gastric cancer: histochemical analysis of cell proliferation, apoptosis, and angiogenesis." J Gastroenterol **35**(2): 150-4.
61. Kondo, Y., S. Arii, et al. (2000). "Enhancement of angiogenesis, tumor growth, and metastasis by transfection of vascular endothelial growth factor into LoVo human colon cancer cell line." Clin Cancer Res **6**(2): 622-30.
62. Kozlow, W. and T. A. Guise (2005). "Breast cancer metastasis to bone: mechanisms of osteolysis and implications for therapy." J Mammary Gland Biol Neoplasia **10**(2): 169-80.
63. Kucia, M., R. Reca, et al. (2005). "Trafficking of normal stem cells and metastasis of cancer stem cells involve similar mechanisms: pivotal role of the SDF-1-CXCR4 axis." Stem Cells **23**(7): 879-94.
64. Kudo-Saito, C., H. Shirako, et al. (2009). "Cancer metastasis is accelerated through immunosuppression during Snail-induced EMT of cancer cells." Cancer Cell **15**(3): 195-206.
65. Kurahara, H., S. Takao, et al. (2004). "Impact of vascular endothelial growth factor-C and -D expression in human pancreatic cancer: its relationship to lymph node metastasis." Clin Cancer Res **10**(24): 8413-20.
66. Liao, D., C. Corle, et al. (2007). "Hypoxia-inducible factor-1alpha is a key regulator of metastasis in a transgenic model of cancer initiation and progression." Cancer Res **67**(2): 563-72.
67. Liao, J., A. Schneider, et al. (2006). "Extracellular calcium as a candidate mediator of prostate cancer skeletal metastasis." Cancer Res **66**(18): 9065-73.
68. Lin, B. R., C. C. Chang, et al. (2005). "Connective tissue growth factor inhibits metastasis and acts as an independent prognostic marker in colorectal cancer." Gastroenterology **128**(1): 9-23.
69. Lin, Y., P. J. Buckhaults, et al. (2009). "Association of the actin-binding protein transgelin with lymph node metastasis in human colorectal cancer." Neoplasia **11**(9): 864-73.
70. Liu, J., M. Ikeguchi, et al. (2002). "Re-expression of the cadherin-catenin complex in lymph nodes with metastasis in advanced gastric cancer: the relationship with patient survival." J Exp Clin Cancer Res **21**(1): 65-71.
71. Loges, S., H. Clausen, et al. (2007). "Determination of microvessel density by quantitative real-time PCR in esophageal cancer: correlation with histologic methods, angiogenic growth factor expression, and lymph node metastasis." Clin Cancer Res **13**(1): 76-80.
72. Luan, F. L., R. Ding, et al. (2003). "Rapamycin is an effective inhibitor of human renal cancer metastasis." Kidney Int **63**(3): 917-26.
73. Luo, J. L., W. Tan, et al. (2007). "Nuclear cytokine-activated IKKalpha controls prostate cancer metastasis by repressing Maspin." Nature **446**(7136): 690-4.
74. Malik, F. A., A. J. Sanders, et al. (2009). "KAI-1/CD82, the molecule and clinical implication in cancer and cancer metastasis." Histol Histopathol **24**(4): 519-30.
75. Maruyama, Y., M. Ono, et al. (2006). "Tumor growth suppression in pancreatic cancer by a putative metastasis suppressor gene Cap43/NDRG1/Drg-1 through modulation of angiogenesis." Cancer Res **66**(12): 6233-42.
76. Matsumori, Y., S. Yano, et al. (2006). "ZD6474, an inhibitor of vascular endothelial growth factor receptor tyrosine kinase, inhibits growth of experimental lung metastasis and production of malignant pleural effusions in a non-small cell lung cancer model." Oncol Res **16**(1): 15-26.
77. Matsusue, R., H. Kubo, et al. (2009). "Hepatic stellate cells promote liver metastasis of colon cancer cells by the action of SDF-1/CXCR4 axis." Ann Surg Oncol **16**(9): 2645-53.
78. Mehrotra, J., M. Vali, et al. (2004). "Very high frequency of hypermethylated genes in breast cancer metastasis to the bone, brain, and lung." Clin Cancer Res **10**(9): 3104-9.
79. Missiaglia, E., E. Blaveri, et al. (2004). "Analysis of gene expression in cancer cell lines identifies candidate markers for pancreatic tumorigenesis and metastasis." Int J Cancer **112**(1): 100-12.
