Bone Cancer Literatures

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Abstract: Cancer is the cells that grow out of control. Cancer cells can also invade other tissues. Growing out of control and invading other tissues are what makes a cell a cancer cell. Involved in more than 100 diseases, cancers can cause serious illness and death. Normally, the cells become cancer cells because of DNA damage. This material is a literature collection of the researches on the cancer and the bone.
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1. Introduction

Cancer is the general name for a group of more than 100 diseases. Although there are many kinds of cancer, all cancers start because abnormal cells grow out of control. Untreated cancers can cause serious illness and death. The body is made up of trillions of living cells. Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace worn-out or dying cells or to repair injuries.

Literatures

Adwan, H., T. J. Bauerle, et al. (2004). "Downregulation of osteopontin and bone sialoprotein II is related to reduced colony formation and metastasis formation of MDA-MB-231 human breast cancer cells." Cancer Gene Ther **11**(2): 109-20.

Osteopontin (OPN), bone sialoprotein (BSPII), and osteonectin (ON) belong to a family of glycoproteins, which have been linked to cancer metastasis and progression. Here, we report on the selection of antisense oligonucleotides (ASOs), which are effective in reducing their protein levels. In human MDA-MB-231 breast cancer cells, the maximum inhibition of protein expression ranged from 84% (OPN) to 75% (BSPII) and 70% (ON). Erucylphospho-NNN-trimethylpropanolamine (ErPC3) was used as positive control and combination partner. Exposure to ErPC3 inhibited colony formation of MDA-MB-231 cells by 11% (10 microM), 45% (14 microM) and 78% (20 microM). The clonogenicity of breast cancer cells was reduced by 15%, 11%, 8% (5 microM), 39%, 19%, 14% (10 microM) and 46%, 39%, 21% (20 microM) in response to ASO-OPN-04, ASO-BSPII-06 and ASO-ON-03, respectively. Combination of ErPC3 with the ASOs caused additive combination effects. Pre-exposure to the ASOs, but not to the NSO, inhibited formation of osteolytic metastasis in three of four (ASO-OPN-04, P<0.03) and two of four (ASO-BSPII-06) nude rats, and reduced metastasis lesions significantly (T/C%=4.3 and 9.1, P=0.05, respectively). We conclude that downregulation of OPN and BSPII reduces colony formation of MDA-MB-231 cells and formation of osteolytic metastasis in nude rats.

Akhtari, M., J. Mansuri, et al. (2008). "Biology of breast cancer bone metastasis." Cancer Biol Ther **7**(1): 3-9.

Breast carcinoma ranks among the most prevalent malignancies in women. Breast carcinoma frequently metastasizes to bone and approximately 70% of patients with breast cancer have bone metastases, which generally are osteolytic lesions. They cause major morbidity and mortality in patients; and the available treatment options are limited. Bone-specific homing and colonization of cancer cells are important and interesting features of metastasis. There are complex and multiple steps in the process of bone metastasis; and the elaborate interaction between breast carcinoma and bone involves various cytokines, growth factors and cellular signals, which results in a vicious cycle and promotes tumor cell accumulation and osteolysis. Recent advances in molecular biology have resulted in major breakthroughs in our understanding of the pathogenesis of bone metastasis in breast cancer, which is critical in preventing metastasis, designing novel and targeted treatments and prolonging survival in this devastating condition.

Bagi, C. M. (2005). "Targeting of therapeutic agents to bone to treat metastatic cancer." Adv Drug Deliv Rev **57**(7): 995-1010.

The three main organs affected by metastasis of all cancers include lungs, liver, and bone. Clinical confirmation of tumor spread to these organs is a negative prognostic sign that marks the stage when

disease is rarely curable. Today, treatment of bone metastases is primarily palliative. The aims of treatment are to relieve pain, prevent development of pathologic fractures, improve mobility and function, and if possible, prolong survival. Significant improvements in our understanding of tumor biology along with early tumor detection has led to the discovery of few innovative approaches aimed to treat bone metastases. The most promising treatment modalities include combination of anti-cancer therapies (surgery, radiation therapy, citostatic therapy) with bone antiresorptive therapies (bisphosphonate) that specifically target osteoclasts, bone resorbing cells. The osteoclast, whose increased activity is induced by the tumor, is responsible for the deterioration of bone mass and structure along with the release of grow factors that feed back and support further tumor growth. The current pharmaceutical approach is to target bone metastases by developing drugs that specifically target tumor cells in bone in addition to bone stroma since skeletal metastases are more resistant to treatment, present the highest bulk of tumor mass in the body, serve as site for secondary spread of tumor cells, and are associated with significant morbidity. There is a real need for a more effective modified release of newer anti-cancer drugs such as gene therapy and immunotherapy by using established and novel delivery platforms that will improve therapy and reduce side effects as a result of more appropriate plasma profiles. Overall, however, developments regarding treatment of cancer metastases to bone are encouraging. The scope of future advancements is immense and includes innovative therapeutics and delivery systems aimed to improve skeletal affinity, selectivity, and efficacy of drugs.

Benoy, I. H., H. Elst, et al. (2006). "Real-time RT-PCR detection of disseminated tumor cells in bone marrow has superior prognostic significance in comparison with circulating tumor cells in patients with breast cancer." Br J Cancer **94**(5): 672-80.

This study assessed the ability of real-time reverse transcription-polymerase chain reaction (RT-PCR) analysis to detect disseminated epithelial cells (DEC) in peripheral blood (PB) and bone marrow (BM) of patients with breast cancer (BC). Detection of DEC in BM is an obvious choice in BC, but blood sampling is more convenient. The aim of this study was to evaluate whether the detection of DEC in either PB or BM predicts overall survival (OS). Peripheral blood and BM samples were collected from 148 patients with primary (stage M0, n=116/78%) and metastatic (stage M+, n=32/21%) BC before the initiation of any local or systemic treatment. Peripheral blood of healthy volunteers and BM of patients with a nonmalignant breast lesion or a haematological malignancy served as the control group. Disseminated epithelial cells was detected by measuring relative gene expression (RGE) for cytokeratin-19 (CK-19) and mammaglobin (MAM), using a quantitative RT-PCR detection method. The mean follow-up time was 786 days (+/- 487). Kaplan-Meier analysis was used for predicting OS. By taking the 95 percentile of the RGE of CK-19 (BM: 26.3 and PB: 58.7) of the control group as cutoff, elevated CK-19 expression was detected in 42 (28%) BM samples and in 22 (15%) PB samples. Mammaglobin expression was elevated in 20% (both PB and BM) of the patients with BC. There was a 68% (CK-19) and 75% (MAM) concordance between PB and BM samples when classifying the results as either positive or negative. Patients with an elevated CK-19 or MAM expression in the BM had a worse prognosis than patients without elevated expression levels (OS: log-rank test, P=0.0045 (CK-19) and P=0.025 (MAM)). For PB survival analysis, no statistical significant difference was observed between patients with or without elevated CK-19 or MAM expression (OS: log-rank test, P=0.551 (CK-19) and P=0.329 (MAM)). Separate analyses of the M0 and M+ patients revealed a marked difference in OS according to the BM CK-19 or MAM status in the M+ patient group, but in the M0 group, only MAM expression was a prognostic marker for OS. Disseminated epithelial cells, measured as elevated CK-19 or MAM mRNA expression, could be detected in both PB and BM of patients with BC. Only the presence of DEC in BM was highly predictive for OS. The occurrence of DEC in the BM is probably less time-dependent and may act as a filter for circulating BC cells. The use of either larger volumes of PB or performing an enrichment step for circulating tumor in blood cells might improve these results.

Benoy, I. H., R. Salgado, et al. (2005). "Relative microvessel area of the primary tumor, and not lymph node status, predicts the presence of bone marrow micrometastases detected by reverse transcriptase polymerase chain reaction in patients with clinically non-metastatic breast cancer." Breast Cancer Res **7**(2): R210-9.

About 50% of patients with breast cancer have no involvement of axillary lymph nodes at diagnosis and can be considered cured after primary locoregional treatment. However, about 20-30% will experience distant relapse. The group of patients at risk is not well characterised: recurrence is probably due to the establishment of micrometastases before treatment. Given the early steps of metastasis in which tumor cells interact with endothelial cells of blood vessels, and, given the independent prognostic value

in breast cancer of both the quantification of tumor vascularisation and the detection of micrometastases in the bone marrow, the aim of this study was to determine the relationship between vascularisation, measured by Chalkley morphometry, and the bone marrow content of cytokeratin-19 (CK-19) mRNA, quantified by real-time reverse transcriptase polymerase chain reaction, in a series of 68 patients with localised untreated breast cancer. The blood concentration of factors involved in angiogenesis (interleukin-6 and vascular endothelial growth factor) and of factors involved in coagulation (D-dimer, fibrinogen, platelets) was also measured. When bone marrow CK-19 relative gene expression (RGE) was categorised according to the cut-off value of 0.77 (95th centile of control patients), 53% of the patients had an elevated CK-19 RGE. Patients with bone marrow micrometastases, on the basis of an elevated CK-19 RGE, had a mean Chalkley count of 7.5 +/- 1.7 (median 7, standard error [SE] 0.30) compared with a mean Chalkley count of 6.5 +/- 1.7 in other patients (median 6, SE 0.3) (Mann-Whitney U-test; P = 0.04). Multiple regression analysis revealed that Chalkley count, not lymph node status, independently predicted CK-19 RGE status (P = 0.04; odds ratio 1.38; 95% confidence interval 1.009-1.882). Blood parameters reflecting angiogenesis and coagulation were positively correlated with Chalkley count and/or CK-19 RGE. Our data are in support of an association between elevated relative microvessel area of the primary tumor and the presence of bone marrow micrometastases in breast cancer patients with operable disease, and corroborate the paracrine and endocrine role of interleukin-6 and the involvement of coagulation in breast cancer growth and metastasis.

Berois, N., M. Varangot, et al. (2000). "Molecular detection of cancer cells in bone marrow and peripheral blood of patients with operable breast cancer. Comparison of CK19, MUC1 and CEA using RT-PCR." Eur J Cancer **36**(6): 717-23.

We have compared three different RT-PCR procedures to measure cytokeratin 19 (CK19), carcinoembryonic antigen (CEA) and mucin MUC1 gene expression in order to determine their diagnostic value in detecting tumor cells in bone marrow aspirates of patients with operable breast cancer. In an experimental model, the best sensitivity was observed for CK19 and MUC1 RT-PCR assays, although only the CEA and CK19 assays showed good specificity. The study of 42 patients showed that a 'CK19 positive/CEA positive' RT-PCR assay in bone marrow correlated positively with a positive axillary lymph node status (N(0) versus N(1-3), P<0.05). Both assays were also positive in 17% of node negative patients. RT-PCR assays were more sensitive in bone marrow than in peripheral blood. Our results suggest that CK19 and CEA RT-PCR assays are powerful methods for detecting disseminated breast cancer cells. A larger study with long-term follow-up is required in order to clarify their clinical usefulness.

Bisanz, K., J. Yu, et al. (2005). "Targeting ECM-integrin interaction with liposome-encapsulated small interfering RNAs inhibits the growth of human prostate cancer in a bone xenograft imaging model." Mol Ther **12**(4): 634-43.

The intricate intracellular communication between stromal and epithelial cells, which involves cell-cell-, cell-insoluble extracellular matrix- (ECM), and cell-soluble factor-mediated signaling processes, is an attractive target for therapeutic intervention in hormone-refractory and bone-metastatic prostate cancer. In the present study we demonstrated that androgen-independent PC3 prostate cancer cells adhered to and migrated on vitronectin (VN), a major noncollagenous ECM in mature bone, through the expression of alphav-containing integrin receptors alphavbeta1 and alphavbeta5 on the cell surface, as determined by antibody function blocking assay and flow cytometry analysis. Small interfering RNAs (siRNAs) targeting human integrin alphav markedly reduced their respective mRNA and protein expression in cells, resulting in nearly complete reduction in VN-mediated cancer progression in vitro. In vivo quantitative bioluminescence analysis of human prostate cancer bone xenografts demonstrated for the first time that intratumoral administration of liposome-encapsulated human alphav-siRNAs significantly inhibits the growth of luciferase-tagged PC3 tumors in skeleton, which was associated with decreased integrin alphav expression and increased apoptosis in tumor cells. This integrin-based gene therapy is particularly suitable for the treatment of prostate cancer bone metastasis.

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

We analyzed expression of candidate genes encoding cell surface or secreted proteins in normal kidney and kidney cancer. This screen identified a bone morphogenetic protein (BMP) antagonist, SOSTDC1 (sclerostin domain-containing-1) as down-regulated in kidney tumors. To confirm screening results, we probed cDNA dot blots with SOSTDC1. The SOSTDC1 message was decreased in 20/20 kidney tumors compared with normal kidney tissue. Immunohistochemistry confirmed significant decrease of SOSTDC1 protein in clear cell renal carcinomas relative to normal proximal renal tubule cells ($p < 0.001$). Expression of SOSTDC1 was not decreased in

papillary and chromophobe kidney tumors. SOSTDC1 was abundantly expressed in podocytes, distal tubules, and transitional epithelia of the normal kidney. Transfection experiments demonstrated that SOSTDC1 is secreted and binds to neighboring cells and/or the extracellular matrix. SOSTDC1 suppresses both BMP-7-induced phosphorylation of R-Smads-1, -5, and -8 and Wnt-3a signaling. Restoration of SOSTDC1 in renal clear carcinoma cells profoundly suppresses proliferation. Collectively, these results demonstrate that SOSTDC1 is expressed in the human kidney and decreased in renal clear cell carcinoma. Because SOSTDC1 suppresses proliferation of renal carcinoma cells, restoration of SOSTDC1 signaling may represent a novel target in treatment of renal clear cell carcinoma.

Bregni, M., K. Fleischhauer, et al. (2006). "Bone marrow mammaglobin expression as a marker of graft-versus-tumor effect after reduced-intensity allografting for advanced breast cancer." Bone Marrow Transplant **37**(3): 311-5.

We assessed mammaglobin (MMG) gene expression in bone marrow (BM) aspirates from patients with advanced breast cancer who had received a reduced-intensity conditioning and stem cell allografting, in order to detect a graft-versus-tumor effect on micrometastatic disease. Nine patients received a reduced-intensity conditioning with fludarabine, cyclophosphamide, and thiotepa, followed by peripheral blood allografting from HLA-identical sibling donors. Nested RT-PCR analysis with sequence-specific primers for MMG was carried out on a monthly basis on BM samples. Three patients had MMG-positive BM, four patients had MMG-negative BM before allografting, and two were undetermined. In two patients, a clinical response after allografting (partial remission) occurred concurrently with the clearance of MMG expression, at a median of 6 months after allografting, following immune manipulation. In two patients, a prolonged stable disease and negative MMG expression occurred after day +360 from allografting. In two patients, progression of the disease was associated with MMG RT-PCR changing from negative to positive. In one case, a disease response occurring after donor lymphocyte infusion and grade II acute GVHD was heralded by negativization of MMG expression. Although preliminary, these data suggest that a graft-versus-breast cancer effect is detectable on micrometastatic BM disease.

Brown, R. S., J. Edwards, et al. (2002). "Amplification of the androgen receptor gene in bone metastases from hormone-refractory prostate cancer." J Pathol **198**(2): 237-44.

The aim of this study was to examine the prevalence of androgen receptor (AR) amplification in metastases to bone and other sites in patients with hormone-refractory prostate cancer (HRPC) and to compare these findings with those in pretreatment primary tumor samples from the same patients. Tissue from 24 patients with HRPC was available for study, together with 13 primary tumor specimens. AR gene amplification and copy number for X-chromosome were assessed by fluorescence in situ hybridization (FISH) using a Spectrum Orange-labelled probe at locus Xq11-13 for the AR gene and a SpectrumGreen-labelled alpha-satellite probe for the X-chromosome (Vysis, UK, Ltd.). A minimum of 20 nuclei were scored in each of three tumor areas by two independent observers. Samples from 18/24 patients with HRPC (12 bone marrow biopsies, three local tumor recurrences, and three lymph nodes) and nine primary tumor specimens were adequate for FISH analysis. Results were expressed as a mean ratio of AR gene copy number: mean X-chromosome number, with a ratio of greater than 1.5 defined as amplification. AR gene amplification was seen in 9/18 (50%) cases of HRPC and in none of the primary (untreated) tumor specimens ($p = 0.0048$, Fisher's exact test). For the 12 bone marrow samples, AR gene amplification occurred in 5/12 (38%) cases. Elevated copy number for chromosome X occurred in 3/18 (17%) HRPC and 4/9 (44%) matched primary tumors. This study shows for the first time that AR gene amplification can be demonstrated by FISH in bone metastases from HRPC patients. Because bone marrow biopsies can be obtained from most patients with HRPC, the findings provide a rational basis for the routine selection of patients who may respond more favourably to second-line anti-androgen therapy.

Bunyaratavej, P., T. G. Hullinger, et al. (2000). "Bone morphogenetic proteins secreted by breast cancer cells upregulate bone sialoprotein expression in preosteoblast cells." Exp Cell Res **260**(2): 324-33.

It is well established that bone metastases comprise bone; however, the exact factors/mechanisms involved remain unknown. We hypothesized that tumor cells secreted factors capable of altering normal bone metabolism. The aims of the present study were to (1) determine the effects of secretory products isolated from HT-39 cells, a human breast cancer cell line, on osteoprogenitor cell (MC3T3-E1 cells) behavior, and (2) identify tumor-derived factor(s) that alters osteoblast activities. Conditioned media (CM) from HT-39 cells were collected following a 24-h serum-free culture. The ability of CM to alter gene expression in MC3T3-E1 cells was determined by Northern analysis. CM effects on cell proliferation and mineralization ability were

determined using a Coulter counter and von Kossa stain, respectively. MC3T3-E1 cells were treated with CM plus noggin, a factor known to block bone morphogenic proteins (BMPs), to determine whether BMPs, shown to be present in CM, were linked with CM effects on MC3T3-E1 cell activity. In addition, inhibitors of MAP kinase kinase (MEK), protein kinase C (PKC), and protein kinase A were used to identify the intracellular signaling pathway(s) by which the active factors in CM regulated osteoblast behavior. CM treatment significantly enhanced BSP mRNA (2.5-fold over control), but had no effect on cell proliferation. Mineralization assay showed that CM enhanced mineral nodule formation compared to controls. Noggin inhibited CM-induced upregulation of BSP mRNA, suggesting that BMPs were responsible for upregulating BSP gene expression in MC3T3-E1 cells. The PKC inhibitor blocked CM-mediated upregulation of BSP, suggesting involvement of the PKC pathway in regulating BSP expression. BMPs secreted by HT-39 cells may be responsible for enhancing BSP expression in MC3T3-E1 cells. Continued studies targeted at determining the role of BMPs in regulating bone metabolism are important for understanding the pathogenesis of bone diseases.

Chanda, D., T. Isayeva, et al. (2008). "Systemic osteoprotegerin gene therapy restores tumor-induced bone loss in a therapeutic model of breast cancer bone metastasis." Mol Ther **16**(5): 871-8.

Enhanced production of receptor activator of nuclear factor-kappaB ligand (RANKL) and its binding to RANK on the osteoclasts have been associated with osteolysis in breast cancer bone metastasis. Osteoprotegerin (OPG) is a decoy receptor that prevents RANKL-RANK interaction. This study determined the effects of sustained expression of OPG using a recombinant adeno-associated viral (rAAV) vector in mouse model of osteolytic breast cancer. Bone metastasis was established by intracardiac injection of the human breast cancer cell line MDA-MB-435. Following this, mice were administered a one-time intramuscular injection of rAAV encoding either OPG.Fc (OPG) or green fluorescent protein (GFP). Mice were killed 1 month later and the effects of therapy on tumor growth and bone remodeling were evaluated. Bioluminescence imaging showed significant reduction of tumor growth in bone of OPG.Fc-treated mice. Micro-computed tomography (microCT) analysis and histomorphometry of the tibia indicated significant protection of trabecular and cortical bones after OPG.Fc therapy. Despite the prevention of bone loss and tumor growth in bone, OPG.Fc therapy failed to provide long-term survival. OPG.Fc-treated mice developed more bone than age-matched normal mice, indicating a requirement for regulated transgene expression. Results of this study indicate the potential of rAAV-OPG therapy for reducing morbidity and mortality in breast cancer patients with osteolytic bone damage.

