Emerging Roles of IL-8 in Cancer Etiology

Introduction to chemokine receptor networks:

Chemokines are chemotactic cytokines belonging to a family of small, secreted molecules that were discovered based on their ability to induce directed leukocyte migration. The principal role of chemokines is to function as chemoattractants, causing the directed migration of cells along a concentration gradient, termed a chemokine gradient, which leads to accumulation of the cells at the source of chemokine production. While some chemokines are homeostatic in nature and are constitutively secreted, others are specifically secreted at sites of infection or in response to a pro-inflammatory stimulus, collectively playing important roles in inflammation and immune surveillance. Chemokines are classified into 4 groups, CXC, CC, C, and CX_3C, defined by the position of the conserved cysteine residues within the amino terminus. There are more than 50 chemokines identified thus far that exert their biological effects by pairing with chemokine receptors. About 19 characterized chemokine receptors have been reported, all of which belong to the G protein-coupled receptor (GPCR) family. These 19 receptors are further divided into 4 distinct groups based on the family of chemokines to which they bind. Although originally identified in leukocytes, chemokines and their receptors are secreted/expressed by several cell types including epithelial cells, endothelial cells, and fibroblasts. Expression of chemokines and their receptors varies greatly between cell types, and is determined by a cell's lineage and differentiation state, as well as by the presence of inflammatory cytokines and the chemokine gradient in the microenvironment. The repertoire of chemokines and chemokine receptors in a tissue determines the cellular signaling potential and physiological response (Balkwill, 2004; Fernandez and Lolis, 2002; Kakinuma and Hwang, 2006).

The chemokine-receptor network evolved to benefit the host, however, deregulation of these proteins is associated with several pathologies. There is mounting evidence for a biological function of chemokines as mediators of tumorigenesis (Bonecchi et al., 2011; Richmond et al., 2009). One of the hallmarks of cancer is immune-cell infiltration, in which leukocytes are recruited to the proximity of tumors in a process orchestrated by chemokines. Additionally, cancer cells themselves can acquire expression of chemokine receptors either by genetic mutations or by changes in the microenvironment (e.g. hypoxia), allowing them to respond to chemokine gradients. Tumor cells exploit chemokine-receptor expression patterns to induce cell growth, survival, and angiogenesis, all processes that contribute to the metastatic potential of tumor cells to target organs (Koizumi et al., 2007). Members of the CXC family were amongst the first chemokines identified as mediators of tumorigenesis. Interleukin-8 (IL-8), also known as CXCL8 belongs to the CXC family of chemokines and its role in the development of tumors and site-specific spread of cancer cells is an active area of investigation (Koizumi et al., 2007; Waugh and Wilson, 2008).

IL-8 Signaling and its role in cancer:

The pro-inflammatory cytokine IL-8 was initially named neutrophil-activating peptide-1 for its activity as a potent chemotactic agent for neutrophils in inflammatory and immune diseases. More recently, a role for IL-8 in cancer has been established whereby it was shown that IL-8 regulates tumor-promoting processes, including cell migration, angiogenesis, and metastasis (Xie, 2001).

IL-8 signals through two cell surface GPCRs, CXCR1 and CXCR2, receptors that are also shared by other chemokine family members to transduce signals. As is characteristic of the GPCR family, CXCR1 and CXCR2 associate with heterotrimeric G proteins, consisting of the α, β, and γ subunits. Ligand binding leads to the exchange of guanosine diphosphate for guanosine triphosphate on the Gα subunit. This catalytic reaction further leads to the dissociation of the Gα subunit from the Gβγ subunits. The Gα and Gβγ subunits activate three primary downstream signaling cascades: phosphatidyl- inositol 3' kinase/Akt (PI3K/Akt), phospholipase C/protein kinase C (PLC/PKC), and Ras/Raf/extracellular signal-regulated protein kinases 1 and 2 (Erk1/2). Other signaling cascades activated in response to IL-8 signaling include the focal adhesion kinase, Rho, Rac, and Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathways. Through these signaling cascades, IL-8 activates a host of effectors and downstream targets that collectively aid in the process of tumorigenesis (Waugh and Wilson, 2008; Xie, 2001).

