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# Molecular Targets and Cancer Therapeutics:

## *Discovery, Biology, and Clinical Applications*

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These results were compared to the expression levels of PDGF A, B and α and β PDGFRs. We find that PDGF C or PDGF D are ubiquitously expressed in all glioblastoma cell lines or human tissues studied. In addition, a functional autocrine PDGF/PDGFR pair was identified in all samples. This is the first study to demonstrate PDGF C and PDGF D expression in glioblastoma and emphasizes the potential significance of these novel growth factors in malignant transformation.

**#538 Antisense KGFR oligonucleotide inhibition of KGF-induced motility in MCF-7 breast cancer cells.** X. P. Zang, S. T. Dunn, J. T. Pento. *Univ of Oklahoma, Health Science Ctr, Oklahoma City, OK.*

The metastasis of breast cancer is known to be directly associated with the motility of breast cancer cells. In a previous study we reported that keratinocyte growth factor (KGF) enhanced the motility of estrogen receptor-positive breast cancer cells (Rajah, T T, et al., Breast Cancer Res. Treat. 57(1):113, 1999) and was associated with cellular levels of KGF receptor (KGFR). Further, we observed that KGF treatment produced a significant upregulation of KGFR gene expression in MCF-7 cells (Zang, X P, et al. Breast Cancer Res. Treat. 64(1):110, 2000). The objective of the present study was to determine the importance of KGFR as a potential anti-metastatic therapeutic target using an antisense KGFR oligonucleotide to reduce the level of KGFR expression in MCF-7 cells. A 19-mer KGFR antisense (5'-GTC CGG TTG GTC AGA CGG A-3') and a complimentary sense phosphorothioate oligonucleotide (P-oligo) were synthesized using sequences unique to KGFR mRNA and purified by HPLC. Cells were treated with antisense or sense P-oligos at a concentration of 10 μM for 48 hours using a lipofectin transfection procedure. Cellular levels of KGFR were quantified by Western blotting and normalized to actin. Cell motility was measured using two methods: first, culture wounding over a period of 48 hours; and secondly, time-lapse videomicroscopy (TLVM; time compression=240:1). The TLVM video images, at various time intervals (5-30 min) over a 4-hour experimental period were analyzed using NIH Image software. We observed that KGFR antisense treatment produced a 74% decrease in the cellular levels of KGFR protein, compared to control levels, while treatment with the KGFR sense P-oligo produced a 14% decrease. Treatment of MCF-7 cells, with the same concentration of KGFR antisense P-oligo, inhibited cell motility by 73% of control levels at 48 hours after KGF (50 ng/ml) treatment using the cell wounding assay. Similar results were obtained using TLVM with a 4-hour observation period. In conclusion, the results of this study indicate that the KGFR antisense treatment employed in this study produces an inhibition of cellular KGFR protein which was interestingly similar to the inhibition of cell motility. These data indicate that the KGF-mediated cell signaling pathway is involved in regulation of the motility of MCF-7 breast cancer cells and suggest that KGF and KGFR may be important biomarkers or therapeutic targets for the treatment of breast cancer and /or metastatic processes. This study was supported in part by grants from NIH/NCI (CA-62117) and DOD (DAMD-170110591).

**#539 Inhibition of insulin-like growth factor receptor-1 function decreases tumor growth and liver metastasis of human colon cancer.** N. Reinmuth, W. Liu, Y. D. Jung, S. A. Ahmad, F. Fan, O. Stoeltzing, C. D. Bucana, R. Radinsky, L. M. Ellis. *M D Anderson Cancer Ctr, Houston, TX.*