Chen, Y. C., D. M. Sosnoski, et al. (2009). "Selenium modifies the osteoblast inflammatory stress response to bone metastatic breast cancer." Carcinogenesis **30**(11): 1941-8.

Breast cancer frequently metastasizes to the skeleton resulting in bone degradation due to osteoclast activation. Metastases also downregulate differentiation and the bone-rebuilding function of osteoblasts. Moreover, cancer cells trigger osteoblast inflammatory stress responses. Pro-inflammatory mediators such as interleukin (IL)-6, monocyte chemoattractant protein-1 (MCP-1), cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), expressed by osteoblasts (MC3T3-E1) stimulated with human breast cancer cell (MDA-MB-231) conditioned medium, are pivotal to osteoclast activation and metastasis. Given that these genes are regulated by nuclear factor-kappaB (NF-kappaB), a redox-sensitive transcription factor, we hypothesized that selenium (Se) could abrogate the inflammatory response to metastatic breast cancer cells by modulating NF-kappaB. Caffeic acid phenethyl ester and parthenolide inhibited NF-kappaB activation, as seen by gel shift assays and immunoblotting for p65 in nuclear fractions, as well as decreased production of IL-6 and MCP-1. Supplementation of MC3T3-E1 with methylseleninic acid (MSA) (0.5 microM to 4 microM) reduced the activation of NF-kappaB leading to a decrease in IL-6, MCP-1, COX-2 and iNOS in response to MDA-MB-231 conditioned medium. Addition of MSA to osteoblasts for as little as 15 min suppressed activation of NF-kappaB suggesting that short-lived active metabolites might be involved. However, brief exposure to MSA also brought about an increase in selenoprotein glutathione peroxidase 1. In summary, our data indicate that the osteoblast response to metastatic breast cancer cells is regulated by NF-kappaB activation, which can be effectively suppressed by MSA either through short-lived active metabolites and/or selenoproteins. Thus, Se supplementation may prevent the osteoblast inflammatory response or dampen the vicious cycle established when breast cancer cells, osteoblasts and osteoclasts interact.

Chu, K., C. J. Cheng, et al. (2008). "Cadherin-11 promotes the metastasis of prostate cancer cells to bone." Mol Cancer Res **6**(8): 1259-67.

Bone is the most common site of metastases from prostate cancer. The mechanism by which

prostate cancer cells metastasize to bone is not fully understood, but interactions between prostate cancer cells and bone cells are thought to initiate the colonization of metastatic cells at that site. Here, we show that cadherin-11 (also known as osteoblast-cadherin) was highly expressed in prostate cancer cell line derived from bone metastases and had strong homophilic binding to recombinant cadherin-11 in vitro. Down-regulation of cadherin-11 in bone metastasis-derived PC3 cells with cadherin-11-specific short hairpin RNA (PC3-shCad-11) significantly decreased the adhesion of those cells to cadherin-11 in vitro. In a mouse model of metastasis, intracardiac injection of PC3 cells led to metastasis of those cells to bone. However, the incidence of PC3 metastasis to bone in this model was reduced greatly when the expression of cadherin-11 by those cells was silenced. The clinical relevance of cadherin-11 in prostate cancer metastases was further studied by examining the expression of cadherin-11 in human prostate cancer specimens. Cadherin-11 was not expressed by normal prostate epithelial cells but was detected in prostate cancer, with its expression increasing from primary to metastatic disease in lymph nodes and especially bone. Cadherin-11 expression was not detected in metastatic lesions that occur in other organs. Collectively, these findings suggest that cadherin-11 is involved in the metastasis of prostate cancer cells to bone.

Clement, J. H., N. Marr, et al. (2000). "Bone morphogenetic protein 2 (BMP-2) induces sequential changes of Id gene expression in the breast cancer cell line MCF-7." J Cancer Res Clin Oncol **126**(5): 271-9.

Bone morphogenetic proteins (BMPs) are involved in the development of various organs including the mammary gland. They are well-regulated and act in a time-, concentration- and cell-type-specific manner. We found that BMP-2 is expressed in primary breast tumor tissue samples and in breast cancer cell lines. Hybridization of labeled cDNA, obtained from the breast cancer cell line MCF-7, against the Atlas human cDNA expression array revealed differential gene expression depending on BMP-2 treatment. The most prominent changes were observed for the helix-loop-helix proteins Id-1, Id-2 and Id-3. Id-1 expression had increased several fold after 4 h and was even higher after 24 h. Id-2 and Id-3 were more strongly induced after 4 h and showed no further significant change after 24 h. Analysis of cell-cycle distribution revealed a marked increase of the sub-G1 phase after 48 h in serum-deprived cells. In the presence of BMP-2 no change was observed over 48 h indicating that BMP-2 does not induce apoptosis. In addition, expression of caspase-3 was reduced in BMP-2-treated cells after 24 h. In summary, our results clearly indicate that BMP-2 is a susceptibility factor keeping the cells ready for the integration of various other signals for cell progression.

Colnot, D. R., E. J. Nieuwenhuis, et al. (2004). "Clinical significance of micrometastatic cells detected by E48 (Ly-6D) reverse transcription-polymerase chain reaction in bone marrow of head and neck cancer patients." Clin Cancer Res **10**(23): 7827-33.

Despite improvements in locoregional treatment of head and neck squamous cell carcinoma (HNSCC), local and distant failure rates remain high. The strongest prognostic indicator of HNSCC is the presence of lymph node metastases in the neck, but the value of this indicator has limitations when using for the individual patient. The presence of micrometastatic cells in bone marrow has been shown to be a putative prognostic indicator in HNSCC and other epithelial malignancies, which might allow more accurate staging and selection of patients for whom adjuvant or experimental therapy is recommended. The gene encoding the E48 antigen is selectively expressed by HNSCC, and the detection of E48 transcripts in bone marrow by reverse transcription-polymerase chain reaction (RT-PCR) presumably represents the presence of micrometastatic cells. The purpose of this study was to determine the association between the presence of micrometastatic cells in bone marrow of HNSCC patients and clinical outcome. A total of 162 patients treated surgically for primary HNSCC underwent a single bone marrow aspiration from the upper iliac crest for detection of micrometastatic cells using E48 RT-PCR. In total, 139 patients were evaluable. The primary statistical endpoints were disease-free survival and distant metastasis-free survival. In addition, bone marrow samples of 30 noncancer controls were evaluated. E48 RT-PCR indicated the presence of micrometastatic cells in the bone marrow in 56 of 139 (40%) of the HNSCC patients and 0 of 30 of the noncancer controls ($P < 0.0001$). The presence of micrometastatic cells had no significant influence on disease-free survival or distant metastasis-free survival for the whole group of HNSCC patients ($P = 0.1460$ and $P = 0.2912$, respectively). For patients with >or=2 lymph node metastases, however, the presence of micrometastatic cells was associated with a poor distant metastasis-free survival ($P = 0.0210$). CONCLUSIONS: The presence of micrometastatic cells in bone marrow of HNSCC patients with >or=2 lymph node metastases is correlated with a poor distant metastasis-free survival. In this subgroup of HNSCC patients, E48 RT-PCR seems to be a valuable tool to identify patients who are at increased risk for development of distant

metastases and therefore might benefit from experimental adjuvant systemic therapy.

Cooper, C. R., J. K. Bhatia, et al. (2002). "The regulation of prostate cancer cell adhesion to human bone marrow endothelial cell monolayers by androgen dihydrotestosterone and cytokines." Clin Exp Metastasis **19**(1): 25-33.

A previous study from our laboratory suggested that prostate cancer metastasis to bone may be mediated, in part, by preferential adhesion to human bone marrow endothelial (HBME) cells. Tumor cell adhesion to endothelial cells may be modulated by the effect of cytokines on cell adhesion molecules (CAMs). Tumor necrosis factor-alpha (TNF-alpha) regulates VCAM expression on the endothelium and this effect is enhanced by dihydrotestosterone (DHT). Transforming growth factor-beta (TGF-beta) stimulates the expression of alpha2beta1 integrin on PC-3 cells. The current study investigated the effects of the above cytokines and DHT (singularly and in various combinations) upon HBME and prostate cancer cell expression of VCAM, alpha2 integrin subunit, and beta1 integrin subunit by flow cytometry. We also monitored the effects of the above treatments on PC-3 cell adhesion to HBME monolayers. The data demonstrate that none of the treatments significantly altered the expression of selected CAMs on HBME cell and neoplastic prostate cell lines. The treatment of HBME monolayers with various combinations of cytokines and DHT prior to performing adhesion assays with PC-3 demonstrates that treatments containing TGF-beta reduced PC-3 cell adhesion to HBME monolayers by 32% or greater ($P < 0.05$). The reduction in PC-3 cell adhesion to TGF-beta-treated HBME monolayers was dose dependent. Interestingly, LNCaP cells but not PC-3 cells treated with TGF-beta had a reduced ability to adhere to untreated HBME monolayers. These results suggest that TGF-beta may reduce tumor cell adhesion to bone marrow microvascular endothelium, in vivo. The biological significance of this observation is discussed.

Cooper, C. R., B. Graves, et al. (2008). "Novel surface expression of reticulocalbin 1 on bone endothelial cells and human prostate cancer cells is regulated by TNF-alpha." J Cell Biochem **104**(6): 2298-309.

An unbiased cDNA expression phage library derived from bone-marrow endothelial cells was used to identify novel surface adhesion molecules that might participate in metastasis. Herein we report that reticulocalbin 1 (RCN1) is a cell surface-associated protein on both endothelial (EC) and prostate cancer (PCa) cell lines. RCN1 is an H/KDEL protein with six EF-hand, calcium-binding motifs, found in the endoplasmic reticulum. Our data indicate that RCN1 also is expressed on the cell surface of several endothelial cell lines, including human dermal microvascular endothelial cells (HDMVECs), bone marrow endothelial cells (BMEC), and transformed human bone marrow endothelial cells (TrHBMEC). While RCN1 protein levels were highest in lysates from HDMVEC, this difference was not statistically significant compared BMEC and TrHBMEC. Given preferential adhesion of PCa to bone-marrow EC, these data suggest that RCN1 is unlikely to account for the preferential metastasis of PCa to bone. In addition, there was not a statistically significant difference in total RCN1 protein expression among the PCa cell lines. RCN1 also was expressed on the surface of several PCa cell lines, including those of the LNCaP human PCa progression model and the highly metastatic PC-3 cell line. Interestingly, RCN1 expression on the cell surface was upregulated by tumor necrosis factor alpha treatment of bone-marrow endothelial cells. Taken together, we show cell surface localization of RCN1 that has not been described previously for either PCa or BMEC and that the surface expression on BMEC is regulated by pro-inflammatory TNF-alpha.

Dai, J., C. L. Hall, et al. (2008). "Prostate cancer induces bone metastasis through Wnt-induced bone morphogenetic protein-dependent and independent mechanisms." Cancer Res **68**(14): 5785-94.

Prostate cancer (PCa) is frequently accompanied by osteosclerotic (i.e., excessive bone production) bone metastases. Although bone morphogenetic proteins (BMP) and Wnts are mediators of PCa-induced osteoblastic activity, the relation between them in PCa bone metastases is unknown. The goal of this study was to define this relationship. Wnt3a and Wnt5a administration or knockdown of DKK-1, a Wnt inhibitor, induced BMP-4 and 6 expression and promoter activation in PCa cells. DKK-1 blocked Wnt activation of the BMP promoters. Transfection of C4-2B cells with axin, an inhibitor of canonical Wnt signaling, blocked Wnt3a but not Wnt5a induction of the BMP promoters. In contrast, Jnk inhibitor I blocked Wnt5a but not Wnt3a induction of the BMP promoters. Wnt3a, Wnt5a, and conditioned medium (CM) from C4-2B or LuCaP23.1 cells induced osteoblast differentiation in vitro. The addition of DKK-1 and Noggin, a BMP inhibitor, to CM diminished PCa CM-induced osteoblast differentiation in a synergistic fashion. However, pretreatment of PCa cells with DKK-1 before collecting CM blocked osteoblast differentiation, whereas pretreatment with Noggin only partially reduced osteoblast differentiation, and pretreatment with both DKK-1 and Noggin had no greater effect

than pretreatment with DKK-1 alone. Additionally, knockdown of BMP expression in C4-2B cells inhibited Wnt-induced osteoblastic activity. These results show that PCa promotes osteoblast differentiation through canonical and noncanonical Wnt signaling pathways that stimulate both BMP-dependent and BMP-independent osteoblast differentiation. These results show a clear link between Wnts and BMPs in PCa-induced osteoblast differentiation and provide novel targets, including the noncanonical Wnt pathway, for therapy of PCa.

Daubine, F., C. Le Gall, et al. (2007). "Antitumor effects of clinical dosing regimens of bisphosphonates in experimental breast cancer bone metastasis." J Natl Cancer Inst **99**(4): 322-30.

Bisphosphonates exhibit direct antitumor activity in animal models, but only at high doses that are incompatible with the clinical dosing regimens approved for the treatment of cancer patients with skeletal metastases. We compared the antitumor effects of clinical dosing regimens of the bisphosphonates zoledronic acid and clodronate in a mouse model of bone metastasis. Mice (n = 6-10 per group) were treated with zoledronic acid, clodronate, or vehicle starting before (preventive protocols) or after (treatment protocols) intravenous injection with human B02/GFP.2 breast cancer cells, which express green fluorescent protein (GFP) and luciferase and metastasize to bone. Zoledronic acid was given as daily, weekly, or single doses at a cumulative dose of 98-100 microg/kg body weight, equivalent to the 4-mg intravenous dose given to patients. Clodronate was given as a daily dose (530 microg/kg/day), equivalent to the daily 1600-mg oral clinical dose given to patients. Bone destruction was measured by radiography, x-ray absorptiometry or tomography, and histomorphometry (as the ratio of bone volume to tissue volume). Skeletal tumor burden was measured by histomorphometry (as the ratio of tumor burden to soft tissue volume [TB/STV]) and luciferase activity. All statistical tests were two-sided. In treatment protocols, daily clodronate was less effective at decreasing the TB/STV ratio than daily (53% versus 87%, difference = 34%, 95% confidence interval [CI] = 16% to 44%, P < .001) or weekly (53% versus 90%, difference = 37%, 95% CI = 19% to 46%, P < .001) zoledronic acid-dosing regimens. Compared with vehicle, a single dose of zoledronic acid decreased tumor burden by only 16% (95% CI = 9% to 22%, P < .001). In preventive protocols, daily clodronate and daily or weekly zoledronic acid decreased the TB/STV ratio by 49% (95% CI = 40% to 57%, P = .006), 83% (95% CI = 68% to 98%, P < .001), and 66% (95% CI = 47% to 84%, P < .001), respectively, compared with vehicle, whereas a single dose of zoledronic acid decreased tumor burden by only 13% (95% CI = -2% to 28%, P = .84). Mice treated with a daily preventive regimen of clodronate or with a daily or weekly preventive regimen of zoledronic acid showed a decreased B02/GFP.2 cell tumor burden compared with vehicle, whereas a single preventive dose of zoledronic acid had no effect. CONCLUSION: Daily or repeated intermittent therapy with clinical doses of bisphosphonates inhibits skeletal tumor growth in a mouse model.

Davies, S. R., G. Watkins, et al. (2008). "Bone morphogenetic proteins 1 to 7 in human breast cancer, expression pattern and clinical/prognostic relevance." J Exp Ther Oncol **7**(4): 327-38.

Bone morphogenetic proteins (BMPs) have a diverse role and they act in a time, concentration and cell type specific manner. They regulate cellular motility and the cells ability to invade. Recently, BMP molecules have further been shown to have an impact on the biological behaviour of breast cancer cells. In this study, we looked, for the first time, at the expression of a panel of BMPs in breast carcinomas. METHOD: Fresh frozen primary human breast cancer tissues (n = 120) and non-neoplastic mammary tissues (n = 32) were used. The distribution and location of BMPs was assessed using immunohistochemical methods and the transcript levels of BMPs (BMP-1, -2, -3, -4, -5, -6, and -7) were determined using quantitative RT-PCR. The results were analysed against the clinical, pathological and follow-up (10 years) data. BMP-2 and BMP-7 exhibited contrasting patterns of expression in normal and tumor tissues, wherein BMP-2 transcript level was lower and BMP-7 was higher in breast tumors than normal tissues. BMP-2 transcript was also significantly lower in tumors from patients with a moderate and poor prognosis than from those with a good prognosis (p = 0.04). BMP-2 and BMP-7 also showed a significant difference between node positive and node negative tumors (p = 0.033 and p = 0.031 respectively). BMPs 1, 3, 4, 5 and 6 showed an inconsistent variation in transcript levels within the cancer subgroups with no statistically significant results. CONCLUSION: This study has demonstrated a differential pattern of expression of BMP molecules in breast cancer and reveals a potential prognostic value of BMP-2 and BMP-7 for patients. The findings also suggest that these BMPs may be potential therapeutic targets.

Deng, X., G. He, et al. (2008). "Adenovirus-mediated expression of TIMP-1 and TIMP-2 in bone inhibits osteolytic degradation by human prostate cancer." Int J Cancer **122**(1): 209-18.

Matrix metalloproteinases (MMPs) are proteolytic enzymes that play critical roles in the

pathogenesis of human cancers. Clinical trials using synthetic small molecule MMP inhibitors have been carried out but with little success. Tissue inhibitors of metalloproteinases (TIMPs) are endogenous inhibitors that block the extracellular matrix-degrading activity of MMPs. Here, we investigated the possibilities of genetically modifying human bones with TIMPs to create a high-TIMP bone microenvironment, which is hostile to metastatic prostate cancer cells using adenovirus-mediated gene transfer technology and SCID-hu end-organ colonization mouse model. Two strategies were used to achieve bone-specific TIMP expression: (i) ex vivo bone adenoviral infection followed by in vivo bone implantation; and (ii) ex vivo BMS cell infection followed by injection into in vivo implanted human fetal bones. PC-3 prostate cancer cells were injected into human fetal bones 4 weeks after implantation in SCID mice. In vitro, adenovirus-mediated expression of TIMP-1 or TIMP-2 in bone fragments inhibited MMP-2 activity, bone turnover and prostate cancer cell-induced proteolytic degradation as determined by gelatin zymography, calcium measurement and DQ protein quenched fluorescence assay, respectively. In vivo, immunohistochemistry confirmed TIMP-2 expression in AdTIMP-2-infected bone implants 4 weeks after implantation in SCID mice. Mice receiving AdTIMP-treated bone fragments showed significantly reduced PC-3-induced osteolysis, osteoclast recruitment and bone turnover in the implanted bones. We propose that adenoviral gene transfer of TIMP-1 and TIMP-2 can prevent the proteolytic activity of prostate cancer cells in bone and that enhancing anti-proteolytic defense mechanisms in target organs represents a promising form of prostate cancer gene therapy.

Deng, X., S. H. Tannehill-Gregg, et al. (2007). "Parathyroid hormone-related protein and ezrin are up-regulated in human lung cancer bone metastases." Clin Exp Metastasis **24**(2): 107-19.

Lung cancer often metastasizes to bone in patients with advanced disease. Identification of the factors involved in the interactions between lung cancer cells and bone will improve the prevention and treatment of bone metastases. We identified changes in metastasis-related gene expression of human HARA lung squamous carcinoma cells co-cultured with neonatal mouse calvariae using a pathway-specific microarray analysis. Nine genes were up-regulated and two genes down-regulated in HARA cells co-cultured with mouse calvariae. Five of the nine up-regulated genes, including caveolin 1, CD44, EphB2, ezrin, and Parathyroid hormone-related protein (PTHrP), and one down-regulated gene, SLPI, were further confirmed by Reverse transcription-polymerase chain reaction (RT-PCR). A mouse model was subsequently used to study the role of PTHrP and ezrin in bone metastasis in vivo. PTHrP (all three isoforms) and ezrin were up-regulated in HARA cells at sites of bone metastasis as detected by RT-PCR and immunohistochemistry. The PTHrP 141 mRNA isoform was increased by the greatest extent (13.9-fold) in bone metastases compared to PTHrP 139 and PTHrP 173 mRNA. We then generated a HARA cell line in which PTHrP expression was inducibly silenced by RNA interference. Silencing of PTHrP expression caused significant reduction of submembranous F-actin and decreased HARA cell invasion. Ezrin up-regulation was confirmed by Western blots on HARA cells co-cultured with adult mouse long bones. Further, Transforming growth factor beta (TGF-beta) was identified as one of the factors in the bone microenvironment that was responsible for the up-regulation of ezrin. The identification of PTHrP and ezrin as important regulators of lung cancer bone metastasis offers new mechanistic insights into the metastasis of lung cancer and provides potential targets for the prevention and treatment of lung cancer metastasis.

