Mechanisms of Tumor Progression and Cancer Therapy

Thursday at 1:25 p.m. in MS&E 0113

Bone Metastatic Microenvironment: Oncostatin M Promotes Osteolytic Bone Degradation and Breast Cancer Metastasis, **KEN TAWARA*, CELESTE BOLIN, CALEB SUTHERLAND, JEFF REDSHAW, PATRICK ARANDA, JIM MOSELY, ROBIN ANDERSON, and CHERYL L. JORCYK** (Department of Biological Sciences, Boise State University, 1910 University Drive, Boise, ID 83725-1515; kentawara@boisestate.edu).

Oncostatin M (OSM) belongs to the interleukin-6 (IL-6) family of inflammatory cytokines. Here we investigate the role of OSM in the formation of bone metastases *in vivo* using the 4T1.2 mouse mammary tumor model in which OSM expression was knocked down using shRNA (4T1.2-OSM). 4T1.2-OSM cells were injected orthotopically into Balb/c mice, resulting in an almost complete elimination of spontaneous metastasis to bone. Intratibial injection of these same 4T1.2-OSM cells also dramatically reduced the osteolytic destruction of trabecular bone volume compared to control cells. Furthermore, in a tumor resection model, mice bearing 4T1.2-OSM tumors showed an increase in survival by a median of 10 days. To investigate the specific cellular mechanisms important for OSM-induced osteolytic metastasis to bone, an *in vitro* model was developed using the RAW 264.7 pre-osteoclast cell line co-cultured with 4T1.2 mouse mammary tumor cells. Treatment of co-cultures with OSM resulted in a 3-fold induction of osteoclastogenesis using the TRAP assay. We identified an OSM induced factor, amphiregulin (AREG) which increased osteoclast differentiation. Our results suggest that one mechanism for OSM-induced osteoclast differentiation is via an AREG autocrine loop, resulting in decreased osteoprotegerin secretion by the 4T1.2 cells. These data provide evidence that OSM might be an important therapeutic target for the prevention of breast cancer metastasis to bone.

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Antitumor Activity of a Polypryidyl Chelating Ligand: In Vitro and In Vivo Inhibition of Glioblastoma, **CLEMENT N. DAVID[1], ELMA S. FRIAS[2], CATHERINE C. ELIX[2], AMEAE M. WALKER[1], JACK F. EICHLER[2], and EMMA H. WILSON[1]*** ([1]Division of Biomedical Sciences, School of Medicine, University of California Riverside, Riverside, CA 92521; [2]Department of Chemistry, College of Natural and Agricultural Sciences, University of California, Riverside, CA USA 92521; emmaw@ucr.edu).

Glioblastoma multiforme is an extremely aggressive and invasive form of central nervous system (CNS) tumor commonly treated with the chemotherapeutic drug Temozolomide (TMZ). Unfortunately, the median survival is still less than 18 months. In this study, we test the anti-tumor capability of a phenanthroline-based ligand, 2,9-di-*sec*-butyl-1,10-phenanthroline (SBP). In an effort to assess the anti-tumor capabilities of SBP, *in-vitro* studies were considered using rodent GL-26 and C6 glioma cells and human U-87, and SW1088 glioblastomas/astrocytomas. *In-vivo* studies were done using mice in which a glioma was established by an intracranial injection of GL-26 cells using a stereotactic mouse frame. SBP injections were given intravenously through the retro-orbital route on day 1, 7 and 13 post tumor implantation. Tumor size and SBP toxicity was quantified. SBP demonstrated strong *in-vitro* activity against GL-26, C6 and SW1088 cells, with little toxicity towards non-tumorigenic primary murine and human astrocytes. *In-vivo* experiments demonstrate a significant reduction in tumor growth with administration of SBP alone, with mild toxicity observed in healthy tissues. Furthermore, *in-vitro* and *in-vivo* TUNEL stain suggests that SBP induces apoptosis in gliomas. These experiments suggest SBP is effective in slowing CNS glioblastoma progression and should be considered as a potential compound for future anticancer drug development.

