Commentary

Roles for Aberrant CXCR3 Signaling in Basal Cell Carcinoma

A Case for Dual Activity

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Cellular growth and proliferation are normally regulated by extracellular stimulation through receptor–ligand interactions at the cell surface. Depending on the cell type, a cell can either express a cell-surface receptor, secrete its cognate ligand, or in some cases both. Cells bearing a specific cell-surface receptor can respond to its cognate ligand secreted by a neighboring cell (paracrine signaling) or a more distant cell (endocrine signaling). If a cell expresses a receptor as well as its ligand, a positive feedback loop is generated (autocrine signaling). Through orchestration of the multitude of heterotypic and homotypic signaling elements, homeostasis of normal cellular proliferation is maintained.

Cancer cells are frequently characterized by aberrant, deregulated receptor signaling, either through prevalent (dominant) autocrine signaling loops or nonautocrine signaling loops, which can drive tumor cell proliferation, angiogenesis, motility, migration, and invasion as well as metastasis.^{1,2} This can involve a number of cell-surface receptor tyrosine kinases (RTKs), several types of extracellular matrix receptors (such as integrins), and different kinds of G protein–coupled cell surface receptors (such as chemokine receptors). In the current issue of *The American Journal of Pathology*, Lo and colleagues describe a role for CXCR3/ligands through autocrine and paracrine-signaling loop in proliferation and tumorigenesis of basal cell carcinoma (BCC).³

BCC develops from the basal layer of the epidermis or from the pilosebaceous adnexa, accounting for 75% of all cases of non-melanoma skin cancer in the United States. Unlike melanoma, BCC is rarely metastatic and is typically characterized by low mortality and highly successful surgical excision. However, BCC commonly causes cosmetic disfigurement because it usually affects sun-exposed skin of the head, neck, and face. BCC has a very high incidence rate and is the most common type of skin

cancer. More than one-fifth of the population of the United States develops BCC. Moreover, the incidence frequency is rising by 2% to 19% per year.³

Aberrant CXCR3 Signaling in Proliferation and Survival of BCC

CXCR3 is a chemokine receptor belonging to the G protein–coupled cell surface receptor family. CXCR3 can bind with high affinity to the interferon- γ -induced chemokines CXCL9, CXCL10, and CXCL11.⁴ Moreover, CXCR3 exists as three alternatively-spliced forms: CXCR3-A, CXCR3-B, and CXCR3-alt.^{4,5} CXCR3-A is the predominant form in activated T cells, natural killer cells, dendritic cells, and B cells,⁴ whereas CXCR3-B has been detected on human microvascular endothelial cells. CXCR3-alt is characterized by a truncated C terminus with loss of the intact second and third extracellular loop, and coexpresses with CXCR3-A. Recent studies have found that CXCR3 is expressed in several types of tumors, including melanoma, breast cancer, and colon cancer.^{6–8} The work of Lo et al now provides new evidence linking CXCR3 to BCC.³

Based on microarray data and qRT-PCR, Lo and colleagues³ showed that expression of both CXCR3 and its ligands was increased significantly in BCC lesions relative to nonlesional skin tissue. In fact, *CXCL11*, *CXCL10*, and *CXCL9* were the three most upregulated genes in the BCC tumor samples. Moreover, all three CXCR3 isoforms were upregulated as well. Upregulation was identified in nodular, superficial, and morpheiform BCC subtypes. However, the RNAs used for these studies were recov-

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ered from tumor masses containing tumor cells, infiltrating lymphocytes, endothelial cells, and other stromal cell types. Therefore, these data alone do not constitute proof that the elevated expression of the CXCR3/ligands was derived from the BCC tumor cell itself.

To begin to determine the cellular origin of the CXCR3/ligands, Lo et al³ used immunohistochemical staining. They found that CXCL10, CXCL11, and CXCR3 were mainly localized to BCC tumor masses, whereas CXCL9 was associated with inflammatory infiltrates within the stromal region. Using double staining with the pan-BCC marker K17, the authors then showed that CXCR3 and its CXCL10 and CXCL11 ligands were expressed predominantly in K17⁺ BCC keratinocytes, but also to a lesser extent in K17⁻ stromal cells, including inflammatory cells. These observations *in situ* strongly suggest the existence in BCC of both autocrine loops, through interactions of CXCR3 with CXCL10 and CXCL11, and paracrine loops, through interactions between CXCR3 and stromal cell-secreted CXCR3 ligands (CXCL9, CXCL10, and CXCL11).