Insulin-like growth factors-I and-II (IGF-I,-II) and its major receptor, IGF-I receptor (IGF-IR), are frequently expressed in human colon cancer and may be involved in enhancing cell proliferation, mediating angiogenesis through induction of vascular endothelial growth factor (VEGF), and preventing apoptosis. To elucidate the in vitro and in vivo effects of the IGF-IR system in human colon cancer, two human colon cancer cell lines (HT29 and KM12L4) were transfected with a truncated dominant-negative form of IGF-IR (IGF-IR-dom-neg) or vector alone (pcDNA3). By Northern blot analysis, IGF-I led to an increase in VEGF expression in parental and pcDNA3 transfected cells while IGF-I induction of VEGF was abrogated in IGF-IR dom-neg cells. In vitro, the IGF-IR dom-neg cells showed impaired growth both in monolayer culture (as determined by MTT assay and BrdU labeling) and in soft agar ($p<0.05$). Subcutaneous injections of IGF-IR dom-neg cells in nude mice led to significantly decreased tumor growth as compared to controls ($p<0.05$). After splenic injections, KM12L4 IGF-IR dom-neg cells failed to induce liver metastases, whereas the control cells formed numerous liver metastases. Following direct injections into the liver, HT29 IGF-IR dom-neg cells exhibited tumors in significantly fewer mice compared to controls ($p<0.05$) while the KM12L4 IGF-IR dom-neg cells did not develop any liver tumors ($p<0.05$). In both experiments, liver weight after injection of IGF-IR dom-neg cells was significantly less than that in control groups ($p<0.05$). Immunohistochemical analyses revealed that subcutaneously grown IGF-I dom-neg tumors demonstrated decreased tumor cell proliferation (PCNA), VEGF expression and vessel count, and enhanced tumor cell apoptosis (TUNEL) ($p<0.05$ for all parameters compared to controls). In addition, markedly decreased staining for the phosphorylated forms of Erk and Akt was noted in IGF-IR dom-neg tumors. These studies demonstrate that 1) the IGF-I system plays an important role in colon cancer growth and metastasis, and 2) inhibition of IGF-IR function leads to a decrease in VEGF that is associated with a decrease in tumor angiogenesis. Targeting the IGF-IR in colon cancer may be a promising therapeutic strategy.

**#540 Signalling via VEGFR-3 is sufficient for lymphangiogenesis in transgenic mice.** L. Jussila, T. Veikkola, M. Jeltsch, G. Thurston, D. McDonald, M. Achen, S. Stacker, K. Alitalo. *Univ of Helsinki, Helsinki, Finland; Univ of CA, San Francisco, CA; Royal Melbourne Hosp, Parkville, Victoria, Australia.*

Vascular endothelial growth factor receptor-3 (VEGFR-3) has an essential role in the development of embryonic blood vessels. However, after midgestation its expression becomes restricted mainly to lymphatic vessels. The VEGFR-3 ligand VEGF-C stimulates lymphangiogenesis in transgenic mice and in chick chorioallantoic membrane. As VEGF-C also binds VEGFR-2, which is expressed in lymphatic endothelia, it is not clear which receptors are responsible for the lymphangiogenic effects of VEGF-C. VEGF-D, which binds to the same receptors, is able to induce angiogenesis, but its lymphangiogenic potential is not known. In order to define the lymphangiogenic signaling pathway we have created transgenic mice overexpressing a VEGFR-3 specific mutant of VEGF-C (VEGF-C156S) or VEGF-D in epidermal keratinocytes under the keratin 14 promoter. Both transgenes induced the growth of lymphatic vessels in the skin, whereas the blood vessel architecture was not affected. Evidence was obtained that these growth factors act in a paracrine manner in vivo. These results demonstrate that stimulation of the VEGFR-3 signal transduction pathway is sufficient to induce specifically lymphangiogenesis in vivo. Future studies will focus on the role of VEGF-C and VEGF-D in tumour lymphangiogenesis and subsequent metastasis during skin carcinogenesis.

**#541 Clinical predictors of survival and the role of determination of HER-2/neu overexpression in pancreatic adenocarcinoma.** V. Koka, A. Potti, M. Koch, R. Levitt, S. Mehdi. *Univ of North Dakota Sch of Medicine and Health Science, Fargo, ND; Roger Maris Cancer Ctr, Fargo, ND; Oncology Section, Veterans Affairs Medical Ctr, Fargo, ND.*