80. Mochizuki, H., A. Matsubara, et al. (2004). "Interaction of ligand-receptor system between stromal-cell-derived factor-1

and CXC chemokine receptor 4 in human prostate cancer: a possible predictor of metastasis." Biochem Biophys Res Commun **320**(3): 656-63.
81. Montel, V., A. Gaultier, et al. (2007). "The low-density lipoprotein receptor-related protein regulates cancer cell survival and metastasis development." Cancer Res **67**(20): 9817-24.
82. Moreau, J. E., K. Anderson, et al. (2007). "Tissue-engineered bone serves as a target for metastasis of human breast cancer in a mouse model." Cancer Res **67**(21): 10304-8.
83. Motoyama, S., M. Miura, et al. (2009). "CRP genetic polymorphism is associated with lymph node metastasis in thoracic esophageal squamous cell cancer." Ann Surg Oncol **16**(9): 2479-85.
84. Muguruma, H., S. Yano, et al. (2005). "Reveromycin A inhibits osteolytic bone metastasis of small-cell lung cancer cells, SBC-5, through an antiosteoclastic activity." Clin Cancer Res **11**(24 Pt 1): 8822-8.
85. Muller-Tidow, C., S. Diederichs, et al. (2005). "Identification of metastasis-associated receptor tyrosine kinases in non-small cell lung cancer." Cancer Res **65**(5): 1778-82.
86. Na, I. K., C. Scheibenbogen, et al. (2008). "Nuclear expression of CXCR4 in tumor cells of non-small cell lung cancer is correlated with lymph node metastasis." Hum Pathol **39**(12): 1751-5.
87. Nagatsuka, I., N. Yamada, et al. (2002). "Inhibitory effect of a selective cyclooxygenase-2 inhibitor on liver metastasis of colon cancer." Int J Cancer **100**(5): 515-9.
88. Nakamura, E. S., K. Koizumi, et al. (2006). "RANKL-induced CCL22/macrophage-derived chemokine produced from osteoclasts potentially promotes the bone metastasis of lung cancer expressing its receptor CCR4." Clin Exp Metastasis **23**(1): 9-18.
89. Narla, G., A. DiFeo, et al. (2008). "KLF6-SV1 overexpression accelerates human and mouse prostate cancer progression and metastasis." J Clin Invest **118**(8): 2711-21.
90. Nicolson, G. L., A. Nawa, et al. (2003). "Tumor metastasis-associated human MTA1 gene and its MTA1 protein product: role in epithelial cancer cell invasion, proliferation and nuclear regulation." Clin Exp Metastasis **20**(1): 19-24.
91. Ninomiya, S., M. Inomata, et al. (2009). "Effect of bevacizumab, a humanized monoclonal antibody to vascular endothelial growth factor, on peritoneal metastasis of MNK-45P human gastric cancer in mice." J Surg Res **154**(2): 196-202.
92. Nishimori, H., T. Yasoshima, et al. (2000). "A novel experimental mouse model of peritoneal dissemination of human gastric cancer cells: different mechanisms in peritoneal dissemination and hematogenous metastasis." Jpn J Cancer Res **91**(7): 715-22.
93. Nishimori, H., T. Yasoshima, et al. (2002). "A novel nude mouse model of liver metastasis and peritoneal dissemination from the same human pancreatic cancer line." Pancreas **24**(3): 242-50.
94. Nogi, H., H. Takeyama, et al. (2003). "Detection of MUC1 and keratin 19 mRNAs in the bone marrow by quantitative RT-PCR predicts the risk of distant metastasis in breast cancer patients." Breast Cancer **10**(1): 74-81.
95. Ohashi, S., S. Okamura, et al. (2007). "Clinicopathological variables associated with lymph node metastasis in submucosal invasive gastric cancer." Gastric Cancer **10**(4): 241-50.
96. Ohta, Y., Z. Nozaki, et al. (2001). "The predictive value of vascular endothelial growth factor and nm23 for the diagnosis of occult metastasis in non-small cell lung cancer." Jpn J Cancer Res **92**(3): 361-6.
97. Ojima, H., S. Sasaki, et al. (2003). "Utility of serosal stamp cytology as an indicator for high-risk peritoneal metastasis in colorectal cancer surgery." Hepatogastroenterology **50**(49): 87-90.
98. Okayama, T., S. Kokura, et al. (2009). "Antitumor effect of pretreatment for colon cancer cells with hyperthermia plus geranylgeranylacetone in experimental metastasis models and a subcutaneous tumor model of colon cancer in mice." Int J Hyperthermia **25**(2): 141-9.