Diel, I. J. and R. J. Cote (2000). "Bone marrow and lymph node assessment for minimal residual disease in patients with breast cancer." Cancer Treat Rev **26**(1): 53-65.

The immunocytological detection of disseminated epithelial cells in bone marrow in patients with breast cancer has been performed at many hospitals and institutes since the early 1980s. Despite numerous publications in this field, it has not been possible to standardize the method and establish the 'ideal' antibody, either nationally or internationally. Molecular biological methods using PCR technology could extend the diagnostic spectrum. However, one of the major problems in breast cancer is the lack of a disease-specific marker gene. As a result, immunocytology is still the standard procedure for tumor cell detection. The detection of disseminated single cells in bone marrow in primary breast cancer (also known as minimal residual disease) is a new prognostic factor for disease-free and overall survival. This has been demonstrated in two large (N > 300) groups and several small to medium groups (N = 50-300). As a marker of dissemination in a target organ for metastasis this prognostic factor corresponds much more closely to the tendency of breast cancer to early haematogenic spread. Tumor cell detection may predict the course of the disease better than the axillary lymph node status. Bone marrow aspiration and detection of disseminated cells might replace lymph node dissection, at least in those patients with small tumors and no clinical signs of lymph node involvement. This strategy will soon be

investigated in appropriate studies. Another possible clinical use might be in deciding on whether or not to give adjuvant systemic therapy to node-negative patients. Patients with positive tumor cell detection are at a higher risk of subsequent metastasis, even if the axillary nodes are histologically normal. The immunohistological or molecular biological detection of tumor cells in axillary lymph nodes might also be very useful, now that it has been shown that a considerable subset of patients determined to be node-negative by means of conventional methods, are positive according to these new techniques. These methods could be a useful supplement to sentinel node biopsy. A further potential use of this method is in monitoring therapy with new treatment modalities such as gene therapy and immunotherapy. Repeated bone marrow aspiration can provide information on the success of therapy in minimal residual disease (cytoreduction). Immunocytochemical investigation of individual cells may be useful in studying the pathogenesis of metastasis, in particular in the skeleton. Phenotyping of cells might allow statements to be made on the metastatic potential of cells and the question of cell dormancy. It remains to be hoped that this aspect of minimal residual disease will be granted more attention in future.

Duivenvoorden, W. C., H. W. Hirte, et al. (1999). "Transforming growth factor beta1 acts as an inducer of matrix metalloproteinase expression and activity in human bone-metastasizing cancer cells." Clin Exp Metastasis **17**(1): 27-34.

Bone metastases are a common complication in prostate and breast cancer patients. It leads to extensive morbidity and eventually mortality. Matrix metalloproteinases (MMPs) are known to be involved in the metastatic process. MMP activity can be down-regulated by transforming growth factor beta1 (TGF-beta1), a growth-modulating factor, found in high concentrations in the bone. TGF-beta1 acts through the TGF-beta1 inhibitory element (TIE) element, a cis-acting element found in the promoter region of most MMP genes, with the exception of MMP-2. We used three human cell lines relevant for bone metastases, namely prostate adenocarcinoma PC-3, breast adenocarcinoma MDA-MB-231, and adenocarcinoma cells of unknown origin, Hs696, and one human osteosarcoma cell line, SAOS-2, and showed that in these cell lines TGF-beta1 partially lost its repressing action on MMP expression. TGF-beta1 was able to induce MMP-9 activity and protein expression in all three bone-metastatic tumor cell types, whereas MMP-9 protein levels were repressed in SAOS-2 cells. In PC-3 cells, TGF-beta1 repressed MMP-1 expression, whereas in MDA-MB-231 and SAOS-2 cells, an increase in the expression of MMP-1 protein was detected. Additionally, an increase in MMP-3 expression was observed in Hs696 cells. Expression and activity of the tissue inhibitors of matrix metalloproteinases, TIMP-1 and TIMP-2, were found increased in both PC-3 and MDA-MB-231 cells. With respect to cell proliferation, TGF-beta1 was able to induce a dose-dependent growth inhibition of up to 50% in primary human mammary epithelial cells. However, in none of the tumor cell lines was TGF-beta1 able to suppress growth substantially. Data presented in this paper support the hypothesis that TGF-beta1 can potentially disrupt the balance existing between osteoclast- and osteoblast-derived MMP activity by inducing altered expression of matrix metalloproteinases and their tissue inhibitors derived from bone-metastasizing cancer cells. This could eventually lead to skeletal destruction in patients with advanced metastatic disease.

Dunn, L. K., K. S. Mohammad, et al. (2009). "Hypoxia and TGF-beta drive breast cancer bone metastases through parallel signaling pathways in tumor cells and the bone microenvironment." PLoS One **4**(9): e6896.

Most patients with advanced breast cancer develop bone metastases, which cause pain, hypercalcemia, fractures, nerve compression and paralysis. Chemotherapy causes further bone loss, and bone-specific treatments are only palliative. Multiple tumor-secreted factors act on the bone microenvironment to drive a feed-forward cycle of tumor growth. Effective treatment requires inhibiting upstream regulators of groups of prometastatic factors. Two central regulators are hypoxia and transforming growth factor (TGF)- beta. We asked whether hypoxia (via HIF-1alpha) and TGF-beta signaling promote bone metastases independently or synergistically, and we tested molecular versus pharmacological inhibition strategies in an animal model. METHODOLOGY/PRINCIPAL FINDINGS: We analyzed interactions between HIF-1alpha and TGF-beta pathways in MDA-MB-231 breast cancer cells. Only vascular endothelial growth factor (VEGF) and the CXC chemokine receptor 4 (CXCR4), of 16 genes tested, were additively increased by both TGF-beta and hypoxia, with effects on the proximal promoters. We inhibited HIF-1alpha and TGF-beta pathways in tumor cells by shRNA and dominant negative receptor approaches. Inhibition of either pathway decreased bone metastasis, with no further effect of double blockade. We tested pharmacologic inhibitors of the pathways, which target both the tumor and the bone microenvironment. Unlike molecular blockade, combined drug treatment decreased bone metastases more than either alone, with effects on bone to decrease osteoclastic bone resorption and increase

osteoblast activity, in addition to actions on tumor cells. CONCLUSIONS/SIGNIFICANCE: Hypoxia and TGF-beta signaling in parallel drive tumor bone metastases and regulate a common set of tumor genes. In contrast, small molecule inhibitors, by acting on both tumor cells and the bone microenvironment, additively decrease tumor burden, while improving skeletal quality. Our studies suggest that inhibitors of HIF-1alpha and TGF-beta may improve treatment of bone metastases and increase survival.

Edlund, M., S. Y. Sung, et al. (2004). "Modulation of prostate cancer growth in bone microenvironments." J Cell Biochem **91**(4): 686-705.

Bone remains one of the major sites, and most lethal host organs, for prostate cancer metastasis. Prostate cell spread and establishment in bone depends on multiple reciprocal modifications of bone stromal and epithelial cancer cell behaviors. This review focuses on recent advances in the characterization of cell-cell and cell-matrix interplay, effects on cell growth, adhesion and invasion, and several therapeutic possibilities for co-targeting prostate cancer cells and bone stroma. We address the topic from three main perspectives: (1) the normal and aging bone stromal environment, (2) the "reactive" bone stromal environment, and (3) the cancerous prostate epithelial cells themselves. First, normal, and especially aging, bones provide uniquely rich and "fertile soil" for roaming cancer cells. The interactions between prostate cancer cells and insoluble extracellular matrices, soluble growth factors, and/or sex steroid hormones trigger bone remodeling, through increased osteoclastogenesis and furthur matrix metalloproteinase activity. Second, after cancer cell arrival and establishment in the bone, host stromal cells respond, becoming "reactive" in a process again involving extracellular matrix remodeling, together with growth factor and steroid receptor signaling this process ultimately enhances cancer cell migration, stromal transdifferentiation, and invasion of the cancer tissues by stromal, inflammatory, and immune-responsive cells. Third, prostate cancer cells also respond to supportive bone microenvironments, where soluble and matrix-associated molecules affect cancer cell growth and gene expression, especially altering cancer cell surface receptor and integrin-mediated cell signaling. We discuss both integrin cell-matrix and gap junctional cell-cell communication between cancer cells and their microenvironments during prostate cancer progression.

Forus, A., H. K. Hoifodt, et al. (1999). "Sensitive fluorescent in situ hybridisation method for the characterisation of breast cancer cells in bone marrow aspirates." Mol Pathol **52**(2): 68-74.

AIM: The presence of malignant cells in the blood and bone marrow of patients with cancer at the time of surgery may be indicative of early relapse. In addition to their numbers, the phenotypes of the micrometastatic cells might be essential in determining whether overt metastases will develop. This study aimed to establish a sensitive method for the detection and characterisation of malignant cells present in bone marrow. In spiking experiments, SKBR3 cells were mixed with mononuclear cells in known proportions to mimic bone marrow samples with micrometastatic cells. Tumor cells were extracted using SAM-M450 Dynabeads coupled to the MOC-31 anti-epithelial antibody, and were further analysed for amplification of erbB2 and int2 by fluorescent in situ hybridisation (FISH). erbB2 and int2 copy numbers were also determined in 15 primary breast cancers, and bone marrow samples from patients with amplification were analysed for micrometastatic cells by immunomagnetic enrichment and FISH. In model experiments, cells with amplification could be detected in bead selected fractions when ratios of tumor cells (SKBR3) to mononuclear cells were as low as 10:10(7). Among the tumor samples, eight showed increased copy numbers of erbB2 and/or int2, and three of these patients had detectable numbers of tumor cells in their bone marrow: 4000, 540, and 26 tumor cells/10(7) mononuclear cells, respectively. The patient with 540 tumor cells/10(7) mononuclear cells showed high level amplification of erbB2 and suffered from a particularly aggressive disease, whereas the patient with 4000 tumor cells/10(7) mononuclear cells had favourable disease progression. CONCLUSION: These results demonstrate the feasibility and advantage of combining immunomagnetic selection and FISH characterisation of cancer cells in bone marrow samples. It is possible that molecular characterisation of such cells could provide prognostically valuable information.

Furuse, S., T. Kawamata, et al. (2009). "Reduction of bone cancer pain by activation of spinal cannabinoid receptor 1 and its expression in the superficial dorsal horn of the spinal cord in a murine model of bone cancer pain." Anesthesiology **111**(1): 173-86.

Bone cancer pain has a strong impact on the quality of life of patients, but it is difficult to treat. Therefore, development of a novel strategy for the treatment of bone cancer pain is needed for improvement of patient quality of life. This study examined whether selective spinal cannabinoid receptor 1 (CB1) activation alleviates bone cancer pain and also examined the spinal expression of CB1. A bone cancer pain model was made by implantation of sarcoma cells into the intramedullary space of the mouse femur. In behavioral experiments, the authors

examined the effects of activation of spinal CB1 and inhibition of metabolism of endocannabinoid on bone cancer-related pain behaviors. Immunohistochemical experiments examined the distribution and localization of CB1 in the superficial dorsal horn of the spinal cord using specific antibodies. Spinal CB1 activation by exogenous administration of a CB1 agonist arachidonyl-2-chloroethylamide reduced bone cancer-related pain behaviors, including behaviors related to spontaneous pain and movement-evoked pain. In immunohistochemical experiments, although mu-opioid receptor 1 expression was reduced in the superficial dorsal horn ipsilateral to the site of implantation of sarcoma cells, CB1 expression was preserved. In addition, CB1 was mainly expressed in the axon terminals, but not in the dendritic process in the superficial dorsal horn. CONCLUSION: Spinal CB1 activation reduced bone cancer-related pain behavior. Presynaptic inhibition may contribute to the analgesic effects of spinal CB1 activation. These findings may lead to novel strategies for the treatment of bone cancer pain.

Gazi, E., J. Dwyer, et al. (2007). "Biomolecular profiling of metastatic prostate cancer cells in bone marrow tissue using FTIR microspectroscopy: a pilot study." Anal Bioanal Chem **387**(5): 1621-31.

Prostate cancer (CaP) cells preferentially metastasise to the bone marrow, a microenvironment that plays a substantial role in the sustenance and progression of the CaP tumor. Here we use a combination of FTIR microspectroscopy and histological stains to increase molecular specificity and probe the biochemistry of metastatic CaP cells in bone marrow tissue derived from a limited source of paraffin-embedded biopsies of different patients. This provides distinction between the following dominant metabolic processes driving the proliferation of the metastatic cells in each of these biopsies: glycerophospholipid synthesis from triacylglyceride, available from surrounding adipocytes, in specimen 1, through significantly high (p < or = 0.05) carbohydrate (8.23 +/- 1.44 cm(-1)), phosphate (6.13 +/- 1.5 cm(-1)) and lipid hydrocarbon (24.14 +/- 5.9 cm(-1)) signals compared with the organ-confined CaP control (OC CaP), together with vacuolation of cell cytoplasm; glycolipid synthesis in specimen 2, through significantly high (p < or = 0.05) carbohydrate (5.51 +/- 0.04 cm(-1)) and high lipid hydrocarbon (17.91 +/- 2.3 cm(-1)) compared with OC CaP, together with positive diastase-digested periodic acid Schiff staining in the majority of metastatic CaP cells; glycolysis in specimen 3, though significantly high (p < or = 0.05) carbohydrate (8.86 +/- 1.78 cm(-1)) and significantly lower (p < or = 0.05) lipid hydrocarbon (11.67 +/- 0.4 cm(-1)) than OC CaP, together with negative diastase-digested periodic acid Schiff staining in the majority of metastatic CaP cells. Detailed understanding of the biochemistry underpinning the proliferation of tumor cells at metastatic sites may help towards refining chemotherapeutic treatment.

Goblirsch, M., C. Lynch, et al. (2005). "Radiation treatment decreases bone cancer pain through direct effect on tumor cells." Radiat Res **164**(4 Pt 1): 400-8.

The most used treatment for bone cancer pain is radiation; however, the mechanism responsible for analgesia after irradiation is unknown. The mechanistic influence of a single, localized 10-, 20- or 30-Gy dose of radiation on painful behaviors, osteolysis, histopathology and osteoclast number was evaluated in mice with painful femoral sarcomas. Dramatic reductions in pain behaviors ($P < 0.05$) and osteolysis ($P < 0.0001$) were seen in mice irradiated with 20 and 30 Gy. Irradiation reduced the tumor area by more than 75% ($P < 0.05$) but did not affect osteoclast frequency per mm2 tumor. Treatment with 20 Gy prior to tumor injection had no effect on tumor growth or pain behaviors, suggesting that radiation reduces osteolysis and pain through direct tumor effects. To demonstrate that tumor elimination was responsible for reduction in osteolysis and pain, sarcoma cells containing the suicide gene cytosine deaminase (CD) were inoculated into femora. After onset of bone cancer pain, mice were treated with the prodrug 5-fluorocytosine (5-FC). 5-FC treatment significantly reduced both osteolysis ($P < 0.0005$) and bone cancer pain ($P < 0.05$). The findings in this study demonstrate that one mechanism through which radiation decreases bone cancer pain is by direct effects on tumor cells.

Goblirsch, M., P. Zwolak, et al. (2006). "Novel cytosine deaminase fusion gene enhances the effect of radiation on breast cancer in bone by reducing tumor burden, osteolysis, and skeletal fracture." Clin Cancer Res **12**(10): 3168-76.

Painful breast carcinoma metastases in bone are a common manifestation of malignant disease. Eradication of these tumors can be evasive, and as a result, skeletal morbidity increases with disease progression. The treatment potential of cytosine deaminase (CD) gene therapy combined with radiation treatment was evaluated in vitro and in vivo using a 4T1 murine breast carcinoma model. 4T1 carcinoma cells were transduced with a fusion gene encoding the extracellular and transmembrane domains of the human nerve growth factor receptor and the cytoplasmic portion of the yeast CD gene (NGFR-CD(y)). CD-expressing tumor cells (4TCD(y)) were highly sensitive to treatment by 5-fluorocytosine

prodrug ($P < 0.0001$). 5-Fluorocytosine treatment of 4TCD(y), but not 4T1 cells, enhanced the effects of radiation in vitro ($P < 0.0001$). 5-Fluorocytosine prodrug treatment also increased the therapeutic potential of radiation in vivo. Mice with 4TCD(y) intrafemoral tumors showed increased effectiveness of radiation based on improved reductions in tumor size, reductions in tumorigenic osteolysis, and a decrease in skeletal fractures ($P < 0.01$).

Goss, J. R., C. F. Harley, et al. (2002). "Herpes vector-mediated expression of proenkephalin reduces bone cancer pain." Ann Neurol **52**(5): 662-5.

We examined whether a herpes simplex virus vector that expresses human proenkephalin could be used to attenuate nociception in a model of bone cancer pain in mice. Osteolytic sarcoma cells were implanted into the medullary space of the right femur, followed by a subcutaneous inoculation of a replication-defective herpes simplex virus vector expressing human proenkephalin (vector SHPE) or a lacZ-expressing control vector (vector SHZ). SHPE-inoculated mice demonstrated a significant, naltrexone-reversible decrease in pain-related behavior assessed during open-field motor activity. These results suggest that gene transfer with an enkephalin-expressing vector may be used to treat pain resulting from cancer in bone.

Guise, T. A. (2009). "Breaking down bone: new insight into site-specific mechanisms of breast cancer osteolysis mediated by metalloproteinases." Genes Dev **23**(18): 2117-23.

Bone metastases are the most common skeletal complication of malignancy. Tumor cells disrupt normal bone remodeling to promote bone destruction and its associated morbidity. In the August 15, 2009, issue of Genes & Development, Lu and colleagues (pp. 1882-1894) propose a novel molecular mechanism by which tumor-produced metalloproteinases release epidermal growth factor (EGF) ligands to activate the central osteoclastogenic pathway receptor activator of NF-kappaB ligand (RANKL) to promote breast cancer osteolysis. This work has important therapeutic applications that may quickly translate to more effective treatment for bone metastases.

Hall, D. C., T. L. Johnson-Pais, et al. (2008). "Maspin reduces prostate cancer metastasis to bone." Urol Oncol **26**(6): 652-8.

Maspin is a serine protease inhibitor with anti-tumor activity, including inhibition of tumor growth, angiogenesis, invasion, motility, and metastasis. Normal mammary and prostate cells express maspin at high levels. In contrast, breast and prostate cancer tissue samples and cell lines exhibit reduced or no expression of the maspin transcript. Previously we have demonstrated that introduction of an intact chromosome 18 into the bone-derived metastatic prostate cancer cell line, PC-3, resulted in reduced in vitro growth and in vivo metastatic potential. The goal of this study was to determine whether maspin is the tumor/metastasis suppressor on chromosome 18 responsible for this phenotype. To investigate whether maspin, when produced at endogenous levels, is capable of inhibiting metastasis to bone we transfected a bacterial artificial chromosome (BAC) genomic clone containing the maspin gene into PC-3 cells that aggressively metastasize to bone in an animal model. The BAC transfected PC-3 cells exhibited an in vitro phenotype consistent with maspin acting as a tumor suppressor. Analysis of the PC-3 maspin transfectants in an in vivo bone metastasis assay resulted in significant reduction of the number and severity of skeletal metastasis, compared with parental PC-3 cells. However, maspin had no effect on the ability of PC-3 cells to metastasize to extra-skeletal sites in this model. These results indicate that maspin expression likely plays a role in reducing the tumor cell's ability to seed to bone or in inhibition of growth in the bone microenvironment. However, it does not affect the ability to metastasize to distant sites.

Hamada, S., K. Satoh, et al. (2007). "Bone morphogenetic protein 4 induces epithelial-mesenchymal transition through MSX2 induction on pancreatic cancer cell line." J Cell Physiol **213**(3): 768-74.