To understand the role of CXCR3/ligand signaling in BCC, Lo and colleagues³ focused on the ligand CXCL11, which was noted to increase the most dramatically in BCC tissues. Human immortalized HaCaT keratinocytes were first used as a model to test the function of CXCL11, which clearly stimulated HaCaT cell proliferation. Lo et al next cultured single cell suspensions of human BCC-derived cells and treated those primary BCC cells with CXCL11, demonstrating that treatment with an optimal concentration (10 nmol/L) of CXCL11 significantly stimulated proliferation of the primary cells. Because the single cell suspension from the tumor mass contains multiple cell types, the authors then used dual labeling of K17 and CXCR3 to distinguish BCC tumor cells from stromal cells in the presence of 10 nmol/L CXCL11. They were able to track three populations of cells: K17⁺/CXCR3⁻, K17⁺/CXCR3⁺, and K17⁻/CXCR3⁺. Notably, over 21 days in culture, CXCL11 caused a gradual and significant change only in the CXCR3⁺ populations: K17⁺/CXCR3⁺ cells numbers were greatly enhanced (from 11% to 65% of the total cells), whereas K17⁻/CXCR3⁺ cells were dramatically diminished (from 60% to less than 2%).

The authors' data clearly show that CXCL11/CXCR3 signaling promotes proliferation and/or survival of BCC cells *in vitro*.³ Perhaps more interesting is the virtual elimination of the K17⁻/CXCR3⁺ stromal population in the presence of CXCL11, an observation that was not explained but may play an important role *in vivo*. The existence of three alternatively spliced forms of CXCR3 may help account for this observation. Although Lo et al³ were unable to distinguish between CXCR3 isoforms in the tumor mass due to the lack of specific antibodies, they did show using qRT-PCR that RNAs encoding all three CXCR3 isoforms were upregulated in BCC tumor tissues, especially the CXCR3-B form (enhanced eightfold). Because the K17⁻/CXCR3⁺ population consisted of infiltrating inflammatory cells (CXCR3-A⁺), endothelial cells (CXCR3-B⁺), and other stromal cell types, it could be postulated that CXCL11 provokes differential responses in cells within the BCC mass expressing different recep-

tor isoforms. Indeed, it has been reported that overexpression of CXCR3-A induces enhanced survival of HMEC-1 human microvascular endothelial cells, whereas CXCR3-B overexpression dramatically reduces DNA synthesis and stimulates apoptosis in these cells.^{9,10} CXCR3-A and CXCR3-B also have opposing functions in breast cancer; CXCR3-B inhibits cell proliferation, whereas CXCR3-A promotes cell growth.¹¹

Does CXCR3 Signaling Promote Tumorigenesis in BCC?

The CXCR3-ligand system is best known for its involvement in two main biological functions (activities): chemoattraction of immune cells to local sites and angiogenesis.⁴ During inflammation, interferon- γ produced by leukocytes induces the production of the three major CXCR3-binding chemokines, CXCL9, CXCL10, and CXCL11, in a wide variety of cells including stromal cells, immune cells, and transformed tumor cells. In response to the presence of pathogens and transformed cells, these ligands attract (recruit) lymphocytes that express CXCR3 (i.e., activated T cells) through CXCR3-ligand paracrine signaling.¹² These chemokines also bind to the alternatively spliced variant CXCR3-B, which is expressed on human microvascular endothelial cells in the tumor microenvironment, thereby activating the p38 pathway and functioning in an angiostatic capacity.^{9,11,13} These two CXCR3-mediated activities would be thought to act as inhibitors of tumor formation; indeed, many studies have shown that activation of CXCR3-ligand loops results in anticancer immune responses through chemoattraction of multiple components, such as activated T cells, natural killer cells, macrophages, and dendritic cells, and through inhibition of angiogenesis.