99. Okuyama, N., A. Matsumine, et al. (2008). "Matrix metalloproteinase-1 is a crucial bone metastasis factor in a human breast cancer-derived highly invasive cell line." Oncol Rep **20**(6): 1497-504.
100. Otsuka, S., M. Hanibuchi, et al. (2009). "A bone metastasis model with osteolytic and osteoblastic properties of human lung cancer ACC-LC-319/bone2 in natural killer cell-depleted severe combined immunodeficient mice." Oncol Res **17**(11-12): 581-91.
101. Park, H. R., S. K. Min, et al. (2003). "Expression of osteoprotegerin and RANK ligand in breast cancer bone metastasis." J Korean Med Sci **18**(4): 541-6.
102. Park, S., A. J. Holmes-Tisch, et al. (2009). "Discordance of molecular biomarkers associated with epidermal growth factor receptor pathway between primary tumors and lymph node metastasis in non-small cell lung cancer." J Thorac Oncol **4**(7): 809-15.
103. Preet, A., R. K. Ganju, et al. (2008). "Delta9-Tetrahydrocannabinol inhibits epithelial growth factor-induced lung cancer cell migration in vitro as well as its growth and metastasis in vivo." Oncogene **27**(3): 339-46.
104. Qian, J., A. Dong, et al. (2007). "Suppression of type 1 Insulin-like growth factor receptor expression by small interfering RNA inhibits A549 human lung cancer cell invasion in vitro and metastasis in xenograft nude mice." Acta Biochim Biophys Sin (Shanghai) **39**(2): 137-47.
105. Qin, L., L. Liao, et al. (2008). "The AIB1 oncogene promotes breast cancer metastasis by activation of PEA3-mediated matrix metalloproteinase 2 (MMP2) and MMP9 expression." Mol Cell Biol **28**(19): 5937-50.
106. Que, H. F., H. F. Chen, et al. (2008). "Effect of runing II on the growth and metastasis of transplanted tumor in mammary cancer-bearing mice and its mechanism." J Tradit Chin Med **28**(4): 293-8.
107. Rahman, K. M., F. H. Sarkar, et al. (2006). "Therapeutic intervention of experimental breast cancer bone metastasis by indole-3-carbinol in SCID-human mouse model." Mol Cancer Ther **5**(11): 2747-56.
108. Rha, S. Y., S. H. Noh, et al. (1999). "Modulation of biological phenotypes for tumor growth and metastasis by target-specific biological inhibitors in gastric cancer." Int J Mol Med **4**(2): 203-12.
109. Rowland-Goldsmith, M. A., H. Maruyama, et al. (2002). "Soluble type II transforming growth factor-beta receptor attenuates expression of metastasis-associated genes and suppresses pancreatic cancer cell metastasis." Mol Cancer Ther **1**(3): 161-7.
110. Sachdev, D., J. S. Hartell, et al. (2004). "A dominant negative type I insulin-like growth factor receptor inhibits metastasis of human cancer cells." J Biol Chem **279**(6): 5017-24.
111. Sakao, Y., H. Miyamoto, et al. (2006). "Prognostic significance of metastasis to the highest mediastinal lymph node in nonsmall cell lung cancer." Ann Thorac Surg **81**(1): 292-7.
112. Satcher, R. L., Jr., K. Dvorkin, et al. (2004). "Gene expression in cancer cells is influenced by contact with bone cells in a novel coculture system that models bone metastasis." Clin Orthop Relat Res(426): 54-63.
113. Sato, F., Y. Shimada, et al. (1999). "Expression of vascular endothelial growth factor, matrix metalloproteinase-9 and E-cadherin in the process of lymph node metastasis in oesophageal cancer." Br J Cancer **80**(9): 1366-72.
114. Sawada, T., K. Kimura, et al. (2006). "TGF-beta1 down-regulates ICAM-1 expression and enhances liver metastasis of pancreatic cancer." Adv Med Sci **51**: 60-5.

115. Shibata, M. A., J. Morimoto, et al. (2008). "Combination therapy with short interfering RNA vectors against VEGF-C and VEGF-A suppresses lymph node and lung metastasis in a mouse immunocompetent mammary cancer model." Cancer Gene Ther **15**(12): 776-86.
116. Shimizu, K., H. Kubo, et al. (2004). "Suppression of VEGFR-3 signaling inhibits lymph node metastasis in gastric cancer." Cancer Sci **95**(4): 328-33.