In our study, we found that bone morphogenetic protein 4 (BMP4) has a novel effect as an inducer of epithelial-mesenchymal transition (EMT) on Panc-1 cells, a human pancreatic carcinoma cell line. BMP4-treated Panc-1 cells showed loose cell contacts and a scattered, fibroblast-like appearance along with E-cadherin downregulation, Vimentin upregulation and enhanced cell migration, which are characteristic of EMT. BMP4 treatment also induced homeobox gene MSX2 expression, which we previously showed to be associated with EMT in pancreatic carcinoma cells. BMP4 treatment activated the Smad signaling pathway, and extracellular signal-related kinase (ERK) and p38 mitogen-activated kinase (MAPK) pathways in these cells. MSX2 was markedly induced by BMP4 through the ERK and p38 MAPK pathways in collaboration with the Smad signaling pathway. The repression of E-cadherin, induction of Vimentin and enhanced cell migration disappeared when siRNA-based MSX2 downregulated pancreatic cancer cells were treated with BMP4. These findings indicate that BMP4 may be involved in

pancreatic carcinoma development through the promotion of EMT and that MSX2 is indispensable to this process.

Haudenschild, D. R., S. M. Palmer, et al. (2004). "Bone morphogenetic protein (BMP)-6 signaling and BMP antagonist noggin in prostate cancer." Cancer Res **64**(22): 8276-84.

It has been proposed that the osteoblastic nature of prostate cancer skeletal metastases is due in part to elevated activity of bone morphogenetic proteins (BMPs). BMPs are osteoinductive morphogens, and elevated expression of BMP-6 correlates with skeletal metastases of prostate cancer. In this study, we investigated the expression levels of BMPs and their modulators in prostate, using microarray analysis of cell cultures and gene expression. Addition of exogenous BMP-6 to DU-145 prostate cancer cell cultures inhibited their growth by up-regulation of several cyclin-dependent kinase inhibitors such as p21/CIP, p18, and p19. Expression of noggin, a BMP antagonist, was significantly up-regulated by BMP-6 by microarray analysis and was confirmed by quantitative reverse transcription-polymerase chain reaction and at the protein level. Noggin protein was present in prostate biopsies and localized to the epithelial components of prostate by immunohistochemistry. Recombinant noggin inhibited the function of BMP-6, suggesting a negative feedback regulation of BMP activity and indicating a strategy for the development of a novel therapeutic target in the treatment of painful osteosclerotic bone metastases of prostate cancer.

Hiraga, T. and H. Nakamura (2009). "Imatinib mesylate suppresses bone metastases of breast cancer by inhibiting osteoclasts through the blockade of c-Fms signals." Int J Cancer **124**(1): 215-22.

Imatinib mesylate (imatinib) is a potent and selective inhibitor of the tyrosine kinases, Bcr-Abl, c-Kit and platelet-derived growth factor receptors (PDGFRs). Recently, it has been reported that imatinib also targets the macrophage colony-stimulating factor (M-CSF) receptor c-Fms. M-CSF signals are essential for the differentiation of osteoclasts. Bone metastases of breast cancer are frequently associated with osteoclastic bone destruction. Furthermore, several lines of evidence suggest that osteoclasts play central roles in the development and progression of bone metastases. Thus, in the present study, we examined the effects of imatinib on bone metastases of breast cancer. Coimmunoprecipitation assays showed that imatinib inhibited the M-CSF-induced phosphorylation of c-Fms in osteoclast precursor cells as well as the PDGF-induced PDGFR phosphorylation in MDA-MB-231 human breast cancer cells. Imatinib also markedly reduced osteoclast formation in vitro. In contrast, those concentrations of imatinib did not affect osteoblast differentiation. We then examined the effects of imatinib on bone metastases of MDA-MB-231 cells in a nude mouse model. Radiographic and histomorphometric analyses demonstrated that imatinib significantly decreased bone metastases associated with the reduced number of osteoclasts. In support of the notion that the inhibition of c-Fms acts to suppress the development of bone metastases, we found that a specific inhibitor of c-Fms Ki20227 also decreased bone metastases. In conclusion, these results collectively suggest that imatinib reduced bone metastases, at least in part, by inhibiting osteoclastic bone destruction through the blockade of c-Fms signals. Our results also suggest that imatinib may have a protective effect against cancer treatment-induced bone loss.

Hsieh, C. L., T. A. Gardner, et al. (2004). "Cotargeting tumor and stroma in a novel chimeric tumor model involving the growth of both human prostate cancer and bone stromal cells." Cancer Gene Ther **11**(2): 148-55.

Stromal-epithelial interaction contributes to local prostate tumor growth, androgen-independent progression and distant metastasis. We have established in vitro coculture and in vivo chimeric tumor models to evaluate the roles of stromal cells isolated from either osteosarcoma or normal bone, a site where prostate cancer cells frequently metastasize, in contributing to the growth and survival of human prostate cancer cells. We have evaluated extensively the effects of toxic gene therapy using luciferase-tagged chimeric human prostate cancer models both in vitro and in vivo. In the in vitro cocultured cell model, we assessed cancer cell growth and residual cellular proteins after targeting either prostate cancer epithelial cells alone or both prostate cancer and bone stromal cells. In the in vivo animal model, we measured tumor volume and serum prostate-specific antigen (PSA) in mice bearing chimeric prostate tumors comprised of human prostate tumor cells and normal bone stromal cells. Our results demonstrated that: (1) The rate of human prostate cancer cell growth in vitro is accelerated by coculturing with human and rat osteosarcoma or normal mouse bone marrow stromal cell lines. No growth stimulation was noted when cocultured with a human prostate epithelial cell line. (2) Disabling the growth of normal bone stromal cells using transgenic targeting with a bystander gene, herpes simplex virus thymidine kinase (hsv-TK), plus the pro-drug ganciclovir (GCV) or acyclovir markedly depressed the growth of cocultured human prostate cancer cells in vitro and human prostate cancer-mouse

normal bone stroma chimeric tumors in vivo. (3) By cotargeting both human prostate cancer and normal mouse bone stromal cells in vitro with an adenoviral construct, Ad-hOC-TK (a replication-defective Ad5 vector with the bystander transgene hsv-TK under the control of a human osteocalcin (hOC) promoter) plus GCV4, we observed greater inhibition of tumor cell growth than by targeting a single cell compartment with Ad-PSA-TK (a vector construct similar to Ad-hOC-TK except that the transgene expression is under regulation by a full-length human PSA promoter). These results, taken together, established a basic principle that cotargeting both tumor and its supporting stroma is more efficacious than targeting a single cell compartment in the treatment of human prostate cancer bone metastasis. This principle can be applied to other clinical conditions of cancer growth where stroma contribute to the overall growth and survival potential of the 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.

Huang, W. C., Z. Xie, et al. (2005). "Human osteocalcin and bone sialoprotein mediating osteomimicry of prostate cancer cells: role of cAMP-dependent protein kinase A signaling pathway." Cancer Res **65**(6): 2303-13.

Osteocalcin and bone sialoprotein are the most abundant noncollagenous bone matrix proteins expressed by osteoblasts. Surprisingly, osteocalcin and bone sialoprotein are also expressed by malignant but not normal prostate epithelial cells. The purpose of this study is to investigate how osteocalcin and bone sialoprotein expression is regulated in prostate cancer cells. Our investigation revealed that (a) human osteocalcin and bone sialoprotein promoter activities in an androgen-independent prostate cancer cell line of LNCaP lineage, C4-2B, were markedly enhanced 7- to 12-fold in a concentration-dependent manner by conditioned medium collected from prostate cancer and bone stromal cells. (b) Deletion analysis of human osteocalcin and bone sialoprotein promoter regions identified cyclic AMP (cAMP)-responsive elements (CRE) as the critical determinants for conditioned medium-mediated osteocalcin and bone sialoprotein gene expression in prostate cancer cells. Consistent with these results, the protein kinase A (PKA) pathway activators forskolin and dibutyryl cAMP and the PKA pathway inhibitor H-89, respectively, increased or repressed human osteocalcin and bone sialoprotein promoter activities. (c) Electrophoretic mobility shift assay showed that conditioned medium-mediated stimulation of human osteocalcin and bone sialoprotein promoter activities occurs through increased interaction between CRE and CRE-binding protein. (d) Conditioned medium was found to induce human osteocalcin and bone sialoprotein promoter activities via increased CRE/CRE-binding protein interaction in a cell background-dependent manner, with marked stimulation in selected prostate cancer but not bone stromal cells. Collectively, these results suggest that osteocalcin and bone sialoprotein expression is coordinated and regulated through cAMP-dependent PKA signaling, which may define the molecular basis of the osteomimicry exhibited by prostate cancer cells.

Ishii, S., S. Tsuji, et al. (2008). "Involvement of bone marrow-derived stromal cells in gastrointestinal cancer development and metastasis." J Gastroenterol Hepatol **23 Suppl 2**: S242-9.

The involvement of bone marrow (BM) in tumor-stroma reactions or tumor development has not been examined in a cancer allograft, which has otherwise been appropriate for assessing therapeutic modalities. We investigated the fate of BM-derived cells in colon cancer allografts and liver metastases in mice. C57BL/6 mice were irradiated and rescued by BM transplantation from green fluorescent protein (GFP)-transgenic mice. MC38 colon cancer cells were stably transfected with the pDsRed gene in order to identify tumor cells by fluorescence. These were inoculated into the mice to generate subcutaneous allografted tumors or liver metastases. The tumors were observed under confocal microscopy and fluorescent immunohistochemistry to determine the

fate of tumor versus BM-derived cells. GFP-positive (GFP(+)) cells were consistently identified as vimentin(+), alpha-smooth muscle actin (alphaSMA)(+), spindle-shaped stromal cells in both the subcutaneous tumors and the liver metastases. GFP(+) cells of leukocyte lineage also infiltrated the tumors. Neither GFP(+) CD31(+) endothelial cells nor GFP(+) DsRed(+) cells were detected in the tumor. CONCLUSIONS: BM-derived cells frequently and consistently infiltrated the tumor allografts and metastases as interstitial cells and leukocytes. Cells derived from the fusion of BM cells and tumor cells were not observed. This model may be appropriate for the clarification of the effects of anticancer therapies and the study of BM-derived cells in tumor-host interactions.

Kaifi, J. T., E. F. Yekebas, et al. (2005). "Tumor-cell homing to lymph nodes and bone marrow and CXCR4 expression in esophageal cancer." J Natl Cancer Inst **97**(24): 1840-7.

The chemokine and bone marrow-homing receptor CXCR4 has been implicated in metastatic dissemination of various cancers. We investigated CXCR4 expression in esophageal cancer specimens and its association with survival, lymph node microinvolvement, and bone marrow micrometastasis. We analyzed frozen tumor specimens from 136 patients with completely resected esophageal cancer for CXCR4 expression by immunohistochemistry. Lymph node microinvolvement and bone marrow micrometastasis were assessed by immunohistochemistry with monoclonal antibodies Ber-EP4 (against epithelial cell adhesion molecule) and pancytokeratin A45-B/B3 (against several cytokeratins), respectively. Associations between CXCR4 expression and clinicopathologic features, including tumor stage, histologic grade, lymph node metastasis and microinvolvement, bone marrow micrometastasis, and survival, were investigated with Fisher's test, log-rank test, and Cox multivariable analysis. All statistical tests were two-sided. CXCR4 protein was expressed in 75 (55%) of 136 esophageal tumors examined. CXCR4 expression was statistically significantly associated with reduced median overall and disease-specific survival, compared with CXCR4 nonexpression ($P < .001$; log-rank test). The median overall survival of patients with CXCR4-positive tumors was 20 months and with CXCR4-negative tumors, 76 months (difference = 56 months, 95% confidence interval [CI] = 4 to 108 months; $P < .001$). The median disease-specific survival of patients with CXCR4-positive tumors was 25 months and with CXCR4-negative tumors was 97 months (difference = 72 months, 95% CI = 34 to 110 months; $P < .001$). CXCR4 expression was statistically significantly associated with increased lymph node microinvolvement ($P < .001$) and with increased bone marrow micrometastasis ($P < .001$). In multivariable analysis, CXCR4 expression, compared with its nonexpression, was identified as the independent variable that was most strongly associated with reduced disease-specific survival (relative risk [RR] of death = 2.03, 95% CI = 1.20 to 3.41; $P = .008$) and overall survival (RR of death = 2.18, 95% CI = 1.33 to 3.59; $P = .002$). CONCLUSION: CXCR4 expression was associated with poor clinical outcome in esophageal cancer patients. CXCR4 may have a role in early metastatic spread because its expression was associated with micrometastases to both the lymph nodes and bone marrow. Thus, CXCR4 should be explored further as a target for adjuvant therapy for micrometastatic disease.

Kleibl, K. and G. P. Margison (1998). "Increasing DNA repair capacity in bone marrow by gene transfer as a prospective tool in cancer therapy." Neoplasma **45**(4): 181-6.

Resistance of tumor cells to alkylating anticancer agents that produce adducts at the O6 position of guanine in DNA, the O6-alkylating agents, correlates with the expression of O6-alkylguanine-DNA alkyltransferase (ATase). O6-benzylguanine and related pseudosubstrates are able to inactivate human ATase in vitro and in vivo and they are being tested as chemotherapeutic adjuvants for enhancing the effectiveness of O6-alkylating drugs. On the other hand, the clinical consequences of ATase depletion may be fatal for some sensitive systems e.g. hematopoiesis. To overcome this problem, strategies for the protection of primary bone marrow cells by targeted transfer of pseudosubstrate-resistant ATase genes have been considered and recently achieved at the laboratory level. This approach could therefore be now extended to a clinical cancer gene therapy program.

Klein, A., C. Olendrowitz, et al. (2009). "Identification of brain- and bone-specific breast cancer metastasis genes." Cancer Lett **276**(2): 212-20.

In breast cancer, metastases are relatively widely distributed, with the most common sites being bone, regional lymph nodes, lung, liver, and brain. The detailed mechanism of organ-specific metastasis is poorly understood. In this study, we initiated a search for genes that are implicated in brain or bone metastasis of primary human breast cancer. We generated gene expression profiles of 18 brain and eight bone metastases derived from primary breast tumors. We identified 73 genes differentially expressed between brain and bone metastases. Visualization of the differential gene expression

profiles by correspondence and cluster analyses shows that the metastases clearly separate into two distinct groups as an exact reflection of their site of metastasis. Moreover, the analysis of this gene set in primary breast tumors relapsing to either bone or brain allowed accurate categorization of the tumors according to their metastatic site. The identified genes may prove to be excellent markers to predict the site of metastasis in breast cancer patients and could lead to tailor-made therapy to an individual patient.

Koc, O. N., J. S. Reese, et al. (1999). "DeltaMGMT-transduced bone marrow infusion increases tolerance to O6-benzylguanine and 1,3-bis(2-chloroethyl)-1-nitrosourea and allows intensive therapy of 1,3-bis(2-chloroethyl)-1-nitrosourea-resistant human colon cancer xenografts." Hum Gene Ther **10**(6): 1021-30.

O6-Benzylguanine (BG) is a potent inhibitor of the DNA repair protein 06-alkylguanine DNA alkyltransferase (AGT), and sensitizes tumors to BCNU in vitro and in xenografts. The combination of BG and BCNU is now undergoing phase I clinical testing. The maximally tolerated dose of BCNU given after BG is expected to be lower then the doses tolerated as a single agent owing to BG sensitization of hematopoietic progenitors. We have previously shown that retroviral expression of G156A mutant MGMT (deltaMGMT) in mouse and human marrow cells results in significant BG and BCNU resistance. In this study we evaluated the effect of deltaMGMT-transduced marrow infusion on the therapeutic index of multiple BG and BCNU treatments in tumor-bearing nude (nu/nu athymic) mice. Prior to subcutaneous implantation of BCNU-resistant SW480 human colon cancer cells, cohorts of mice were given intraperitoneal injections of nonablative doses of BG (30 mg/kg) and BCNU (10 mg/kg, one-half of the LD10) and then infused with 1-2 x 10(6) isogeneic deltaMGMT (n = 29 mice) or lacZ-transduced (n = 20 mice) marrow cells. The xenograft-bearing mice were treated with multiple cycles of BG (30 mg/kg) and BCNU (10-25 mg/kg). After three cycles, deltaMGMT mouse bone marrow was repopulated with CFU containing the provirus, and demonstrated a 2.7-fold increase in AGT activity and a 5.5-fold increase in BCNU IC90 compared with LacZ mice. After five cycles, the BCNU IC90 of CFU cells increased nine-fold over control cells, indicating selective enrichment of CFU precursor cells expressing high levels of deltaMGMT. Starting with the third cycle of therapy, tolerance to BG and BCNU was significantly improved in deltaMGMT mice compared with LacZ mice, as evidenced by preserved peripheral blood counts, bone marrow cellularity, and CFU content 1 and 2 weeks posttreatment and a significantly higher survival rate. Xenograft growth was significantly delayed in mice tolerating multiple cycles and higher dose intensity of BG and BCNU as compared with mice receiving less intensive therapy. We conclude that deltaMGMT-transduced marrow cells can improve the therapeutic index of BG and BCNU by selectively repopulating the marrow and providing significant marrow tolerance to this combination, allowing intensive therapy of a BCNU-resistant tumor.

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

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

Koeneman, K. S., C. Kao, et al. (2000). "Osteocalcin-directed gene therapy for prostate-cancer bone metastasis." World J Urol **18**(2): 102-10.

Osteocalcin (OC) is a major noncollagenous bone protein whose expression is limited almost exclusively to osteotropic tumors and mature calcified tissue (differentiated osteoblasts). The function of OC, a highly conserved gamma-carboxyglutamic acid-

containing protein, relies in part on its ability to bind hydroxyapatite and act as a chemoattractant for bone-resorbing cells. Serum osteocalcin levels are used clinically as an index of active bone turnover. Research in our laboratory has revealed that OC is expressed in several solid tumors, including osteosarcoma and ovarian, lung, brain, and prostate cancers. Evidence arising from reverse-transcription polymerase chain reaction (RT-PCR; detection of OC mRNA), immunohistochemical staining (detection of OC protein), and transient transfection and reporter assay (detection of OC mRNA transcription) reveals that OC expression is up-regulated in numerous solid tumors, with its expression being further elevated in androgen-independent prostate cancers. A recombinant, replication-defective adenovirus, Ad-OC-TK (OC promoter-driven herpes-simplex-virus thymidine kinase) was constructed and, when combined with the appropriate prodrug, either ganciclovir (GCV) or acyclovir (ACV), was found to be effective at destroying prostate-cancer cell lines in vitro and prostate tumor xenografts in vivo in both subcutaneous and bone sites. Additionally, via use of the OC promoter the supporting bone stromal cells are cotargeted when the prostate cancer interdigitates with bone stroma at the metastatic skeletal sites. Thus, maximal tissue-specific cell toxicity is achieved by the interruption of cellular communication between the prostate cancer and the bone stroma. We describe herein the preclinical foundation as well as the design and implementation of an ongoing phase I clinical trial at the University of Virginia that targets androgen-independent metastatic prostate cancer using the Ad-OC-TK vector.

Koeneman, K. S., F. Yeung, et al. (1999). "Osteomimetic properties of prostate cancer cells: a hypothesis supporting the predilection of prostate cancer metastasis and growth in the bone environment." Prostate **39**(4): 246-61.