Antimaia Inhibits Breast Cancer Metastasis through Effects on Both Tumor and Immune Cells, **KUAN-HUI E. CHEN, TOMOHIRO YONEZAWA, MRINAL K. GHOSH, and AMEAE M. WALKER*** (Division of Biomedical Sciences, University of California, Riverside, CA 92521; ameae.walker@ucr.edu)

Several lines of evidence support a role for prolactin (PRL) in the development and progression of cancers of the ovary, prostate and breast. PRL receptors (PRLR) are differentially spliced to produce a variety of transmembrane proteins that initiate different signaling cascades. Previous work has implicated the long form of the PRLR in cancer progression. We have developed a splice-modulating oligomer that specifically and dose-dependently knocks down the long PRLR *in vivo*. For ease of description, we have named the oligomer, Antimaia. Using both the 4T1 mouse syngeneic and a human xenograft breast cancer model, we have shown that Antimaia markedly inhibits metastatic spread to the liver and lungs. Some inhibition is due to direct effects of Antimaia on tumor cells, including a reduction in the number of cancer stem cells. Other effects are on immune cell recruitment to metastatic sites, resulting in reduced monocytes and increased tumor-specific cytotoxic T cells. Despite increased numbers of cytotoxic T cells, inflammatory damage in metastatic sites was reduced with Antimaia treatment, perhaps the result of cytokine-specific effects in tumor T regulatory (Treg) cells *viz.* inhibition of production of TGFβ, but not IL-10 or IL-2. When tumor cells were co-cultured with Tregs from control tumors, PRL induced a more mesenchymal phenotype. When Tregs from Antimaia-treated animals were used, PRL induced a more epithelial phenotype, as it did in the absence of Tregs. We conclude that Antimaia has great potential for the treatment and prevention of metastatic disease.

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Immune Responses and Racial Disparities in Colon Cancer, **KATHLEEN L. McGUIRE[1], MOHAMMAD W. KHAN[1]*, and JOHN M. CARETHERS[2]** ([1]Department of Biology, Molecular Biology Institute, San Diego State University, 5500 Campanile Drive, San Diego, CA 92182-4614; [2]Division of Gastroenterology, Department of Internal Medicine, University of Michigan, Ann Arbor, MI; kmcguire@mail.sdsu.edu).

Immune responses have been recently shown to have a significant impact on cancer development, progression and metastasis. Patient outcome may be dependent upon the balance of pro- and anti-tumor immune responses in malignancy. That anti-tumor immunity can significantly protect patients has been well demonstrated in colorectal cancer (CRC). CRC is the third leading cause of cancer deaths in the US and African Americans have more CRC and higher fatality than Caucasians. CRC can be caused by chromosomal instability (CIN) or microsatellite instability (MSI). MSI is the result of mismatch repair defects leading to tumors that contain strong immune responses and are less aggressive than CIN tumors. We now have evidence that African Americans have fewer

MSI tumors than Caucasians. EMAST, an instability of specific tetranucleotide repeats, appears to be a marker for aggressive CRC, and we have evidence that African Americans have more EMAST^{positive} rectal cancer. EMAST^{positive} tumors are also characterized by heavy immune infiltrate, but it is presumably pro-tumor, leading to the increased aggressiveness. A more recent study of over two hundred CIN tumors has shown that African Americans overall have poorer anti-tumor immune responses in their colon tumors than Caucasians. Taken all together, these data suggest genetic changes lead to more aggressive disease in African Americans and show that immune responses may contribute to the racial disparity observed in CRC.

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Promoting Breast Cancer Metastasis: A Role for the Inflammatory Cytokine Oncostatin M, **CHERYL L. JORCYK (**Department of Biological Sciences, Biomolecular Sciences Program, Boise State University, 1910 University Drive, Boise, ID 83725-1515; cjorcyk@boisestate.edu).

Oncostatin M (OSM) is an interleukin-6 (IL-6)-family cytokine that has been implicated in a number of biological processes including inflammation, hematopoiesis, immune responses, and development. It is produced by multiple cell types, including activated T cells, macrophages, neutrophils, and tumor cells such as breast. OSM was initially shown to inhibit the proliferation of breast cancer cells *in vitro*, and was therefore evaluated as a potential cancer therapy. Evidence in the literature and data from our laboratory; however, suggests that OSM promotes tumor invasion and metastasis. In breast cancer cells, OSM induces secretion of proteases important for breakdown of the extracellular matrix during invasion and metastasis, promotes expression of angiogenic factors such as vascular endothelial growth factor (VEGF) and hypoxia-inducible factor 1alpha (HIF1alpha), and induces expression of pro-metastatic inflammatory factors such as cyclooxygenase-2 (COX2). The results from our novel *in vivo* studies will be presented and may provide evidence that OSM is an important therapeutic target for the prevention of breast cancer metastasis.

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