IL-8 exerts its tumorigenic effects through both autocrine and paracrine signaling. Secretion of IL-8 by tumor cells can enhance cell survival and proliferation of the cancer cells through autocrine signaling. IL-8 also plays an important role in the tumor microenvironment, which is composed of a diverse group of cells including fibroblasts, endothelial cells, dendritic cells, and tumor-associated macrophages. A complex network of cytokines, chemokines, and other inflammatory molecules drives communication between the microenvironment and the malignant tumor cells (Todorovic-Rakovic and Milovanovic, 2013). Importantly, IL-8 is implicated in the initiation of leukocyte infiltration, neovascularization, and angiogenesis, all processes that precede invasion and metastasis of tumor cells. Cancer cells secrete IL-8, triggering the infiltration of leukocytes to the site of chemokine production. Leukocytes in turn secrete cytokines and growth factors promoting angiogenesis and cell survival (Gales et al., 2013). In addition, tumor-secreted IL-8 acts on endothelial cells and other components of the microenvironment that express the IL-8 receptors, leading to the production of other cytokines and growth factors that influence the invasiveness and metastatic potential of the primary tumor. Specifically, a recent study showed that IL-8 activates vascular endothelial growth factor (VEGF) expression in endothelial cells through autocrine signaling, thereby promoting angiogenesis (Martin et al., 2009). Furthermore, IL-8 also enhances the production of matrix metalloproteinases like MMP-2 and MMP-9 in tumor cells, thereby promoting tumor dissemination (Luca et al., 1997) (Fig.1).
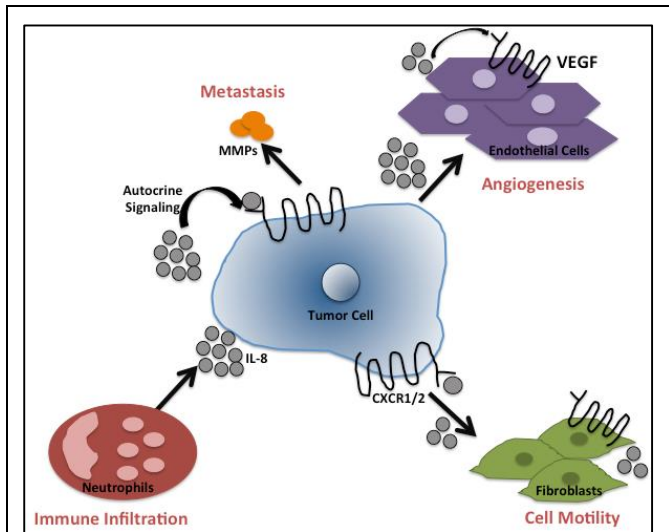
Figure 1: Role of IL-8 in tumorigenesis

IL-8 exerts profound effects on tumor cells and the surrounding microenvironment through both autocrine and paracrine signaling. Multiple cell types express CXCR1/2 and are responsive to IL-8. IL-8 signaling can lead to activation of immune cells, growth factors, cytokines and other molecules, which activate tumor-promoting processes such as cell motility, angiogenesis, and metastasis.

IL-8 as a prognostic marker and therapeutic target in cancer:

Given the wide array of biological functions IL-8 exerts on tumor cells and the surrounding microenvironment, it is not surprising that high IL-8 expression is characteristic of patients with melanoma, breast carcinomas, pancreatic cancers, and ovarian cancers (Chen et al., 2012; De Larco et al., 2001; Singh et al., 1994). Interestingly, all these tumor types have a high propensity to metastasize. IL-8 expression has been studied as a potential prognostic marker in several different preclinical and clinical tumor specimens (Kotyza, 2012). In pancreatic cancer patients, high IL-8 expression was detected both in the tumors themselves as well as in patient serum (Chen et al., 2012). Tumors from patients with high IL-8 serum levels grew faster and were more aggressive compared to the tumors from patients with low IL-8 serum levels when transplanted into nude mice (Matsuo et al., 2012). High IL-8 expression also correlates with high metastatic potential of human melanoma cell lines in a xenograft mouse model (Singh et al., 1994). Furthermore, in non-human models of cancer, IL-8 serves as a putative indicator of tumorigenic potential as high IL-8 expression is associated with a canine model of human inflammatory mammary cancer (de Andres et al., 2013). Elevated expression of IL-8 is also a recurrent feature of hemangiosarcoma, a highly metastatic form of cancer that occurs commonly in dogs.

The role of IL-8 in cancer has predominantly been established based on correlative studies such as those mentioned above. However, the precise mechanism(s) underlying the role of IL-8 in promoting

tumorigenesis remain unclear. The establishment of the molecular mechanism of IL-8 in cancer has proven difficult due to the redundancy in functions of chemokines that signal through the CXCR1 and CXCR2 receptors (Balkwill, 2004). In addition, the absence of a human IL-8 orthologue in mice has largely hindered research into the role of IL-8 in cancer, underscoring the importance of developing alternative genetic models to advance our knowledge of IL-8 in the pathogenesis of cancer (Bozic et al., 1994). There are several IL-8-relevant reagents available for use in a host of species, including bovine, canine, and feline, which express IL-8 orthologues. These reagents will be useful for gaining further insight into the mechanisms of IL-8 function in tumor promotion.