Unlike most other malignancies, prostate cancer metastasizes preferentially to the skeleton and elicits osteoblastic reactions. We present a hypothesis, based upon results obtained from our laboratory and others, on the nature of progression of prostate cancer cells and their predilection to growth and metastasis in the bone microenvironment. We propose the hypothesis that osseous metastatic prostate cancer cells must be osteomimetic in order to metastasize, grow, and survive in the skeleton. The reciprocal interaction between prostate cancer and bone stromal growth factors, including basic fibroblast growth factor (bFGF), hepatocyte growth factor/scatter factor (HGF/SF), and especially the insulin growth factor (IGF) axis initiates bone tropism, and is enhanced by prostate secreted endothelin-1 (ET-1) and urokinase-type plasminogen activator (uPA). Growth factors and peptides that have differentiating activity, such as transforming growth factor beta (TGF-beta), parathyroid hormone-related protein (PTH-rp), and the bone morphogenetic proteins (BMPs), can shift local homeostasis to produce the characteristic blastic phenotype, via interaction with prostate-secreted human kalikrein 2 (hK2), and prostate-specific antigen (PSA). This proposal asserts that altering the expression of certain critical transcription factors, such as Cbfa and MSX in prostate cancer cells, which presumably are under the inductive influences of prostate or bone stromal cells, can confer profiles of gene expression, such as osteopontin (OPN), osteocalcin (OC), and bone sialoprotein (BSP), that mimic that of osteoblasts. Elucidation of common proteins, presumably driven by the same promoters, expressed by both prostate cancer and bone stromal cells, could result in the development of novel preventive and therapeutic strategies for the treatment of prostate cancer skeletal metastasis. Agents developed using these strategies could have the potential advantage of interfering with growth and enhancing apoptosis in both prostate cancer and bone stromal compartments. The selective application of gene therapy strategy, driven by tissue-specific and tumor-restricted promoters for the safe delivery and expression of therapeutic genes in experimental models of prostate cancer metastasis, is discussed.

Kominsky, S. L. and N. E. Davidson (2006). "A "bone" fide predictor of metastasis? Predicting breast cancer metastasis to bone." J Clin Oncol **24**(15): 2227-9.

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.

Kumagai, T., K. Tomari, et al. (2006). "Alteration of gene expression in response to bone morphogenetic protein-2 in androgen-dependent human prostate cancer LNCaP cells." Int J Mol Med **17**(2): 285-91.

Bone morphogenetic protein (BMP)-2, a multifunctional member of the transforming growth factor (TGF)-beta superfamily with powerful osteoinductive effects, has various biological activities in a variety of cells. We observed that BMP-2 inhibits cell proliferation in the androgen-dependent human prostate cancer cell line, LNCaP. To investigate the mechanism of inhibition of androgen-dependent growth by BMP-2, we compared the gene expression in LNCaP cells treated with dihydrotestosterone (DHT) to that of LNCaP cells treated with DHT and BMP-2, using DNA microarray analysis. Of 8,400 human genes on the gene chip, 38 genes were up-regulated by >2.0-fold and 48 genes were down-regulated by <0.5-fold by treatment with BMP-2. These genes were involved in a variety of cellular functions, including signal transduction, transcription regulation, enzymes, transporters, structural molecules and translation. RT-PCR analysis showed that CH1CL and BMX were up-regulated and DACH1 and WNT5A were down-regulated by treatment with BMP-2. Furthermore, we detected an increase of WNT5A protein in the medium by DHT and inhibition of the increase by BMP-2. In the present study, we identified several BMP-2-responsive genes in LNCaP cells. Further studies of the roles of these genes may clarify the mechanisms underlying the inhibition of cell proliferation by BMP-2 and identify better approaches for the prevention and treatment of prostate cancer.

Lang, S. I., T. Kottke, et al. (2007). "Unbiased selection of bone marrow derived cells as carriers for cancer gene therapy." J Gene Med **9**(11): 927-37.

There is currently great interest in development of cell-based carriers for delivery of viral vectors to metastatic tumors. To date, several cell carriers have been tested based largely upon their predicted tumor-localizing properties. However, cell types may exist which can be mobilized from the circulation by a tumor which have not yet been identified. Here we use an unbiased screen of bone marrow (BM) cells to identify cells which localize to tumors and which might serve as effective candidate cell carriers without any prior prediction or selection. Unsorted BM cells from green fluorescent protein (GFP)-transgenic donor mice were adoptively transferred into C57Bl/6 mice bearing pre-established subcutaneous B16 melanoma tumors. Forty-eight hours and eight days later, tumors, organs and blood were analyzed for GFP-expressing cells by flow cytometry. The phenotype of GFP cells in organs was determined by co-staining with specific cell surface markers. CD45(+) hematopoietic cells were readily detected in tumor, spleen, bone marrow, blood and lung at both time points. Within these CD45(+) cell populations, preferential accumulation in the tumor was observed of cells expressing Sca-1, c-kit, NK1.1, Thy1.2, CD14, Mac-3 and/or CD11c. Lymphodepletion increased homing to spleen and bone marrow, but not to tumors. CONCLUSIONS: We have used an in vivo screen to identify populations of BM-derived donor cells which accumulate within tumors. These studies will direct rational selection of specific cell types which can be tested in standardized assays of cell carrier efficiency for the treatment of metastatic tumors.

Li, B. (2008). "Bone morphogenetic protein-Smad pathway as drug targets for osteoporosis and cancer therapy." Endocr Metab Immune Disord Drug Targets **8**(3): 208-19.

Bone morphogenetic proteins (BMPs) are members of the TGF-beta superfamily. Engaging of BMPs to BMP receptors on the cell surface leads to activation of the receptor kinase activity, which phosphorylates Smad1/5/8. Smad1, 5, or 8, with Smad4, forms a complex, which is translocated to the nucleus, where it binds to the consensus DNA sequence to regulate the transcription of BMP target genes. BMP-Smad signaling regulates stem cell renewal, cell proliferation, differentiation, migration, and apoptosis, and controls embryo development and postnatal tissue homoeostasis. Both human and mouse genetic studies have demonstrated that BMPs play positive roles in postnatal bone homeostasis including osteoblast expansion, differentiation, and bone formation. Defects in BMP-Smad signaling cause bone-related disorders such as osteoporosis, a disease that affects hundreds of millions of people. In addition, BMP-Smad signaling has been shown to play an important role in tumorigenesis. Mounting evidence indicates that in many tissues, BMP-Smad signaling has a tumor-suppressing activity and that BMPs can repress tumor growth. These findings suggest that BMP-Smad pathway can be a potential

target not only for osteoporosis therapy but also for cancer therapy.

Li, Y., M. Che, et al. (2004). "Regulation of gene expression and inhibition of experimental prostate cancer bone metastasis by dietary genistein." Neoplasia **6**(4): 354-63.

Prostate cancer frequently metastasizes to the bone, and the treatment outcome for metastatic prostate cancer has been disappointing so far. Dietary genistein, derived primarily from soy product, has been proposed to be partly responsible for the low rate of prostate cancer in Asians. Our previous studies have shown that genistein elicits pleiotropic effects on prostate cancer cells, but there are no studies documenting comprehensive gene expression profiles and antitumor effects of dietary genistein on human prostate cancer grown in human bone environment. In this study, we investigated the effects of genistein on PC3 prostate cancer cells and experimental PC3 bone tumors created by injecting PC3 cells into human bone fragments previously implanted in severe combined immunodeficient (SCID) mice (SCID human model). We found that genistein significantly inhibited PC3 bone tumor growth using both prevention and intervention strategies. By using microarray and real-time polymerase chain reaction technology, we found that genistein regulated the expression of multiple genes involved in the control of cell growth, apoptosis, and metastasis both in vitro and in vivo. For example, the expression of various metalloproteinases (MMPs) in PC3 bone tumors was inhibited by genistein treatment, whereas osteoprotegerin was upregulated. MMP immunostaining and transfection experiments also demonstrated that MMP-9 expression was inhibited in PC3 cells in vitro and PC3 bone tumors in vivo after genistein treatment. These results, particularly the in vivo results, demonstrate that dietary genistein may inhibit prostate cancer bone metastasis by regulating metastasis-related genes. Genistein may thus be a promising agent for the prevention and/or treatment of prostate cancer.

Loberg, R. D., C. J. Logothetis, et al. (2005). "Pathogenesis and treatment of prostate cancer bone metastases: targeting the lethal phenotype." J Clin Oncol **23**(32): 8232-41.

Traditionally, prostate cancer treatment, as well as all cancer treatment, has been designed to target the tumor cell directly via various hormonal and chemotherapeutic agents. Recently, the realization that cancer cells exist in complex microenvironments that are essential for the tumorigenic and metastatic potential of the cancer cells is starting the redefine the paradigm for cancer therapy. The propensity of prostate cancer cells to metastasize to bone is leading to the design of novel therapies targeting both the cancer cell as well as the bone microenvironment. Tumor cells in the bone interact with the extracellular matrix, stromal cells, osteoblasts, osteoclasts, and endothelial cells to promote tumor-cell survival and proliferation leading to a lethal phenotype that includes increased morbidity and mortality for patients with advanced prostate cancer. Several strategies are being developed that target these complex tumor cell-microenvironment interactions and target the signal transduction pathways of other cells important to the development of metastases, including the osteoclasts, osteoblasts, and endothelial cells of the bone microenvironment. Current and new therapies in metastatic prostate cancer will comprise a multitargeted approach aimed at both the tumor cell and the tumor microenvironment. Here, we review the current therapeutic strategies for targeting the prostate cancer-bone microenvironment and several single- and multiagent targeted approaches to the treatment of advanced prostate cancer that are under development.

Loh, K., J. A. Chia, et al. (2008). "Bone morphogenic protein 3 inactivation is an early and frequent event in colorectal cancer development." Genes Chromosomes Cancer **47**(6): 449-60.

Bone morphogenic proteins (BMPs) are members of the TGFB growth factor superfamily with well-described functions in bone formation. Although disrupted BMP signalling in tumor development has more recently been investigated, a role for BMP3 in colorectal cancer (CRC) has remained largely unexplored. The aim of this study was to investigate BMP3 disruption in CRCs in relation to both the traditional and serrated pathways of tumor progression. BMP3 was down-regulated as assessed by real-time PCR in 50 of 56 primary tumors (89%). Bisulfite sequencing of the putative promoter revealed extensive hypermethylation in the cell line HT29, in which expression could be restored by treatment with a methyltransferase inhibitor. Aberrant hypermethylation was observed in 33/60 (55%) tumors and was highly correlated with microsatellite instability ($P < 0.01$), the CpG Island Methylator Phenotype ($P < 0.01$), BRAF oncogene mutation ($P < 0.01$), and proximal location ($P < 0.001$). Methylation was also frequently observed in serrated and traditional adenomatous polyps (22/29, 76%). Re-introduction of BMP3 into cell lines revealed marked growth suppression supporting the functional relevance of this alteration in colorectal tumor development. This study provides molecular and functional data supporting the importance of BMP3 silencing as an early and frequent event in colorectal tumors progressing via the serrated and traditional pathways.

Malerba, I., L. Gribaldo, et al. (2005). "Induction of apoptosis and inhibition of telomerase activity in human bone marrow and HL-60 p53 null cells treated with anti-cancer drugs." Toxicol In Vitro **19**(4): 523-32.

Telomerase plays a key role in the maintenance of chromosomal stability in tumors, and the ability of anti-cancer agents to inhibit telomerase activity is under investigation. In this study, we evaluated the effect of etoposide and taxol, on the telomerase activity and telomere length in human leukaemia p53 null cells and human bone marrow cells, as well as apoptosis and cell cycle modulation. Results showed that after exposure to the drugs, HL-60 cells as well as the human progenitors underwent a block in G2 and subsequently apoptosis, whereas stromal cells from bone marrow did not undergo a block in G2 or enter apoptosis after etoposide exposure. Telomere length increased in stromal cells after treatment with both etoposide and taxol whereas in HL-60 cells only after etoposide treatment with. Bax, bcl-2 and bcl-x change their expression in stromal cells, whereas bcl-x was induced after drug treatment and bcl-2 down regulated in progenitor cells. Our data suggest that telomerase activity and apoptosis are correlated and they seem to be modulated by a common gene, bcl-2.

Margheri, F., S. D'Alessio, et al. (2005). "Effects of blocking urokinase receptor signaling by antisense oligonucleotides in a mouse model of experimental prostate cancer bone metastases." Gene Ther **12**(8): 702-14.

An important factor implicated in tumor cell predisposition for invasion and metastasis is the malignancy-related upregulation of urokinase plasminogen activator receptor (uPAR). uPAR signals by activating different tyrosine kinases in different cells. We examined the effects of inhibiting uPAR signaling by inhibition of uPAR expression with antisense oligonucleotides (aODNs) in PC3 human prostate cancer cells and evaluated aODN effect in a mouse model of prostate cancer bone metastasis. Following uPAR aODN treatment, PC3 cells exhibited a strong decrease in uPAR expression, evaluated by flow cytometry and by polymerase chain reaction, and of FAK/JNK/Jun phosphorylation. The synthesis of cyclins A, B, D1 and D3 was inhibited, as shown by Western blotting, flow cytometry and polymerase chain reaction, and PC3 cells accumulated in the G2 phase of the cell cycle. PC3 cells' adhesion was unaffected, while proliferation and invasion of Matrigel were impaired. A total of 60 mice were subjected to intracardiac injection of PC3 cells and were randomly assigned to three groups: aODN (treated with 0.5 mg intraperitoneum/mouse/day), dODN (treated with the same amounts of a degenerated ODN) and control (injected with a saline solution). At 28 days after heart injection, mice were subjected to a digital scan of total body radiography, which revealed 80% reduction in mice affected by bone metastasis. The use of uPAR aODNs produced a substantial prophylactic effect against prostate cancer bone metastasis, which has to be ascribed to downregulation of uPAR expression.

Masuda, T. A., A. Kataoka, et al. (2005). "Detection of occult cancer cells in peripheral blood and bone marrow by quantitative RT-PCR assay for cytokeratin-7 in breast cancer patients." Int J Oncol **26**(3): 721-30.

The clinical significance of occult micrometastasis (O.M) remains unknown. We investigated it in peripheral blood (P.B.) and bone marrow (B.M.) in breast cancer patients with surgery. First, we investigated the expression levels of 7 representative molecular markers for detecting O.M (CEA, CK-7, CK-18, CK-19, CK-20, MAM and MUC-1) in 27 cancer and 8 non-epithelial cell lines using quantitative RT-PCR (QRT-PCR), and showed that the expression level of CK-7 was higher in every cancer cell line than in the non-epithelial cell lines. Next, we studied the clinical significance of O.M in P.B. and B.M. by QRT-PCR for CK-7 in breast cancer patients with surgery. Based on comparison with 17 non-cancer controls, 37 (18.0%) and 100 (48.5%) of the 206 patients were positive for CK-7 in P.B. and B.M., respectively. In 98 cases observed over 24 months after surgery, the CK-7-positive group in P.B. had poorer disease-free survival (DFS) than the negative group ($p<0.01$). The CK-7-positive group in P.B. showed poorer DFS than the negative group in 132 lymph node-negative cases ($p=0.01$), and moreover, in 61 lymph node-negative cases observed over 24 months after surgery, the CK-7-positive group in P.B. showed poorer DFS than the negative group ($p<0.0001$). In B.M., no significant difference in DFS was found between the CK-7-positive and CK-7-negative groups. QRT-PCR for CK-7 could be a useful and universal method for detecting O.M, and the quantitative detection of CK-7 in P.B. would have a prognostic value as a marker of early recurrence in breast cancer patients with surgery.

Matsubara, S., Y. Wada, et al. (2001). "A conditional replication-competent adenoviral vector, Ad-OC-E1a, to cotarget prostate cancer and bone stroma in an experimental model of androgen-independent prostate cancer bone metastasis." Cancer Res **61**(16): 6012-9.

Prostate cancer has a high propensity to metastasize to bone, which often resists hormone,

radiation, and chemotherapies. Because of the reciprocal nature of the prostate cancer and bone stroma interaction, we designed a cotargeting strategy using a conditional replication-competent adenovirus to target the growth of tumor cells and their associated osteoblasts. The recombinant Ad-OC-E1a was constructed using a noncollagenous bone matrix protein osteocalcin (OC) promoter to drive the viral early E1a gene with restricted replication in cells that express OC transcriptional activity. Unlike Ad-PSE-E1a, Ad-OC-E1a was highly efficient in inhibiting the growth of PSA-producing (LNCaP, C4-2, and ARCaP) and nonproducing (PC-3 and DU145) human prostate cancer cell lines. This virus was also found to effectively inhibit the growth of human osteoblasts and human prostate stromal cells in vitro. Athymic mice bearing s.c. androgen receptor-negative and PSA-negative PC-3 xenografts responded to a single intratumoral administration of 2 x 10(9) plaque-forming unit(s) of Ad-OC-E1a. In SCID/bg mice, intraosseous growth of androgen receptor-positive and PSA-producing C4-2 xenografts responded markedly to i.v. administrations of a single dose of Ad-OC-E1a. One hundred percent of the treated mice responded to this systemic Ad-OC-E1a therapy with a decline of serum PSA to an undetectable level, and 80% of the mice with PSA rebound responded to the second dose of systemic Ad-OC-E1a. Forty percent of the mice were found to be cured by systemic Ad-OC-E1a without subsequent PSA rebound or tumor cells found in the skeleton. This cotargeting strategy shows a broader spectrum and appears to be more effective than systemic Ad-PSE-E1a in preclinical models of human prostate cancer skeletal metastasis.

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.

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. 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). 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.

Moreau, J., K. M. Anderson, et al. (2007). "Studies of osteotropism on both sides of the breast cancer-bone interaction." Ann N Y Acad Sci **1117**: 328-44.

While important advances have been made in the treatment of breast cancer (BrCa), little progress has been made in developing therapies for metastasis to bone, a complication that signals entry of the disease into an incurable phase. The process of identifying genes and gene signatures of BrCa associated with metastasis has begun. In contrast, knowledge of the contributions of bone to tumor-stroma interaction is still rudimentary. We are performing research designed to elucidate the mechanisms by which human BrCa metastasizes to bone (osteotropism). With evidence mounting that there is mutual recognition of BrCa and bone, we are investigating osteotropism from both sides of the tumor-stroma interface. We created a novel "all human" model in which human bone is transplanted into immunodeficient (NOD/SCID) mice. Human BrCa cells are injected into the mammary fat pad. Metastases later appear as metastases in the human bone, but not mouse skeleton. The model recapitulates the metastatic sequence occurring in patients. Using DNA microarrays, we plan to identify putative osteotropic genes expressed by metastatic BrCa cells. We will test the hypothesis that distinct "tool kits" are used by BrCa metastasizing to human bone. In addition, using human tissue-engineered bone, we are identifying components within bone stroma essential for metastasis, and osteotropism genes expressed by bone in response to the presence of BrCa. We recently demonstrated that tissue-engineered bone based on a silk sponge platform is a target for human BrCa metastasis, even in preference to the mouse skeleton.

Mouchess, M. L., Y. Sohara, et al. (2006). "Multimodal imaging analysis of tumor progression

and bone resorption in a murine cancer model." J Comput Assist Tomogr **30**(3): 525-34.

OBJECTIVE: This study evaluates the use of multimodal imaging to qualitatively and quantitatively measure tumor progression and bone resorption in a xenotransplanted tumor model of human neuroblastoma. Human neuroblastoma cells expressing a luciferase reporter gene were injected into the femur of nu/nu mice. Tumor progression with and without zoledronic acid treatment was monitored using radiographs, D-luciferin-induced luminescence, micro-computer tomography (CT) and micro-magnetic resonance imaging (MRI). We observed a gradual increase in D-luciferin-based bioluminescence concomitant with detectable osteolytic lesions. Tumor growth was inhibited (P=0.003-0.07) with zoledronic acid treatment. Micro-CT analysis in vivo provided a method to quantify bone loss, and its prevention by zoledronic acid. High-resolution MRI images allowed the observation of tumor cells within the bone marrow cavity, as well as distant metastasis. CONCLUSION: Multimodal imaging allows to measure tumor growth and bone resorption simultaneously in vivo and also proved useful in the detection distant metastasis.

Nam, J. S., A. M. Suchar, et al. (2006). "Bone sialoprotein mediates the tumor cell-targeted prometastatic activity of transforming growth factor beta in a mouse model of breast cancer." Cancer Res **66**(12): 6327-35.

Transforming growth factor betas (TGF-beta) play a dual role in carcinogenesis, functioning as tumor suppressors early in the process, and then switching to act as prometastatic factors in late-stage disease. We have previously shown that high molecular weight TGF-beta antagonists can suppress metastasis without the predicted toxicities. To address the underlying mechanisms, we have used the 4T1 syngeneic mouse model of metastatic breast cancer. Treatment of mice with a monoclonal anti-TGF-beta antibody (1D11) significantly suppressed metastasis of 4T1 cells to the lungs. When metastatic 4T1 cells were recovered from lungs of 1D11-treated and control mice, the most differentially expressed gene was found to be bone sialoprotein (Bsp). Immunostaining confirmed the loss of Bsp protein in 1D11-treated lung metastases, and TGF-beta was shown to regulate and correlate with Bsp expression in vitro. Functionally, knockdown of Bsp in 4T1 cells reduced the ability of TGF-beta to induce local collagen degradation and invasion in vitro, and treatment with recombinant Bsp protected 4T1 cells from complement-mediated lysis. Finally, suppression of Bsp in 4T1 cells reduced metastasis in vivo. We conclude that Bsp is a plausible mediator of at least some of the tumor cell-targeted prometastatic activity of TGF-beta in this model and that Bsp expression in metastases can be successfully suppressed by systemic treatment with anti-TGF-beta antibodies.