The role of HER-2/*neu*, a 185kd protein, as a predictor of metastatic potential/disease progression in breast cancer has been clearly defined. Preliminary reports suggest a similar role in pancreatic adenocarcinoma and phase II trials testing the efficacy of Trastuzumab (Herceptin) have begun. We performed a retrospective analysis to validate immunohistochemical testing for HER-2/*neu* overexpression and to determine other potential predictors of survival in patients with a biopsy-proven diagnosis of adenocarcinoma of the pancreas. Data collection included age, sex, family history, symptoms at presentation, histologic grade, survival (disease free and total) and treatment modality. HER-2/*neu* testing was performed on paraffin-embedded archival tumor tissue using immunohistochemistry (IHC)(DAKO - Hercep Test). Tumors were classified as HER-2 positive for an IHC score of greater than/equal to 2+. In addition, we evaluated HER-2 positive specimens for variability of overexpression on at least three different sections of each malignant pancreatic tissue sample. One pathologist, blinded from the results of thr first HER-2/*neu* testing, performed all the repeat IHC testing. 308 patients, 160 men and 148 women with mean ages of 68 and 73 respectively (range: 34-96 years) were identified. Mean survival was 7.5 months (range: 0-97 months). After adjustment for age, performance scores and grade of tumor, among the clinical factors evaluated, smoking ($p = 0.01$) and a history of diabetes ($p = 0.4$) were the only negative predictors of survival. Positive associations were observed with a history of surgery ($p < 0.01$) and/or chemotherapy ($p < 0.01$), tumor location in the head ($p < 0.01$) and a family history of pancreatic cancer ($p = 0.4$); although these may all not have reached statistical significance. No definite relationship was found between clinical symptoms at presentation and survival. In our study, 37/308 (12%) patients revealed HER-2/*neu* overexpression. The distribution of HER-2/*neu* positivity did not vary with histologic grade and overexpression was not an independent predictor of survival in our patients with pancreatic adenocarcinoma. Of the 37 patients with HER-2/*neu* overexpression, 28 patients had adequate malignant tissue available to assess for variability in overexpression (on the same resected specimen). Interestingly, 16/28 (57.1%) samples that were initially HER-2 positive showed variable HER-2/*neu* overexpression, ranging from 0 - 2+/3+. There seems to be significant variation in the degree of HER-2/*neu* overexpression in different samples of malignant pancreatic tissue obtained from the same patient. Unless the role of HER-2/*neu* amplification is validated using fluorescence in-situ hybridization and awaiting further definitive studies on the utility of IHC, HER-2/*neu* seems to be an unreliable target for treatment (with Herceptin) in pancreatic adenocarcinoma.

**#542 Studies with CWR22 xenograft models in nude mice suggest that ZD1839 (Iressa) may have a role in the treatment of both androgen-dependent and androgen-independent human prostate cancer.** F. M. Sirotnak, Y. She, F. Lee, H. I. Scher. *Program in Molecular Pharmacology and Experimental Therapeutics, Memorial Sloan Kettering Cancer Ctr, New York, NY; Genitourinary Oncology Service, Memorial Sloan Kettering Cancer Ctr, New York, NY.*

These studies examined the effect of the epidermal growth factor receptor tyrosine kinase inhibitor ZD1839 ('Iressa') against both androgen-dependent and androgen-independent human CWR22 prostate tumors in nude mice. Two variants (CWR22LD1 and CWR22RV1) with different degrees of androgen independence were selected. Growth of CWR22, but not CWR22LD1 or CWR22RV1, requires testosterone supplementation in male mice. Although both CWR22LD1 and CWR22RV1 are able to grow in male mice without testosterone, only CWR22RV1 is able to grow in female or castrated male mice. Daily oral administration (qd x 5 for 2 consecutive weeks) of ZD1839 at its MTD (150 mg/kg; Sirotnak et al. Clin Cancer Res 2000; 6: 4885-4892) inhibited the growth of the androgen-dependent CWR22 by 54% and the growth of CWR22LD1 and CWR22RV1 by 76%. The two variants responded similarily to ZD1839 despite fourfold higher levels of EGFR expression in CWR22RV1 than in CWR22LD1 and CWR22, as determined by real-time RT-PCR. Levels of expression of androgen receptor and CyclinD1 genes were 8- and 30-fold higher, respectively, in CWR22RV1 than in the other tumors; levels of Her-2/neu gene expression were similar in all three tumors. Coadministration of ZD1839