However, arising evidence now suggests that the CXCR3 signaling network can influence tumor cell growth, survival, and migration, as well as angiogenesis. CXCR3 and its ligand CXCL10 are expressed in many human glioma cell lines, and CXCL10 treatment activates MAPK, stimulating cell proliferation.¹⁴ Many breast cancer cell lines, such as MDA-MB-231, MCF-7, and T37D, harbor CXCR3/ligand autocrine loops that influence cell proliferation.^{15,16} In a murine model of breast cancer, CXCR3 has been associated with poor survival and promotion of metastasis⁷; moreover, antagonism of CXCR3 by a small molecule inhibitor blocks pulmonary metastasis of a highly metastatic breast cancer.¹⁵ In addition, CXCR3 has been found to be upregulated in human melanoma, correlating with tumor progression⁶ and contributing to the regulation of cell proliferation, survival, migration, and metastasis in these cells.¹⁷ Furthermore, CXCL10/CXCR3 signaling has been reported to promote invasion-related properties as well as metastasis in human colorectal carcinoma cells.⁸

It is noteworthy that the determination of CXCR3's role in tumorigenesis is complicated by the fact that many cells in the tumor microenvironment potentially express CXCR3 and ligands. The new data of Lo et al³ suggest that aberrant CXCR3/ligand signaling promotes prolifer-

ation and survival in BCC through both autocrine and paracrine pathways. However, in their article Lo et al³ did not directly test the tumorigenic property of the CXCR3 networks. Further experiments based on grafting assays *in vivo* and soft agar assays *in vitro* will need to be performed in BCC. Moreover, RNAi-based loss-of-function assays should be used to test the specific roles of the CXCR3 isotypes and their ligands.

The Multiple Roles of CXCR3 Signaling and the Nature of BCC

Until fairly recently, the lack of appropriate model systems and the difficulty in culturing BCC cells have greatly hindered the identification of mechanisms underpinning BCC. However, analysis of the basal cell nevus syndrome, linked to basal cell carcinoma, led to the discovery that *PTCH*, homologous with the *Drosophila* segment polarity gene *patched*, experiences loss of heterozygosity and mutations in both inherited and sporadic BCC.¹⁸ The *PTCH* gene encodes a transmembrane receptor that can interact with its ligand Sonic Hedgehog (SHH) to repress the oncogenic activation of Smoothened (SMO) and the downstream transcription factor Gli1, thereby regulating cell proliferation.^{19,20} Multiple studies have shown that abnormalities within the PTCH-SHH-Gli1 pathway are associated with more than 70% of BCC, and a mutation in any one gene within this pathway is thought to be capable of inciting BCC.¹⁹ In addition to the PTCH-SHH-Gli1 pathway, other pathways or factors also contribute to the development of BCC, including mutations of p53, inactivation of 13-3-3 σ , and infection by HPV38.

The new study by Lo et al³ implicates the upregulation of CXCR3 isotypes and their ligands, the chemokines CXCL9, CXCL10, and CXCL11, in BCC genesis through autocrine and/or paracrine mechanisms, which may be opposing. The dual activities of CXCR3 signaling pathways might actually contribute to the poorly aggressive and rarely metastatic nature of BCC through both the recruitment of T-cells into the tumor microenvironment and the inhibition of endothelial cells carrying high levels of CXCR3-B, resulting in angiostatic activity.

Lo et al's study³ revealed that IL8, CXCL12, CXCR4, and CXCL4 were down-regulated in BCC as well. Down-regulation of CXCR12 and IL8 may also contribute to the inhibition of angiogenesis. CXCL4 is another high-affinity ligand of CXCR3-B, and the inhibitory function of CXCR3-B is also dependent on the presence of CXCL4. Reduced CXCL4 may therefore act to balance the pro-tumorigenic effects of enhanced expression of CXCR3-B and its ligands CXCL9, CXCL10, and CXCL11.

In summary, the work by Lo et al³ further extends our understanding of the mechanisms of BCC progression, particularly the role of CXCR3 signaling in the regulation of BCC cell proliferation. However, further studies will be needed to answer the following key questions: is deregulated CXCR3 signaling sufficient to confer tumorigenic properties to keratinocytes; how is CXCR3 expression regulated and is the PTCH-SHH-Gli1 pathway involved; do BCC tumors with deregulated CXCR3 signaling also

harbor mutations in the PTCH-SHH-Gli1 pathway; is there cross talk between the CXCR3 and PTCH pathways? The future will reveal whether this CXCR3 discovery will improve therapeutic approaches to treatment of advanced BCC.

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