Regardless of the molecular mechanisms of IL-8 function in carcinogenesis, multiple reports suggest that IL-8 and its receptors serve as valid therapeutic targets in different forms of cancer. To this end, a humanized antibody against IL-8 as well as a small-molecule inhibitor, repertaxin, of the IL-8 receptor have been tested in several xenograft tumor models with promising effects in the context of their ability to inhibit tumor growth, angiogenesis, and metastasis (Gales et al., 2013). It is important to note that, apart from cancer, IL-8 plays an active role in the etiology of several inflammatory pathologies such as psoriasis, rheumatoid arthritis, and inflammatory bowel disease (Qazi et al., 2011). There is a striking similarity in the contribution of chemokines to the pathogenesis of inflammatory conditions and cancer. Thus, further research could lead to enhanced knowledge of the specific functions of IL-8 in diseases, opening up new avenues for therapeutic intervention.

References:

Balkwill, F. (2004). Cancer and the chemokine network. Nat Rev Cancer *4*, 540-550.

Bonecchi, R., Locati, M., and Mantovani, A. (2011). Chemokines and cancer: a fatal attraction. Cancer Cell *19*, 434-435.

Bozic, C.R., Gerard, N.P., von Uexkull-Guldenband, C., Kolakowski, L.F., Jr., Conklyn, M.J., Breslow, R., Showell, H.J., and Gerard, C. (1994). The murine interleukin 8 type B receptor homologue and its ligands. Expression and biological characterization. J Biol Chem *269*, 29355-29358.

Chen, Y., Shi, M., Yu, G.Z., Qin, X.R., Jin, G., Chen, P., and Zhu, M.H. (2012). Interleukin-8, a promising predictor for prognosis of pancreatic cancer. World J Gastroenterol *18*, 1123-1129.

de Andres, P.J., Illera, J.C., Caceres, S., Diez, L., Perez-Alenza, M.D., and Pena, L. (2013). Increased levels of interleukins 8 and 10 as findings of canine inflammatory mammary cancer. Vet Immunol Immunopathol *152*, 245-251.

De Larco, J.E., Wuertz, B.R., Rosner, K.A., Erickson, S.A., Gamache, D.E., Manivel, J.C., and Furcht, L.T. (2001). A potential role for interleukin-8 in the metastatic phenotype of breast carcinoma cells. Am J Pathol *158*, 639-646.

Fernandez, E.J., and Lolis, E. (2002). Structure, function, and inhibition of chemokines. Annu Rev Pharmacol Toxicol *42*, 469-499.

Gales, D., Clark, C., Manne, U., and Samuel, T. (2013). The Chemokine CXCL8 in Carcinogenesis and Drug Response. ISRN Oncol *2013*, 859154.

Kakinuma, T., and Hwang, S.T. (2006). Chemokines, chemokine receptors, and cancer metastasis. J Leukoc Biol *79*, 639-651.

Koizumi, K., Hojo, S., Akashi, T., Yasumoto, K., and Saiki, I. (2007). Chemokine receptors in cancer metastasis and cancer cell-derived chemokines in host immune response. Cancer Sci *98*, 1652-1658.

Kotyza, J. (2012). Interleukin-8 (CXCL8) in tumor associated non-vascular extracellular fluids: its diagnostic and prognostic values. A review. Int J Biol Markers *27*, 169-178.

Luca, M., Huang, S., Gershenwald, J.E., Singh, R.K., Reich, R., and Bar-Eli, M. (1997). Expression of interleukin-8 by human melanoma cells up-regulates MMP-2 activity and increases tumor growth and metastasis. Am J Pathol *151*, 1105-1113.

Martin, D., Galisteo, R., and Gutkind, J.S. (2009). CXCL8/IL8 stimulates vascular endothelial growth factor (VEGF) expression and the autocrine activation of VEGFR2 in endothelial cells by activating NFkappaB through the CBM (Carma3/Bcl10/Malt1) complex. J Biol Chem *284*, 6038-6042.

Matsuo, Y., Takeyama, H., and Guha, S. (2012). Cytokine network: new targeted therapy for pancreatic cancer. Curr Pharm Des *18*, 2416-2419.

Qazi, B.S., Tang, K., and Qazi, A. (2011). Recent advances in underlying pathologies provide insight into interleukin-8 expression-mediated inflammation and angiogenesis. Int J Inflam *2011*, 908468.

Richmond, A., Yang, J., and Su, Y. (2009). The good and the bad of chemokines/chemokine receptors in melanoma. Pigment Cell Melanoma Res *22*, 175-186.

Singh, R.K., Gutman, M., Radinsky, R., Bucana, C.D., and Fidler, I.J. (1994). Expression of interleukin 8 correlates with the metastatic potential of human melanoma cells in nude mice. Cancer Res *54*, 3242-3247.

Todorovic-Rakovic, N., and Milovanovic, J. (2013). Interleukin-8 in breast cancer progression. J Interferon Cytokine Res *33*, 563-570.

Waugh, D.J., and Wilson, C. (2008). The interleukin-8 pathway in cancer. Clin Cancer Res *14*, 6735-6741.

Xie, K. (2001). Interleukin-8 and human cancer biology. Cytokine Growth Factor Rev *12*, 375-391.