Niitsu, Y., Y. Takahashi, et al. (1998). "A proof of glutathione S-transferase-pi-related multidrug resistance by transfer of antisense gene to cancer cells and sense gene to bone marrow stem cell." Chem Biol Interact **111-112**: 325-32.

In order to directly prove the involvement of GST-pi in drug resistance, it's antisense gene was transduced into human colorectal cancer cell line which has been shown to express high level of GST-pi and the sensitivity of this cell line to anticancer drugs were assessed. The transfectant showed higher sensitivity to adriamycin (3.3-fold), Cisplatnum (2.3-fold), Melphalan (2.2-fold), Etoposode (2.2-fold) than the parental cell, while the sensitivity to vincristine, mitomicin C, 5-fluorouracil was unchanged by transfection. When the transfectant and parental cells were innoculated in nude mice and treated with adriamycin, a significant suppression of tumor growth was observed with the transfectant as compared to the parental cell. On the basis of this observation, we then transduced sense GST-pi gene into human bone marrow stem cells (CD34+ cells) to protect them from toxicity of anticancer drug. The gene transduced CD34+ cells formed more CFU-GM than nontransduced CD34+ cell in the presence of adriamycin (30 ng/ml). Thus, the autotransplantation of GST-pi gene transduced cell into cancer patients to protect the bone marrow from subsequent highdose chemotherapy is considered to be a new strategy for cancer gene therapy.

Niiyama, Y., T. Kawamata, et al. (2007). "Bone cancer increases transient receptor potential vanilloid subfamily 1 expression within distinct subpopulations of dorsal root ganglion neurons." Neuroscience **148**(2): 560-72.

Bone cancer pain has a strong impact on the quality of life of patients but is difficult to treat. Therefore, the mechanisms of bone cancer pain require elucidation for the purpose of development of new therapeutics. A recent study showed that activation of transient receptor potential vanilloid subfamily 1 (TRPV1) was involved in bone cancer pain. In this study, we re-evaluated the analgesic effects of pharmacological blockade of TRPV1 using the potent TRPV1 antagonist 5-iodoresiniferatoxin (I-RTX) and examined whether bone cancer can change TRPV1 expression and distribution in the primary sensory neurons in a mouse model of bone cancer pain. Implantation of osteosarcoma into the femur induced ongoing and movement-evoked bone cancer-related pain behaviors. These behaviors were

significantly reduced by i.p. administration of I-RTX, compared with vehicle. Western blot and reverse transcription-polymerase chain reaction (RT-PCR) analyses revealed that TRPV1 level was significantly increased in dorsal root ganglions (DRGs) ipsilateral to sarcoma implantation. Immunohistochemical analysis showed that implantation of osteosarcoma induced not only an increase in the percentage of TRPV1-positive neurons among DRG neurons (24.3+/-1.3% in sham mice and 31.2+/-1.3% in mice with osteosarcoma implantation, P<0.05) but also an overall shift in the distribution of area of profiles to the right. Colocalization study showed that the percentages of colocalization of TRPV1 with neurofilament 200 kD (NF200) and calcitonin gene-related peptide (CGRP) but not isolectin B4 (IB4) among DRG neurons in mice with osteosarcoma implantation were increased compared with those in sham mice (from 0.8+/-0.1% to 2.1+/-0.3% for TRPV1 and NF200 and from 21.1+/-1.3% to 26.5+/-0.2% for TRPV1 and CGRP). In conclusion, TRPV1 activation plays a critical role in the generation of bone cancer pain, and bone cancer increases TRPV1 expression within distinct subpopulation of DRG neurons. These findings may lead to novel strategies for the treatment of bone cancer pain.

Nishanian, T. G., J. S. Kim, et al. (2004). "Suppression of tumorigenesis and activation of Wnt signaling by bone morphogenetic protein 4 in human cancer cells." Cancer Biol Ther **3**(7): 667-75.

Aberrations in BMP signaling have recently been implicated as a cause of human cancer. Here we demonstrate and define the tumor suppressive properties of BMP4. Consistent with its potential role in a tumor suppressor pathway, BMP4 treatment eliminated the tumorigenic potential of an undifferentiated human cancer cell line. This loss of tumorigenicity was accompanied by an increase in apoptosis, alterations in cell cycle profile, and an increase in cell size. Interestingly, human colon cancer cells were resistant to the growth-suppressive properties of BMP4. To identify putative downstream mediators of BMP4-mediated tumor suppression, Affymetrix Genechips were employed to identify BMP4-regulated genes. The human BMP4 transcriptome was characterized by the modulation of many genes well known to play important roles in differentiation and development, including the induction of numerous genes involved in Wnt signaling. Modulation of Wnt gene expression by BMP4 had several functional consequences--BMP4 treatment led to activation of TCF reporters; complete activation of at least one BMP4-responsive gene required TCF sites; and treatment with a Wnt ligand was sufficient to mimic several of the phenotypic effects of BMP4 treatment. These data demonstrate the tumor suppressive properties of BMP4 signaling, show that colon cancer cells are resistant to BMP4-induced differentiation and growth suppression, further define the BMP4 transcriptome, and raise the intriguing possibility that interactions between the Wnt and BMP signaling pathways may play an important role in differentiation and tumor suppression.

Nunez, N. P., D. Jelovac, et al. (2004). "Effects of the antiestrogen tamoxifen and the aromatase inhibitor letrozole on serum hormones and bone characteristics in a preclinical tumor model for breast cancer." Clin Cancer Res **10**(16): 5375-80.

The purpose of this study was to evaluate and compare the effects of the antiestrogen tamoxifen and the aromatase inhibitor letrozole on tumor growth, serum hormones, uterine weight, body composition, and bone characteristics in mice. Human estrogen-dependent breast cancer cells stably transfected with the aromatase gene (MCF-7CA cells) were inoculated in Matrigel subcutaneously into ovariectomized nude mice. This model represents postmenopausal breast cancer in many respects, including the fact that estrogen is no longer produced by the ovaries and is not under feedback regulation by gonadotropins. Mice that received subcutaneously implanted MCF-7CA cancer cells were then treated with tamoxifen or letrozole for 7 weeks. As reported previously, tumor growth was markedly inhibited by both tamoxifen (100 microg/day) and letrozole (10 microg/day). Tamoxifen treatment led to increased bone mineral density (BMD) and hyperplastic uteri. Mice treated with letrozole had significantly smaller uteri than the controls and tamoxifen-treated mice. Letrozole did not affect BMD. There was no significant difference in systemic leptin and insulin-like growth factor I levels as a result of tamoxifen or letrozole treatment. CONCLUSIONS: Tamoxifen treatment inhibited breast cancer cell growth and increased BMD but caused uterine hypertrophy in this preclinical model of postmenopausal breast cancer. Letrozole inhibited tumor growth without inducing uterine hypertrophy. In addition, letrozole had no effect on BMD. These findings provide experimental evidence that letrozole is an effective and safe (in terms of risk of endometrial cancer risk and osteoporosis) alternative or complement to tamoxifen treatment for breast cancer.

Pariente, N., K. Morizono, et al. (2007). "A novel dual-targeted lentiviral vector leads to specific transduction of prostate cancer bone metastases in vivo after systemic administration." Mol Ther **15**(11): 1973-81.

Targeted gene transduction to organs and tissues of interest is the ultimate goal of therapeutic gene delivery. Lentiviral vectors (LVs) are powerful tools for stable gene delivery but their integration into undesired cell types poses a serious safety concern for their use in the clinic. Here we report the development of a new dual-targeted LV that can preferentially home to and express in prostate cancer bone metastases in vivo after systemic delivery. Transductional targeting is mediated by a modified Sindbis virus envelope that interacts with the prostate stem cell antigen (PSCA) expressed by prostate cancer cells, and transcriptional targeting is mediated by a prostate cell specific promoter. Homing to prostate tumors was achieved in 70% of the animals. Importantly, tumors could be detected in some cases by molecular imaging prior to X-ray detection. The dual-targeted vector presents enhanced specificity with respect to individual transcriptional or transductional targeted vectors. Transgene expression in the liver was 190 times lower than the expression associated with solely transductionally targeted vectors, and there was 12 times less vector DNA than the amount present with solely transcriptionally targeted vectors. The LV presented here is a powerful tool for obtaining stable and site-specific gene expression and can be easily modified for its use in other diseases.

Park, B. K., H. Zhang, et al. (2007). "NF-kappaB in breast cancer cells promotes osteolytic bone metastasis by inducing osteoclastogenesis via GM-CSF." Nat Med **13**(1): 62-9.

Advanced breast cancers frequently metastasize to bone, resulting in osteolytic lesions, yet the underlying mechanisms are poorly understood. Here we report that nuclear factor-kappaB (NF-kappaB) plays a crucial role in the osteolytic bone metastasis of breast cancer by stimulating osteoclastogenesis. Using an in vivo bone metastasis model, we found that constitutive NF-kappaB activity in breast cancer cells is crucial for the bone resorption characteristic of osteolytic bone metastasis. We identified the gene encoding granulocyte macrophage-colony stimulating factor (GM-CSF) as a key target of NF-kappaB and found that it mediates osteolytic bone metastasis of breast cancer by stimulating osteoclast development. Moreover, we observed that the expression of GM-CSF correlated with NF-kappaB activation in bone-metastatic tumor tissues from individuals with breast cancer. These results uncover a new and specific role of NF-kappaB in osteolytic bone metastasis through GM-CSF induction, suggesting that NF-kappaB is a potential target for the treatment of breast cancer and the prevention of skeletal metastasis.

Peters, C. M., T. H. Lindsay, et al. (2004). "Endothelin and the tumorigenic component of bone cancer pain." Neuroscience **126**(4): 1043-52.

Tumors including sarcomas and breast, prostate, and lung carcinomas frequently grow in or metastasize to the skeleton where they can induce significant bone remodeling and cancer pain. To define products that are released from tumors that are involved in the generation and maintenance of bone cancer pain, we focus here on endothelin-1 (ET-1) and endothelin receptors as several tumors including human prostate and breast have been shown to express high levels of ETs and the application of ETs to peripheral nerves can induce pain. Here we show that in a murine osteolytic 2472 sarcoma model of bone cancer pain, the 2472 sarcoma cells express high levels of ET-1, but express low or undetectable levels of endothelin A (ETAR) or B (ETBR) receptors whereas a subpopulation of sensory neurons express the ETAR and non-myelinating Schwann cells express the ETBR. Acute (10 mg/kg, i.p.) or chronic (10 mg/kg/day, p.o.) administration of the ETAR selective antagonist ABT-627 significantly attenuated ongoing and movement-evoked bone cancer pain and chronic administration of ABT-627 reduced several neurochemical indices of peripheral and central sensitization without influencing tumor growth or bone destruction. In contrast, acute treatment (30 mg/kg, i.p.) with the ETBR selective antagonist, A-192621 increased several measures of ongoing and movement evoked pain. As tumor expression and release of ET-1 has been shown to be regulated by the local environment, location specific expression and release of ET-1 by tumor cells may provide insight into the mechanisms that underlie the heterogeneity of bone cancer pain that is frequently observed in humans with multiple skeletal metastases.

Pinthus, J. H., T. Waks, et al. (2004). "Adoptive immunotherapy of prostate cancer bone lesions using redirected effector lymphocytes." J Clin Invest **114**(12): 1774-81.

Prostate cancer is currently the most commonly diagnosed noncutaneous malignancy in American men. When metastatic, usually to the bone, the disease is no longer curable and is usually treated palliatively with androgen ablation. However, after conversion to androgen-independent disease, there is no effective therapy currently available. The "T body" approach, which uses genetically reprogrammed lymphocytes derived from the patient and expressing chimeric receptor genes, combines the effector functions of T lymphocytes and NK cells with the ability of antibodies to recognize predefined surface antigens with high specificity and in a non-MHC-

restricted manner. We show here the therapeutic efficacy of human lymphocytes bearing erbB2-specific chimeric receptors on human prostate cancer BM lesions in a SCID mouse model after conditioning of the recipient to allow homing and persistent functioning of the adoptively transferred cells. Induction of stromal cell-derived factor-1 production within the BM using low-dose irradiation or cyclophosphamide combined with IL-2 administration enhanced the homing of systemically delivered T bodies, resulting in decreased tumor growth and prostate-specific antigen secretion, prolongation of survival, and even cure of the treated mice. These preclinical studies strongly support the idea that the T body approach has therapeutic potential in disseminated prostate cancer.

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.

Ramnaraine, M. L., W. E. Mathews, et al. (2006). "Osteoclasts direct bystander killing of bone cancer." Cancer Res **66**(22): 10929-35.

Primary and metastatic bone cancers are difficult to eradicate and novel approaches are needed to improve treatment and extend life. As bone cancer grows, osteoclasts, the principal bone-resorbing cells of the body, are recruited to and activated at sites of cancer. In this investigation, we determined if osteoclast lineage cells could function as a cell-based gene delivery system to bone cancers. We used the cytosine deaminase (CD) 5-fluorocytosine (5-FC) enzyme/prodrug system and studied bone marrow and bones from transgenic mice expressing a novel CD gene regulated by the osteoclast tartrate-resistant acid phosphatase (TRAP) gene promoter (Tg/NCD). DsRed2-labeled 2472 sarcoma cells were placed in Tg/NCD osteoclastogenic cultures and treated with 5-FC. 5-FC treatment resulted in profound bystander killing (90%; $P < 0.05$). The effect of 5-FC treatment on osteoclast lineage cells was most dramatic when administered at the beginning of the 7-day cultures, suggesting that mature osteoclasts are less sensitive to 5-FC. Evaluation of osteoclast-directed bystander killing in vivo revealed dramatic killing of bone cancer with only a modest effect on osteoclast number. Specifically, 5-FC treatment of tumor-bearing Tg/NCD mice or Tg/NCD bone marrow transplanted C3H mice (Tg/NCD-C3H) resulted in 92% and 44% reductions in tumor area, respectively ($P < 0.05$). Eight of ten 5-FC-treated Tg/NCD mice had complete bone tumor killing and five of six 5-FC-treated Tg/NCD-C3H mice had reduced tumor compared with controls. In addition, Tg/NCD osteoclasts were resistant to 5-FC treatment in vivo, a very important feature, as it identifies osteoclasts as an ideal CD gene delivery system.

Roato, I., E. Gorassini, et al. (2008). "Spontaneous osteoclastogenesis is a predictive factor for bone metastases from non-small cell lung cancer." Lung Cancer **61**(1): 109-16.

Lung cancer is a widespread disease and its incidence is growing. Since therapies have increased the life expectancy of lung cancer patients, the development of bone osteolytic metastases is becoming a common cause of morbidity. Osteolysis is

caused by an increased osteoclast activity and may be reduced by inhibiting their formation and activity. We studied 60 male patients affected by NSCLC, divided in early and advanced stage disease. Patients' blood and urinary samples were collected at tumor diagnosis and at follow-up. PBMCs were cultured to investigate the spontaneous osteoclastogenesis. IL-7 was dosed in serum and its quantitative gene expression was evaluated on tumor and healthy tissues by RQ-PCR. Both at diagnosis and follow-up, osteolytic bone patients showed high spontaneous osteoclastogenesis level compared to non-bone metastatic and healthy controls. The presence of spontaneous osteoclastogenesis correlated with urinary crosslinks increase. Serum IL-7 levels were higher in bone metastatic patients than in patients without bone lesions and healthy controls. The serum IL-7 increase correlated with the osteoclastogenesis and, at least in part, depended on an increased IL-7 production by tumor cells. At follow-up, patients with increased osteoclastogenesis and serum IL-7 levels, were subjected to standard clinical analysis, which showed early secondary bone lesions. The in vitro assay for spontaneous osteoclastogenesis and serum IL-7 dosage could be useful for diagnostic purposes and it might be able to monitor cancer patients with a high risk to develop osteolytic metastases at follow-up, especially after a curative treatment.

Saha, B., A. Arase, et al. (2008). "Overexpression of E-cadherin and beta-catenin proteins in metastatic prostate cancer cells in bone." Prostate **68**(1): 78-84.

The expression of E-cadherin in the intercellular adhesion of metastatic prostate cancer cells in bone, which is the most prevalent site of metastatic growth, remains elusive. The aim of the study was to compare the concurrent membranous expression of E-cadherin and beta-catenin proteins, the state which is known to be associated with the cellular adhesion function of E-cadherin, in prostate biopsy tissue specimens by immunohistochemical staining method. The expression patterns of E-cadherin or beta-catenin were classified as homogeneous (most cells exhibiting positively), heterogeneous (a few scattered patches of cells with positivity) or negative. Benign prostate hyperplasia cells exhibited homogeneous expression of both E-cadherin and beta-catenin in 9 of 11 (82%), whereas the primary prostate cancer cells were homogeneously positive for both proteins only in 4 of 22 (18%) of the cases. The results are similar to those reported in literature. However, in contrast to the primary cancer, a significantly increased frequency of the metastatic prostate cancer cells in bone exhibited homogeneous expression of E-cadherin and beta-catenin in 12 of 17 (71%) of the cases. A statistically significant association was observed between the overexpression of both proteins and the metastatic prostate cancer cells in bone (Fisher's exact $P < 0.001$). CONCLUSIONS: The result of the study demonstrated for the first time that the membranous overexpression of E-cadherin and beta-catenin are significantly associated with the metastatic prostate cancer cells in bone and that the high frequency of expression suggest their involvement in the intercellular adhesion of the metastatic cells in bone.

Saha, B., B. Chaiwun, et al. (2007). "Overexpression of E-cadherin protein in metastatic breast cancer cells in bone." Anticancer Res **27**(6B): 3903-8.

AIM: The aim of the present study was to evaluate E-cadherin, whose expression remains poorly understood in the intercellular adhesion of metastatic breast cancer cells in bone, the most prevalent site for metastatic growth. An immunohistochemical staining method was used for the localization of E-cadherin protein in tissue biopsy specimens of normal breast ($n = 9$) and well- ($n = 8$), moderately ($n = 8$) or poorly ($n = 14$) differentiated invasive primary breast cancer and metastatic breast cancer in bone ($n = 17$). The expression patterns of E-cadherin were classified as homogeneous (most cells exhibiting positivity), heterogeneous (a few scattered patches of cells with positivity) or negative (cells with undetectable positivity). Normal breast epithelial cells showed homogeneous overexpression of E-cadherin in all cases. A progressive and statistically significant reduction of E-cadherin expression was detected in the histologically well- to moderately to poorly differentiated breast cancer cells ($p < 0.001$). The clumps of invasive primary breast cancer cells in CD-31-positive blood vessels exhibited E-cadherin expression. Moreover, as compared to the poorly differentiated breast cancer cells, a significantly increased frequency of the metastatic breast cancer cells in bone exhibited homogeneous expression of E-cadherin in 15 out of 17 and heterogeneous expression in the remaining 2 cases (McNemar Exact $p < 0.001$). This is the first demonstration of membranous overexpression of E-cadherin on metastatic breast cancer cells in bone; the high frequency of its expression may have a role in the intercellular adhesion of metastatic cells in bone.

Saika, T., T. Satoh, et al. (2004). "Route of administration influences the antitumor effects of bone marrow-derived dendritic cells engineered to produce interleukin-12 in a metastatic mouse prostate cancer model." Cancer Gene Ther **11**(5): 317-24.