117. Shimo, T., S. Kubota, et al. (2006). "Pathogenic role of connective tissue growth factor (CTGF/CCN2) in osteolytic metastasis of breast cancer." J Bone Miner Res **21**(7): 1045-59.
118. Shinohara, T., T. Miki, et al. (2001). "Nuclear factor-kappaB-dependent expression of metastasis suppressor KAI1/CD82 gene in lung cancer cell lines expressing mutant p53." Cancer Res **61**(2): 673-8.
119. Shintani, S., C. Li, et al. (2003). "Gefitinib ('Iressa'), an epidermal growth factor receptor tyrosine kinase inhibitor, mediates the inhibition of lymph node metastasis in oral cancer cells." Cancer Lett **201**(2): 149-55.
120. Shintani, S., T. Ishikawa, et al. (2004). "Growth-regulated oncogene-1 expression is associated with angiogenesis and lymph node metastasis in human oral cancer." Oncology **66**(4): 316-22.
121. Shintani, Y., S. Higashiyama, et al. (2004). "Overexpression of ADAM9 in non-small cell lung cancer correlates with brain metastasis." Cancer Res **64**(12): 4190-6.
122. Singh, D., D. D. Joshi, et al. (2000). "Increased expression of preprotachykinin-I and neurokinin receptors in human breast cancer cells: implications for bone marrow metastasis." Proc Natl Acad Sci U S A **97**(1): 388-93.
123. Singh, L. S., M. Berk, et al. (2007). "Ovarian cancer G protein-coupled receptor 1, a new metastasis suppressor gene in prostate cancer." J Natl Cancer Inst **99**(17): 1313-27.
124. Stathopoulos, G. T., T. P. Sherrill, et al. (2008). "Host nuclear factor-kappaB activation potentiates lung cancer metastasis." Mol Cancer Res **6**(3): 364-71.
125. Stathopoulos, G. T., T. P. Sherrill, et al. (2008). "Use of bioluminescent imaging to investigate the role of nuclear factor-kappaBeta in experimental non-small cell lung cancer metastasis." Clin Exp Metastasis **25**(1): 43-51.
126. Su, J. L., P. C. Yang, et al. (2006). "The VEGF-C/Flt-4 axis promotes invasion and metastasis of cancer cells." Cancer Cell **9**(3): 209-23.
127. Suarez-Cuervo, C., M. A. Merrell, et al. (2004). "Breast cancer cells with inhibition of p38alpha have decreased MMP-9 activity and exhibit decreased bone metastasis in mice." Clin Exp Metastasis **21**(6): 525-33.
128. Sulzer, M. A., M. P. Leers, et al. (1998). "Reduced E-cadherin expression is associated with increased lymph node metastasis and unfavorable prognosis in non-small cell lung cancer." Am J Respir Crit Care Med **157**(4 Pt 1): 1319-23.
129. Sumiyoshi, Y., Y. Yamashita, et al. (2000). "Expression of CD44, vascular endothelial growth factor, and proliferating cell nuclear antigen in severe venous invasional colorectal cancer and its relationship to liver metastasis." Surg Today **30**(4): 323-7.
130. Sun, Y. X., E. A. Pedersen, et al. (2008). "CD26/dipeptidyl peptidase IV regulates prostate cancer metastasis by degrading SDF-1/CXCL12." Clin Exp Metastasis **25**(7): 765-76.
131. Sweeney, C. J., S. Mehrotra, et al. (2005). "The sesquiterpene lactone parthenolide in combination with docetaxel reduces metastasis and improves survival in a xenograft model of breast cancer." Mol Cancer Ther **4**(6): 1004-12.
132. Tahir, S. A., G. Yang, et al. (2001). "Secreted caveolin-1 stimulates cell survival/clonal growth and contributes to metastasis in androgen-insensitive prostate cancer." Cancer Res **61**(10): 3882-5.
133. Takanami, I. (2006). "Lymphatic microvessel density using D2-40 is associated with nodal metastasis in non-small cell lung cancer." Oncol Rep **15**(2): 437-42.
134. Takayama, T., K. Miyanishi, et al. (2006). "Colorectal cancer: genetics of development and metastasis." J Gastroenterol **41**(3): 185-92.
135. Takizawa, H., K. Kondo, et al. (2006). "The balance of VEGF-C and VEGFR-3 mRNA is a predictor of lymph node metastasis in non-small cell lung cancer." Br J Cancer **95**(1): 75-9.
136. Tamura, M., M. Oda, et al. (2004). "Chest CT and serum vascular endothelial growth factor-C level to diagnose lymph node metastasis in patients with primary non-small cell lung cancer." Chest **126**(2): 342-6.
137. Tamura, M., M. Oda, et al. (2004). "The combination assay with circulating vascular endothelial growth factor (VEGF)-C, matrix metalloproteinase-9, and VEGF for diagnosing lymph node metastasis in patients with non-small cell lung cancer." Ann Surg Oncol **11**(10): 928-33.
138. Tanaka, S., S. C. Pero, et al. (2006). "Specific peptide ligand for Grb7 signal transduction protein and pancreatic cancer metastasis." J Natl Cancer Inst **98**(7): 491-8.
139. Tanaka, Y., H. Kobayashi, et al. (2004). "Genetic downregulation of pregnancy-associated plasma protein-A (PAPP-A) by bikunin reduces IGF-I-dependent Akt and ERK1/2 activation and subsequently reduces ovarian cancer cell growth, invasion and metastasis." Int J Cancer **109**(3): 336-47.
140. Taylor, A. P. and D. M. Goldenberg (2007). "Role of placenta growth factor in malignancy and evidence that an antagonistic PlGF/Flt-1 peptide inhibits the growth and metastasis of human breast cancer xenografts." Mol Cancer Ther **6**(2): 524-31.
141. Tokunaga, T., M. Nakamura, et al. (1999). "Thrombospondin 2 expression is correlated with inhibition of angiogenesis and metastasis of colon cancer." Br J Cancer **79**(2): 354-9.
142. Tokunaga, T., Y. Abe, et al. (2002). "Ribozyme mediated cleavage of cell-associated isoform of vascular endothelial growth factor inhibits liver metastasis of a pancreatic cancer cell line." Int J Oncol **21**(5): 1027-32.
143. Tsuruga, T., S. Nakagawa, et al. (2007). "Loss of Hugl-1 expression associates with lymph node metastasis in endometrial cancer." Oncol Res **16**(9): 431-5.
144. Tsutsumi, S., T. Yanagawa, et al. (2004). "Autocrine motility factor signaling enhances pancreatic cancer metastasis." Clin Cancer Res **10**(22): 7775-84.
145. Uchima, Y., T. Sawada, et al. (2003). "Identification of a trypsinogen activity stimulating factor produced by pancreatic cancer cells: its role in tumor invasion and metastasis." Int J Mol Med **12**(6): 871-8.
146. Versteeg, H. H., C. A. Spek, et al. (2004). "Tissue factor and cancer metastasis: the role of intracellular and extracellular signaling pathways." Mol Med **10**(1-6): 6-11.
147. Von Marschall, Z., A. Scholz, et al. (2005). "Vascular endothelial growth factor-D induces lymphangiogenesis and lymphatic metastasis in models of ductal pancreatic cancer." Int J Oncol **27**(3): 669-79.
148. Wang, J., Y. Cai, et al. (2006). "Increased expression of the metastasis-associated gene Ehm2 in prostate cancer." Prostate **66**(15): 1641-52.
149. Wang, W. B., S. Boing, et al. (2002). "Identification of metastasis-associated genes in early stage non-small cell lung cancer by subtractive hybridization." Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao (Shanghai) **34**(3): 273-8.
150. Wegiel, B., A. Bjartell, et al. (2008). "Multiple cellular mechanisms related to cyclin A1 in prostate cancer invasion and metastasis." J Natl Cancer Inst **100**(14): 1022-36.
151. Wen, J., K. Matsumoto, et al. (2004). "Hepatic gene expression of NK4, an HGF-antagonist/angiogenesis

inhibitor, suppresses liver metastasis and invasive growth of colon cancer in mice." Cancer Gene Ther **11**(6): 419-30.
152. Wen, J., K. Matsumoto, et al. (2007). "Inhibition of colon cancer growth and metastasis by NK4 gene repetitive delivery in mice." Biochem Biophys Res Commun **358**(1): 117-23.
153. Whang, P. G., E. M. Schwarz, et al. (2005). "The effects of RANK blockade and osteoclast depletion in a model of pure osteoblastic prostate cancer metastasis in bone." J Orthop Res **23**(6): 1475-83.
154. Wiesner, C., S. M. Nabha, et al. (2008). "C-kit and its ligand stem cell factor: potential contribution to prostate cancer bone metastasis." Neoplasia **10**(9): 996-1003.