Gene-modified dendritic cells (DC) provide unique therapeutic strategies for prostate cancer; however, the comparative evaluation of specific

delivery options using appropriate preclinical models has not been described. In this study, bone marrow-derived DC were genetically engineered to express high levels of interleukin-12 (IL-12) with or without the costimulatory molecule B7-1, by ex vivo infection with recombinant adenoviral vectors. We used an orthotopic metastatic mouse prostate cancer preclinical model (178-2 BMA) to compare two therapeutic protocols for DC delivery, in situ and subcutaneous. DC were generated from bone marrow of syngeneic 129/Sv mice by culturing in the presence of GM-CSF and IL-4. In vitro DC/IL-12 or DC/IL-12/B7 produced high levels of biologically active IL-12. In situ delivery of DC/IL-12 or DC/IL-12/B7 induced a significant suppression of primary tumor growth compared to DC/beta gal controls (P=.0328 and P=.0019, respectively), as well as reduced numbers of spontaneous lung metastatic nodules (P=.1404 and P=.0335, respectively). In survival experiments, in situ DC/IL-12 injection demonstrated a small but statistically significant advantage (P=.0041). Subcutaneous, tumor lysate pulsed DC/IL-12 significantly decreased tumor size (P=.0152) and increased survival (P=0.0433) compared to HBSS controls but the decrease in the number of spontaneous lung metastases did not achieve statistical significance. Both in situ and subcutaneous treatments enhanced cytolytic activities of natural killer (NK) cells and cytotoxic T lymphocytes (CTL). In this preclinical model, gene-modified DC-based intratumoral immunotherapy was shown to be an effective therapeutic strategy for locally advanced prostate cancer based on tumor growth suppression, inhibition of metastasis and survival improvement.

Saito, H., T. Tsunenari, et al. (2005). "Humanized monoclonal antibody against parathyroid hormone-related protein suppresses osteolytic bone metastasis of human breast cancer cells derived from MDA-MB-231." Anticancer Res **25**(6B): 3817-23.

Parathyroid hormone-related protein (PTHrP) has been implicated in bone metastasis. However, the effects on bone metastasis of blocking the PTHrP function have not been tested in the clinic. Here, the effects of a humanized anti-PTHrP monoclonal antibody (mAb) on bone metastasis in a human xenograft model are shown. Subline MDA-5a, with high bone metastatic activity, was established from the human breast cancer cell line MDA-MB-231. Mice were injected with MDA-5a and an anti-PTHrP monoclonal antibody (mAb) raised against human PTHrP (1-34); bone metastasis was evaluated by X-ray photography. MDA-5a produced elevated levels of PTHrP, Interleukin 8 (IL-8), IL-6 and matrix metalloproteinase 1 (MMP-1) and frequently metastasized to the bone. Administration of the humanized anti-PTHrP mAb significantly suppressed osteolytic bone metastasis of MDA-5a and caused osteogenesis at the sites of metastasis. CONCLUSION: The humanized anti-PTHrP mAb was effective against bone metastasis by inducing osteogenesis and, therefore, will provide a new treatment option for bone metastasis in breast cancer.

Schwaninger, R., C. A. Rentsch, et al. (2007). "Lack of noggin expression by cancer cells is a determinant of the osteoblast response in bone metastases." Am J Pathol **170**(1): 160-75.

Prostate and mammary cancer bone metastases can be osteoblastic or osteolytic, but the mechanisms determining these features are unclear. Bone morphogenetic and Wnt proteins are osteoinductive molecules. Their activity is modulated by antagonists such as noggin and dickkopf-1. Differential expression analysis of bone morphogenetic and Wnt protein antagonists in human prostate and mammary cancer cell lines showed that osteolytic cell lines constitutively express in vitro noggin and dickkopf-1 and at least one of the osteolytic cytokines parathyroid hormone-related protein, colony-stimulating factor-1, and interleukin-8. In contrast, osteoinductive cell lines express neither noggin nor dickkopf-1 nor osteolytic cytokines in vitro. The noggin differential expression profile observed in vitro was confirmed in vivo in prostate cancer cell lines xenografted into bone and in clinical samples of bone metastasis. Forced noggin expression in an osteoinductive prostate cancer cell line abolished the osteoblast response induced in vivo by its intraosseous xenografts. Basal bone resorption and tumor growth kinetics were marginally affected. Lack of noggin and possibly dickkopf-1 expression by cancer cells may be a relevant mechanism contributing to the osteoblast response in bone metastases. Concomitant lack of osteolytic cytokines may be permissive of this effect. Noggin is a candidate drug for the adjuvant therapy of bone metastasis.

Seong, J., H. C. Park, et al. (2004). "Radiation-induced alteration of pain-related signals in an animal model with bone invasion from cancer." Ann N Y Acad Sci **1030**: 179-86.

Although radiotherapy is highly effective in relieving bone pain from cancer invasion, the mechanism of pain relief remains unclear. To explore the mechanism of radiotherapy-induced analgesia, we have developed an animal model of bone pain resulting from cancer invasion. Using this animal model system, radiation-induced pain response and pain-related signals in the spinal cord were analyzed. The hind paw model of bone pain from cancer

invasion was developed by injecting transplantable hepatocellular carcinoma, HCa-1, into the periosteal membrane of the foot dorsum in C3H/HeJ mice. Bony invasion from HCa-1 cells was confirmed by histopathological examinations. We also measured the development of pain-associated behaviors. In this model, changes in the objective level of pain response after irradiation of the tumor were analyzed. Expression of pain-related host signals in the spinal cord, such as calcitonin gene-related peptide (CGRP), substance P, and c-fos, was investigated with immunohistochemical staining. In the histopathological examinations, bone invasion from HCa-1 cells was seen from day 7 and was evident at day 14 after injection. Measurable pain-associated behaviors were developed from day 7. In this model, mice treated with radiotherapy showed decreased objective levels of pain with a higher threshold to graded mechanical stimulation than did control mice from day 3 after irradiation. After irradiation of tumors, significant decreases in the expression of CGRP were shown in the spinal cord, whereas neither substance P nor c-fos showed any alteration. We developed a novel hind paw model of bone pain from cancer invasion that was confirmed by histopathological examination and measurable pain-associated behaviors. Radiotherapy decreased the objective level of pain and the underlying mechanism involved in the alteration of pain-related host signal, CGRP, in the spinal cord.

Shepherd, T. G. and M. W. Nachtigal (2003). "Identification of a putative autocrine bone morphogenetic protein-signaling pathway in human ovarian surface epithelium and ovarian cancer cells." Endocrinology **144**(8): 3306-14.

Bone morphogenetic proteins (BMPs) are members of the TGFbeta superfamily of cytokines that are involved in development, differentiation, and disease. In an analysis of normal ovarian surface epithelium (OSE) and ovarian cancer (OC) cells, we observed BMP4 mRNA expression and found that primary OC cells produce mature BMP4. In addition, each member of the downstream signaling pathway was expressed in primary OSE and OC cells. Smad1 was phosphorylated and underwent nuclear translocation in normal OSE and OC cells upon treatment with BMP4. Interestingly, the BMP target genes ID1 and ID3 were up-regulated 10- to 15-fold in primary OC cells, compared with a 2- to 3-fold increase in normal OSE. The growth of several primary OC cells was relatively unaltered by BMP4 treatment; however, long-term BMP4 treatment of primary OC cells resulted in decreased cell density as well as increased cell spreading and adherence. These data demonstrate the existence and putative function of BMP signaling in normal OSE and OC cells, and thus the continued examination of BMP4 signaling in the regulation of these two processes will be critical to further our current understanding of the role of BMP biology in OC pathogenesis.

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.

Sloan, E. K. and R. L. Anderson (2002). "Genes involved in breast cancer metastasis to bone." Cell Mol Life Sci **59**(9): 1491-502.

Metastasis to bone occurs frequently in advanced breast cancer and is accompanied by debilitating skeletal complications. Current treatments are palliative and new therapies that specifically prevent the spread of breast cancer to bone are

urgently required. While our understanding of interactions between breast cancer cells and bone cells has greatly improved, we still know little about the molecular determinants that regulate specific homing of breast cancer cells to the bone. In this review, we focus on genes that have been implicated in migration and adhesion of breast cancer cells to bone, as well as genes that promote tumor cell proliferation in the bone microenvironment. In addition, the review discusses new technologies, including better animal models, that will further assist with the identification of the molecular determinants of bone metastasis and will guide the development of new therapies.

Smid, M., Y. Wang, et al. (2006). "Genes associated with breast cancer metastatic to bone." J Clin Oncol **24**(15): 2261-7.

The biology of tumors relapsing to bone is poorly understood. In this study, we initiated a search for genes that are implicated in tumors relapsing to bone in breast cancer. We analyzed 107 primary breast tumors in patients who were all lymph node negative at the time of diagnosis and all had experienced relapse. Total RNA isolated from frozen tumor samples was used to gather gene expression data using oligo microarrays. A panel of 69 genes was found significantly differentially expressed between patients who experienced relapse to bone versus those who experienced relapse elsewhere in the body. The most differentially expressed gene, TFF1, was confirmed by quantitative reverse transcriptase polymerase chain reaction in an independent cohort (n = 122; P = .0015). Our differentially expressed genes, combined with a recently reported gene set relevant to tumors relapsing to bone in an animal model system, pointed to the involvement of the fibroblast growth factor receptor signaling pathway in preference of tumor cells that relapse to bone. Given that patients who experience relapse to bone may benefit from bisphosphonate therapy, we developed a classifier of 31 genes, which in an independent validation set correctly predicts all tumors relapsing to bone with a specificity of 50%. CONCLUSION: Our study identifies a panel of genes relevant to bone metastasis in breast cancer. The subsequently developed classifier of tumors relapsing to bone could, after thorough confirmation on an extended number of independent samples, and in combination with our previously developed high-risk profile, provide a diagnostic tool for the recommendation of adjuvant bisphosphonate therapy in addition to endocrine therapy or chemotherapy.

Soos, G., G. P. Haas, et al. (2003). "Differential gene expression in human prostate cancer cells adapted to growth in bone in Beige mice." Urol Oncol **21**(1): 15-9.

OBJECTIVE: A metastasis model was used to identify genes potentially related to the growth of human prostate cancer in the bone. Injection of the human prostate cancer line PC3 into the femurs of Beige mice induced tumors that ruptured the femurs in 4 to 6 weeks. The subline PC3a was cultured in vitro from one of these PC3 bone tumors. PC3a cells were reinjected into femurs, and the subline PC3b was then cultured from a resulting PC3a tumor. Likewise, PC3c was derived from a PC3b bone tumor. The PC3 tumors were osteolytic, invasive and metastatic. Analysis of gene expression in these PC3 sublines by differential-display RT-PCR identified two groups of transcripts whose steady state levels differed substantially from the original PC3 line. One group of transcripts increased with progressive adaptation to tumor formation in bone. The second group showed the reverse pattern. They progressively diminished in subsequent sublines, and were virtually absent in PC3b and PC3c. Two in this group were fibroblast growth factor receptor-2 and caveolin-1. They were strongly expressed in non-malignant prostate tissue. CONCLUSION: These two downregulated genes, which have been reported to play a role in the development of androgen independence and malignant progression, may reflect molecular changes in growth regulation of PC3 cells during readaptation to an intra-osseal environment.

Steinert, S., T. C. Kroll, et al. (2008). "Differential expression of cancer-related genes by single and permanent exposure to bone morphogenetic protein 2." J Cancer Res Clin Oncol **134**(11): 1237-45.

Bone morphogenetic proteins (BMPs) are multifunctional regulators of various cell functions. The BMP-signalling network plays a pivotal role during embryogenesis and tumorigenesis. BMPs, e.g. BMP-2 exert their biological function in a time and concentration-dependent manner but also modulated by the context of the cellular microenvironment. In this study, we investigated the effect of a steady high level of BMP-2 versus a single application of BMP-2 on the breast cancer cell line MCF-7. The effect of the incubation regimes was analysed by DNA microarray expression profiling. Data were verified by real-time PCR. The protein expression of apoptosis-related genes was studied by western blot analysis. We found a clear difference in the altered gene expression between the constant high level and the single application of BMP-2. After grouping the genes of interest into the biological processes of Gene Ontology, the group of apoptosis-related genes like BAX, BAG5 or PKR, was predominantly affected under the single-application regime of BMP-2.

Among these protein kinase R was the most prominently regulated. Further studies on the protein level showed activation of PKR after 4 h with a subsequent enhanced phosphorylation of the PKR substrate eIF2alpha for several hours. CONCLUSIONS: The duration of treatment and the concentration of BMP-2 affect the global expression pattern of MCF-7 cells. Among the regulated cancer-related genes, the cohort of the apoptosis-related genes showed the pronounced alterations. Our data point to a novel role of BMP-2 in the regulation of the PKR pathway in tumorigenesis.

Tomari, K., T. Kumagai, et al. (2005). "Bone morphogenetic protein-2 induces hypophosphorylation of Rb protein and repression of E2F in androgen-treated LNCaP human prostate cancer cells." Int J Mol Med **15**(2): 253-8.

Bone morphogenetic protein-2 (BMP-2), a multifunctional member of the transforming growth factor (TGF)-beta, superfamily, has powerful osteoinductive effects and causes cell cycle arrest in a variety of transformed cell lines. We have observed BMP-2-induced inhibition of cell proliferation in an androgen-dependent human prostate cancer cell line (LNCaP). To investigate the mechanism of inhibition of androgen-dependent growth by BMP-2, we examined the effect of dihydrotestosterone (DHT) and/or BMP-2 on cell cycle-related proteins in LNCaP cells. BMP-2 decreased the phosphorylation of retinoblastoma (Rb) protein induced by treatment with DHT. DHT-induced expression of cyclin A and cyclin-dependent kinase 2 (CDK2) protein was also inhibited by co-treatment with BMP-2. Furthermore, BMP-2 induced expression of p21(WAF1/CIP1), a CDK inhibitor. These results indicate that changes in expression of these proteins lead to modulation of the phosphorylation state of Rb. DHT-induced E2F-1 protein and mRNA expressions was also inhibited by BMP-2, suggesting that BMP-2 inhibits DHT-induced growth of LNCaP cells through a decrease in E2F protein expression and suppression of E2F activity by hypophosphorylation of Rb.

Uehara, H., S. J. Kim, et al. (2003). "Effects of blocking platelet-derived growth factor-receptor signaling in a mouse model of experimental prostate cancer bone metastases." J Natl Cancer Inst **95**(6): 458-70.

Expression of platelet-derived growth factor (PDGF) and activation (by autophosphorylation) of its receptor (PDGF-R), a tyrosine kinase, are associated with the growth of metastatic prostate tumor cells in the bone parenchyma. The tyrosine kinase inhibitor STI571 blocks the PDGF signaling pathway by inhibiting PDGF-R autophosphorylation. We examined the effects of STI571, given alone or with paclitaxel (Taxol), on tumor growth in a mouse model of prostate cancer metastasis. Human prostate cancer PC-3MM2 cells were injected into the tibias of male nude mice. Three days later the mice (20 per group) were randomly assigned to 5 weeks of treatment with oral and injected water (control), daily oral STI571, weekly injected paclitaxel, or STI571 plus paclitaxel. Lesions in bone and the surrounding muscles were then harvested and analyzed by histology, western blotting (for PDGF-R phosphorylation), immunohistochemistry (for expression of proangiogenic molecules), and double immunofluorescence (to identify endothelial cells and apoptotic tumor cells). Growth of bone lesions was monitored by digital radiography. Bone lesions from control mice were used to establish short-term cell cultures for analysis of PDGF-R phosphorylation. All statistical tests were two-sided. PC-3MM2 cells cultured from bone lesions and treated in vitro with STI571 had less phosphorylated PDGF-R than untreated cells. In control mice, bone lesions expressed high levels of PDGF and activated (i.e., phosphorylated) PDGF-R, whereas lesions in the adjacent musculature did not. Activated PDGF-R was present on the surface of endothelial cells within the bone lesions but not in endothelial cells of uninjected bone. Mice treated with STI571 or STI571 plus paclitaxel had a lower tumor incidence, smaller tumors, and less bone lysis and lymph node metastasis than mice treated with water or paclitaxel alone ($P<.001$ for all). Mice treated with STI571 or STI571 plus paclitaxel had less phosphorylated PDGF-R on tumor cells and tumor-associated endothelial cells, less tumor cell proliferation, statistically significantly more apoptotic tumor cells (all $P<.001$), and fewer tumor-associated endothelial cells ($P<.001$) than control mice. CONCLUSIONS: Endothelial cells appear to express phosphorylated PDGF-R when they are exposed to tumor cells that express PDGF. Using STI571 to inhibit PDGF-R phosphorylation may, especially in combination with paclitaxel, produce substantial therapeutic effects against prostate cancer bone metastasis.

Wang, G., R. K. Chopra, et al. (1998). "A T cell-independent antitumor response in mice with bone marrow cells retrovirally transduced with an antibody/Fc-gamma chain chimeric receptor gene recognizing a human ovarian cancer antigen." Nat Med **4**(2): 168-72.

In order to treat common cancers with immunotherapy, chimeric receptors have been developed that combine the tumor specificity of antibodies with T-cell effector functions. Previously, we demonstrated that T cells transduced with a

chimeric receptor gene against human ovarian cancer were able to recognize ovarian cancer cells in vitro and in vivo. We now report that recipients of bone marrow cells transduced with these genes exhibited significant antitumor activity in vivo. Moreover, in vivo depletion of T cells in reconstituted mice did not affect antitumor activity, suggesting that other immune cells expressing the chimeric receptor gene may play an important role in tumor rejection.

Wang, H. and T. C. Thompson (2008). "Gene-modified bone marrow cell therapy for prostate cancer." Gene Ther **15**(10): 787-96.

There is a critical need to develop new and effective cancer therapies that target bone, the primary metastatic site for prostate cancer and other malignancies. Among the various therapeutic approaches being considered for this application, gene-modified cell-based therapies may have specific advantages. Gene-modified cell therapy uses gene transfer and cell-based technologies in a complementary fashion to chaperone appropriate gene expression cassettes to active sites of tumor growth. In this paper, we briefly review potential cell vehicles for this approach and discuss relevant gene therapy strategies for prostate cancer. We further discuss selected studies that led to the conceptual development and preclinical testing of IL-12 gene-modified bone marrow cell therapy for prostate cancer. Finally, we discuss future directions in the development of gene-modified cell therapy for metastatic prostate cancer, including the need to identify and test novel therapeutic genes such as GLIPR1.

Wang, H., G. Yang, et al. (2007). "IL-12 gene-modified bone marrow cell therapy suppresses the development of experimental metastatic prostate cancer." Cancer Gene Ther **14**(10): 819-27.

To investigate the immunomodulatory effects of interleukin-12 (IL-12) for treatment of metastatic prostate cancer, we administered adult bone marrow cells (BMC) that were genetically modified by retroviral vector-mediated IL-12 gene transduction in an experimental mouse model of prostate cancer metastasis. This therapy produced significant anti-metastatic effects in bone and lung and prolonged animal survival. Flow cytometric analysis indicated donor BMC could effectively home to bone and lung after treatment. Intensive infiltration of CD4 and CD8T cells in lung metastases and increased systemic natural killer and cytotoxic T lymphocyte activities indicated induction of a significant anti-metastatic immune response after treatment with IL-12 transduced BMC. Our results demonstrate the therapeutic potential of gene-modified BMC gene therapy.

Wang, R., J. Xu, et al. (2005). "Three-dimensional co-culture models to study prostate cancer growth, progression, and metastasis to bone." Semin Cancer Biol **15**(5): 353-64.

Cancer-stromal interaction results in the co-evolution of both the cancer cells and the surrounding host stromal cells. As a consequence of this interaction, cancer cells acquire increased malignant potential and stromal cells become more inductive. In this review we suggest that cancer-stromal interaction can best be investigated by three-dimensional (3D) co-culture models with the results validated by clinical specimens. We showed that 3D culture promoted bone formation in vitro, and explored for the first time, with the help of the astronauts of the Space Shuttle Columbia, the co-culture of human prostate cancer and bone cells to further understand the interactions between these cells. Continued exploration of cancer growth under 3D conditions will rapidly lead to new discoveries and ultimately to improvements in the treatment of men with hormonal refractory prostate cancer.

Wang, T., D. Xia, et al. (2005). "Bone marrow stromal cell-derived growth inhibitor inhibits growth and migration of breast cancer cells via induction of cell cycle arrest and apoptosis." J Biol Chem **280**(6): 4374-82.