155. Wu, D., H. E. Zhau, et al. (2007). "cAMP-responsive element-binding protein regulates vascular endothelial growth factor expression: implication in human prostate cancer bone metastasis." Oncogene **26**(35): 5070-7.
156. Xue, C., F. Liang, et al. (2006). "ErbB3-dependent motility and intravasation in breast cancer metastasis." Cancer Res **66**(3): 1418-26.
157. Yamamoto, M., H. Kikuchi, et al. (2008). "TSU68 prevents liver metastasis of colon cancer xenografts by modulating the premetastatic niche." Cancer Res **68**(23): 9754-62.
158. Yamashita, H., J. Kitayama, et al. (2007). "Tissue factor expression is a clinical indicator of lymphatic metastasis and poor prognosis in gastric cancer with intestinal phenotype." J Surg Oncol **95**(4): 324-31.
159. Yamauchi, T., M. Watanabe, et al. (2003). "The potential for a selective cyclooxygenase-2 inhibitor in the prevention of liver metastasis in human colorectal cancer." Anticancer Res **23**(1A): 245-9.
160. Yano, S., H. Nokihara, et al. (2003). "Multifunctional interleukin-1beta promotes metastasis of human lung cancer cells in SCID mice via enhanced expression of adhesion-, invasion- and angiogenesis-related molecules." Cancer Sci **94**(3): 244-52.
161. Yasuoka, H., M. Tsujimoto, et al. (2008). "Cytoplasmic CXCR4 expression in breast cancer: induction by nitric oxide and correlation with lymph node metastasis and poor prognosis." BMC Cancer **8**: 340.
162. Yeh, K. Y., J. W. Chang, et al. (2003). "Ovarian metastasis originating from bronchioloalveolar carcinoma: a rare presentation of lung cancer." Jpn J Clin Oncol **33**(8): 404-7.
163. Yi, B., P. J. Williams, et al. (2002). "Tumor-derived platelet-derived growth factor-BB plays a critical role in osteosclerotic bone metastasis in an animal model of human breast cancer." Cancer Res **62**(3): 917-23.
164. Yokoi, H., M. Nakata, et al. (2001). "Intraglomerular metastasis from pancreatic cancer." Am J Kidney Dis **37**(6): 1299-303.
165. Yonemura, Y., Y. Endo, et al. (1999). "Role of vascular endothelial growth factor C expression in the development of lymph node metastasis in gastric cancer." Clin Cancer Res **5**(7): 1823-9.
166. Yoshikawa, R., H. Yanagi, et al. (2006). "ECA39 is a novel distant metastasis-related biomarker in colorectal cancer." World J Gastroenterol **12**(36): 5884-9.
167. Yoshitake, N., H. Fukui, et al. (2008). "Expression of SDF-1 alpha and nuclear CXCR4 predicts lymph node metastasis in colorectal cancer." Br J Cancer **98**(10): 1682-9.
168. Zhang, G., B. He, et al. (2003). "Growth factor signaling induces metastasis genes in transformed cells: molecular connection between Akt kinase and osteopontin in breast cancer." Mol Cell Biol **23**(18): 6507-19.
169. Zhang, H., L. C. Stephens, et al. (2006). "Metastasis tumor antigen family proteins during breast cancer progression and metastasis in a reliable mouse model for human breast cancer." Clin Cancer Res **12**(5): 1479-86.
170. Zhang, H., S. Yano, et al. (2003). "A novel bisphosphonate minodronate (YM529) specifically inhibits osteolytic bone metastasis produced by human small-cell lung cancer cells in NK-cell depleted SCID mice." Clin Exp Metastasis **20**(2): 153-9.
171. Zhao, S., K. Venkatasubbarao, et al. (2008). "Inhibition of STAT3 Tyr705 phosphorylation by Smad4 suppresses transforming growth factor beta-mediated invasion and metastasis in pancreatic cancer cells." Cancer Res **68**(11): 4221-8.
172. Zheng, J. (2008). "Is SATB1 a master regulator in breast cancer growth and metastasis?" Womens Health (Lond Engl) **4**(4): 329-32.
173. Zheng, R., S. Yano, et al. (2005). "CD9 overexpression suppressed the liver metastasis and malignant ascites via inhibition of proliferation and motility of small-cell lung cancer cells in NK cell-depleted SCID mice." Oncol Res **15**(7-8): 365-72.
174. PubMed (2012). http://www.ncbi.nlm.nih.gov/pubmed.
175. Cancer. Wikipedia. (2012) http://en.wikipedia.org/wiki/Cancer.

8/9/2012