Genes encoding growth-inhibitory proteins are postulated to be candidate tumor suppressors. The identification of such proteins may benefit the early diagnosis and therapy of tumors. Here we report the cloning and functional characterization of a novel human bone marrow stromal cell (BMSC)-derived growth inhibitor (BDGI) by large scale random sequencing of a human BMSC cDNA library. Human BDGI cDNA encodes a 477-amino acid residue protein that shares high homology with rat and mouse pregnancy-induced growth inhibitors. The C-terminal of BDGI is identical to a novel human pregnancy-induced growth inhibitor, OKL38. BDGI is also closely related to many other eukaryotic proteins, which together form a novel and highly conserved family of BDGI-like proteins. BDGI overexpression inhibits the proliferation, decreases anchorage-dependent growth, and reduces migration of MCF-7 human breast cancer cells, whereas down-regulation of BDGI expression promotes the proliferation of MCF-7 and HeLa cervix epitheloid carcinoma cells. Interestingly, the inhibitory effect of BDGI on MCF-7 cells is more potent than that of OKL38. We demonstrate that BDGI induces cell cycle arrest in S phase and subsequent apoptosis of MCF-7 cells,

which is likely to account for the antiproliferative effects of BDGI. This process may involve up-regulation of p27Kip1 and down-regulation of cyclin A, Bcl-2, and Bcl-xL. The inhibitory effect of BDGI on cell proliferation and the induction of apoptosis were also observed in A549 lung cancer cells but not HeLa cells. These results indicate that BDGI might be a growth inhibitor for human tumor cells, especially breast cancer cells, possibly contributing to the development of new therapeutic strategies for breast cancer.

Watson, M. A., L. R. Ylagan, et al. (2007). "Isolation and molecular profiling of bone marrow micrometastases identifies TWIST1 as a marker of early tumor relapse in breast cancer patients." Clin Cancer Res **13**(17): 5001-9.

Micrometastatic cells detected in the bone marrow have prognostic significance in breast cancer. These cells are heterogeneous and likely do not exhibit uniform biological behavior. To understand the molecular diversity of disseminated cancer cells that reside in bone marrow, we enriched this cell population and did global gene expression profiling in the context of a prospective clinical trial involving women with clinical stage II/III breast cancer undergoing neoadjuvant chemotherapy. Enrichment of TACSTD1 (EpCAM)-expressing cells from bone marrow of breast cancer patients was achieved using immunomagnetic beads. Gene expression profiles were compared between enriched cell populations and whole bone marrow from 5 normal volunteers and 23 breast cancer patients after neoadjuvant chemotherapy treatment. Enriched cells from bone marrow samples of breast cancer patients before treatment or at 1 year follow-up were also analyzed (total of 87 data sets). The expression of transcripts specifically detected in enriched cell populations from breast cancer patients was correlated with 1-year clinical outcome using quantitative reverse transcription-PCR in an independent cohort of bone marrow samples. Analysis of EpCAM-enriched bone marrow cells revealed specific expression of a subgroup of transcripts, including the metastasis regulator, TWIST1. Most transcripts identified, including TWIST1, were not expressed in enriched populations of bone marrow from normal volunteers, suggesting that this expression profile reflects a signature of breast cancer bone marrow micrometastases that persist after chemotherapy. In an independent set of bone marrow samples obtained before any treatment, TWIST1 expression correlated with early disease relapse. CONCLUSIONS: Disseminated breast cancer cells present in bone marrow after chemotherapy possess unique transcriptional signatures. Genes whose expression is overrepresented in these cell populations, such as TWIST1, may prove to be excellent markers of early distant relapse in breast cancer patients.

Whitfield, J. F. (2006). "Parathyroid hormone: a novel tool for treating bone marrow depletion in cancer patients caused by chemotherapeutic drugs and ionizing radiation." Cancer Lett **244**(1): 8-15.

Between 1958 and the late 1970s it was learned that PTH (the parathyroid hormone) could directly stimulate the initiation of DNA replication by murine CFU-S (colony-forming unit-spleen) cells via cyclic AMP, stimulate the proliferation of normal and X-irradiated murine and rat bone marrow cells, control hematopoiesis, and increase the survival of X-irradiated mice and rats when injected any time between 18h before and 3h after X-irradiation. Since then, it has been shown that the hematopoietic stem cell niche consists of PTH receptor-bearing, osteoblastic trabecular bone-lining cells that maintain the stem cells' (HSCs') proliferatively quiescent 'stemness' by various gene up-regulating and down-regulating signals caused by the tight adhesion of the HSCs to the osteoblastic niche-lining cells. Stimulating the osteoblastic lining cells with recombinant human PTH-(1-34) (Forteo) causes a cyclic AMP-mediated enlargement of the HSC pool and promotes bone marrow transplant engraftment and growth and the survival of lethally irradiated mice. But this is only the beginning of the exploitation of the PTHs for marrow engraftment. It must now be determined whether the marrow engraftment-enhancing action of this potent bone growth-stimulating PTH can be extended from mice to rats and monkeys. It must be determined whether two other PTH peptides, rhPTH-(1-84) [Preos]and [Leu(27)]cyclo(Glu(22)-Lys(26))hPTH-(1-31)NH(2) [Ostabolin-C]) are as effective as or better than rhPTH-(1-34)(Forteo). Since, all three peptides are on the market, or nearing the market, for safely and strongly stimulating bone growth and treating osteoporosis one or all of them may become valuable tools for safely promoting the engraftment of peripherally harvested HSCs in cancer patients whose bone marrows have been 'emptied' by chemotherapeutic drugs or ionizing radiation.

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.

Wu, J. M., D. Bensen-Kennedy, et al. (2005). "The effects of interleukin 10 and interferon gamma cytokine gene polymorphisms on survival after autologous bone marrow transplantation for patients with breast cancer." Biol Blood Marrow Transplant **11**(6): 455-64.

Several clinical trials evaluating the induction of autoimmune graft-versus-host disease (GVHD) after autologous bone marrow transplantation (BMT) as antitumor immunotherapy have shown that autologous GVHD is associated with increased production of interleukin (IL)-10. The induction of autologous GVHD also segregated with single nucleotide polymorphisms in the IL-10 promoter region (IL-10 -592 and IL-10 -1082) and with CA repeats in the first intron of the interferon (IFN)-gamma gene. Polymorphisms within these promoter regions can significantly modify the cytokine response because of differential transcription factor efficiency. This study evaluated the relationship between inheritance of polymorphisms within the IL-10 promoter and in the IFN-gamma gene and the overall survival of patients who received autologous BMT for metastatic breast cancer. Peripheral mononuclear cells from 87 women enrolled in 3 autologous BMT (plus induction of autologous GVHD) clinical trials were examined. By using a Cox proportional hazard model, trends in survival after autologous BMT were analyzed. The model included inheritance polymorphisms of IL-10 -592 , IL-10 -1082 , CA repeats within the first intron of the IFN-gamma gene, estrogen and progesterone receptor status, and stage of disease. Increased survival was significantly associated with patients having the IL-10 -592 promoter allele associated with high IL-10 production (hazard ratio, 0.23; 95% confidence interval, 0.09-0.55; P = .001). The effect of the strong IL-10 promoter allele on survival seems to be independent of the development of clinical autologous GVHD. However, decreased survival was significantly associated with patients having CA repeats associated with higher IFN-gamma transcription (hazard ratio, 2.34; 95% confidence interval, 1.21-4.54; P = .011). Inheritance of specific alleles that modify IL-10 and IFN-gamma production may have unexpected effects on the efficacy of immune-based strategies after autologous BMT. Additional studies are necessary to further define the influence of IL-10 and IFN-gamma on the immune response after BMT.

Wu, W. K., J. J. Sung, et al. (2008). "Bone morphogenetic protein signalling is required for the anti-mitogenic effect of the proteasome inhibitor MG-132 on colon cancer cells." Br J Pharmacol **154**(3): 632-8.

BACKGROUND AND Inhibition of proteasome has been emerging as a promising approach in pathway-directed cancer therapy. Bone morphogenetic protein (BMP) signalling, which is known to be regulated by the ubiquitin-proteasome pathway in osteoblasts, plays a crucial role in the suppression of gastrointestinal carcinogenesis. Here we sought to elucidate the anti-mitogenic effect of a proteasome inhibitor in relation to BMP signalling in colon cancer. The effects of the proteasome inhibitor MG-132 on proliferation of SW1116 and HT-29 colon cancer cells were determined by [(3)H]-thymidine incorporation and colony-formation assay. The involvement of BMP signalling in the action of MG-132 was elucidated by western blot, real-time PCR, immunofluorescence and RNA interference. KEY MG-132 significantly suppressed the proliferation of colon cancer SW1116 and HT-29 cells. In this regard, MG-132 activated BMP signalling and this was manifested as an increase in Smad1/5/8 phosphorylation and upregulation of p21(Waf1/Cip1) and p27(Kip1) expression. Knockdown of BMP receptor II abolished Smad1/5/8 phosphorylation, the induction of p21(Waf1/Cip1) and p27(Kip1) and inhibition of cell proliferation induced by MG-132. Further analysis revealed that MG-132 upregulated the expression of BMP1 and BMP2, which are secreted members of the BMP superfamily. Moreover, the expression of Smad6, an intracellular inhibitor of BMP signalling, was suppressed by MG-132. CONCLUSIONS AND IMPLICATIONS: These findings suggest that inhibition of proteasome suppresses the proliferation of colon cancer cells via activation of BMP signalling. They also demonstrate a novel aspect of proteasome function in the regulation of colon cancer cell proliferation.

Xu, J., R. Wang, et al. (2006). "Prostate cancer metastasis: role of the host microenvironment in promoting epithelial to mesenchymal transition and

increased bone and adrenal gland metastasis." Prostate **66**(15): 1664-73.

The ARCaP cell line was established from the ascites fluid of a patient with metastatic prostate cancer. This study characterized the host microenvironmental role in cancer progression, epithelial to mesenchymal transition (EMT), and bone and adrenal metastasis in parental ARCaP and its derived cell subclones. Cytogenetic profiles, growth, migration, invasion, cellular interaction, drug sensitivities, and gene expression of ARCaP cell subclones were compared. In vivo gene expression, behavior, and metastasis of ARCaP subclones were analyzed by serial intracardiac injections into SCID mice. ARCaP(E) cells, with cobblestone morphology, underwent EMT through cellular interaction with host bone and adrenal gland. Lineage-derived ARCaP(M) cells, with spindle-shape fibroblastic morphology, exhibited decreased cell adhesion and increased metastasis to bone and adrenal gland. Cytogenetic analyses of parental and ARCaP subclones confirmed their clonality. CONCLUSIONS: ARCaP uniquely models the molecular basis of prostate cancer bone and adrenal metastases and epithelial to mesenchymal transition.

Yamamoto, J., T. Kawamata, et al. (2008). "Down-regulation of mu opioid receptor expression within distinct subpopulations of dorsal root ganglion neurons in a murine model of bone cancer pain." Neuroscience **151**(3): 843-53.

Although micro opioid receptor (MOR) agonists are used for treatment of most types of pain, a recent study has suggested that the sensitivity of bone cancer pain to systemic morphine was lower than that of inflammatory pain. However, the reasons for this have remained unclear. In this study, MOR expression and the analgesic effects of morphine in a bone cancer model were compared with those in an inflammatory pain model. A bone cancer pain model and an inflammatory pain model were made by implantation of sarcoma cells into the intramedullary space of the femur and hind-paw injection of complete Freund's adjuvant (CFA), respectively. In a behavioral study, sarcoma-implanted mice showed flinching behavior of magnitude comparable to that induced by CFA injection. The flinching behavior of sarcoma-implanted mice was less sensitive to intrathecal morphine than that of CFA-injected mice. Western blot analysis showed that MOR expression in the dorsal root ganglion (DRG) ipsilateral to sarcoma implantation was significantly reduced, while that in the DRG ipsilateral to CFA injection was increased. In sarcoma-implanted mice, the percentage of MOR-positive DRG neuronal profiles was lower than that in control mice (30.3% vs. 45.2%). In particular, MOR expression was reduced among calcitonin gene-related peptide- and transient receptor potential vanilloid subfamily 1-positive DRG neuronal profiles, which are considered to be involved in the generation of bone cancer pain (from 61.5% to 41.5% and from 72.1% to 48.4%, respectively). These results suggest that down-regulation of MOR in the distinct populations of DRG neurons contributes to the fact that higher doses of morphine are needed to produce analgesia in bone cancer as compared with those used in non-malignant inflammatory situations.

Yang, M., P. Jiang, et al. (1999). "A fluorescent orthotopic bone metastasis model of human prostate cancer." Cancer Res **59**(4): 781-6.

Here, we report a fluorescent spontaneous bone metastatic model of human prostate cancer developed by surgical orthotopic implantation of green fluorescent protein (GFP)-expressing prostate cancer tissue. Human prostate cancer PC-3 cells were transduced with the pLEIN expression retroviral vector containing the enhanced GFP and neomycin resistance genes. Stable GFP high-expression PC-3 clones were selected in vitro with G418, which were then combined and injected s.c. in nude mice. For metastasis studies, fragments of a single highly fluorescent s.c. growing tumor were implanted by surgical orthotopic implantation in the prostate of a series of nude mice. Subsequent micrometastases and metastases were visualized by GFP fluorescence throughout the skeleton, including the skull, rib, pelvis, femur, and tibia The central nervous system, including the brain and spinal cord, was also involved with tumor, as visualized by GFP fluorescence. Systemic organs, including the lung, plural membrane, liver, kidney, and adrenal gland, also had fluorescent metastases. The metastasis pattern in this model reflects the bone and other metastatic sites of human prostate cancer. Thus, this model should be very useful for the study and development of treatment for metastatic androgen-independent prostate cancer.

Yang, S., L. K. Pham, et al. (2008). "A novel bone morphogenetic protein signaling in heterotypic cell interactions in prostate cancer." Cancer Res **68**(1): 198-205.

We examined the effect of the extracellular bone morphogenetic protein (BMP) 2 and 7, which are up-regulated in the prostate adenocarcinomas of the conditional Pten deletion mouse model, on primary cultures of cancer-associated fibroblasts (CAF) derived from these tumors. In the CAF, we show that BMP2 or BMP7, but not transforming growth factor beta-1, can strikingly stimulate secretion of stromal cell-derived factor-1 (SDF-1), also known as CXCL12. The CAF cells express type I and type II

BMP receptors as well as the receptor for SDF-1, CXCR4. SDF-1 activation is associated with BMP-induced Smad phosphorylation, and the stimulatory effect is blocked by BMP antagonist, noggin. The findings that BMP treatment can increase SDF-1 pre-mRNA levels in a time-dependent manner and actinomycin D treatment can abolish stimulatory effect of BMP suggest a transcriptional modulation of SDF-1 by BMP signaling. Using a human microvascular endothelial cell line, we show that SDF-1 present in the conditioned medium from the stimulated CAF can significantly induce tube formation, an effect relating to angiogenic function. Furthermore, we found that BMP2 can also protect the CAF from serum starvation-induced apoptosis independent of SDF-1, implying that BMP may induce other factors to sustain the survival of these cells. In short, this report establishes a novel BMP-SDF-1 axis in the prostate tumor along with a new prosurvival effect of BMP that when considered together with our previously described oncogenic properties of BMP indicate a circuitry for heterotypic cell interactions potentially critical in prostate cancer.

Zhang, M., Q. Wang, et al. (2007). "Epigenetic regulation of bone morphogenetic protein-6 gene expression in breast cancer cells." J Steroid Biochem Mol Biol **105**(1-5): 91-7.

Bone morphogenetic protein-6 (BMP-6) is closely correlated with tumor differentiation and skeletal metastasis. Our previous research found that BMP-6 gene expression can be activated dose-dependently by estrogen in estrogen receptor positive (ER(+)) breast cancer cell line MCF-7, but not in ER negative (ER(-)) cell line MDA-MB-231. This experiment is designed to investigate the epigenetic regulatory mechanism of the BMP-6 gene expression in breast cancer cell lines MDA-MB-231, MCF-7 and T47D with regard to the methylation status in the 5' flanking region of the human BMP-6 gene. The endogenous level of BMP-6 mRNA in ER(-) cell line MDA-MB-231 was relatively lower than that in ER(+) MCF-7 and T47D cell lines. After the treatment with 5-aza-2'-deoxycytidine (5-aza-dC, especially in the concentration of 10 microM), the BMP-6 mRNA expression in MDA-MB-231 was obviously up-regulated. However, 5-aza-dC treatment failed to regulate the expression of BMP-6 in MCF-7 and T47D cells. Using enzyme restriction PCR (MSRE-PCR), as well as bisulfite sequencing (BSG), methylation of human BMP-6 gene promoter was detected in MDA-MB-231; while in MCF-7 and T47D, BMP-6 gene promoter remained demethylated status. In 33 breast tumor specimens, promoter methylation of BMP-6 was detected by methylation-specific PCR, hypermethylation of BMP-6 was observed in ER negative cases (16 of 16 cases (100%)), while obviously lower methylation frequency were observed in ER positive cases (3 of 17 cases (18%)), indicating that BMP-6 promoter methylation status is correlated with ER status in breast cancer.

Zhang, R. X., A. Li, et al. (2007). "Electroacupuncture attenuates bone cancer pain and inhibits spinal interleukin-1 beta expression in a rat model." Anesth Analg **105**(5): 1482-8, table of contents.

Although pain affects the quality of life of cancer patients, current medical treatments are either ineffective or have side effects. In the present study we investigated the effect of electroacupuncture (EA) on cancer-induced hyperalgesia and expression of interleukin-1beta (IL-1beta), upregulation of which is related to the maintenance of persistent pain, in a rat model of bone cancer pain. Cancer was induced by injecting AT-3.1 prostate cancer cells into the tibia of male Copenhagen rats. The resulting pain was treated with 10 Hz/2 mA/0.4 ms pulse EA for 30 min daily at the equivalent of the human acupoint GB30 (Huantiao) between Days 14 and 18 after cancer cell inoculation. For sham control, EA needles were inserted into GB30 without stimulation. Thermal hyperalgesia, a decrease in paw withdrawal latency to a noxious thermal stimulus, was measured at baseline and 20 min after EA treatment. IL-1beta and its mRNA were respectively determined by immunohistochemistry and reverse transcription-polymerase chain reaction analysis. Thermal hyperalgesia developed between Days 12 and 18 after cancer cell inoculation. EA significantly ($P < 0.05$) attenuated this hyperalgesia, increasing paw withdrawal latency from 7.0 +/- 0.3 s to 9.2 +/- 0.4 s, and inhibited the upregulation of IL-1beta and its mRNA compared to the sham control. Intrathecal injection of IL-1 receptor antagonist (IL-1ra, 0.1 mg/rat) also significantly inhibited cancer-induced thermal hyperalgesia. CONCLUSION: The data suggest that EA alleviates bone cancer pain, at least in part by suppressing IL-1beta expression. The results support the clinical use of EA in the treatment of cancer pain.

Zhu, R., R. Xu, et al. (2007). "Expression profile of cancer-related genes in human adult bone marrow-derived neural stemlike cells highlights the need for tumorigenicity study." J Neurosci Res **85**(14): 3064-70.

Human adult bone marrow-derived neural stemlike cells (MDNSCs) may serve as ideal seed cells for cell replacement therapy for human neurological disorders and injuries. However, the long-term safety of this cell population after

transplantation must be thoroughly explored before clinical application, and tumorigenicity is a major concern. In this study, we generated MDNSCs capable of forming neurospherelike aggregates and with the potency to differentiate into neural lineage cells in vitro and investigated hundreds of cancer-related genes in MDNSCs in order to determine whether there were any characteristics that could help in the evaluation of their tumorigenic potential. According to the results of testing by PCR and DNA sequencing, there were no mutations at the frequent mutation sites of tumor-suppressor genes p53, p16, and Rb1. Of the 440 cancer-related genes covered by Oligo GEArray Human Cancer Microarray OHS-802, 63 were found to be significantly overexpressed compared with that in fresh normal human adult bone marrow depleted of red blood cells (RBCs). In particular, the overexpressed genes included those promoting cell proliferation and cell invasion and metastasis and members of several oncogenic signaling pathways. The overexpression of MYC, MMP2, Notch2, STC1, ITGA3, STAT5b, RhoC, and Wnt1 was also revealed by quantitative real-time RT-PCR. Because it has been shown that activation of some of these genes promote tumorigenesis, our findings highlight the need for further studies of long-term tumorigenicity in MDNSCs.

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