The Interleukin-8 Pathway in Cancer

David J.J. Waugh and Catherine Wilson

Abstract Interleukin-8 (IL-8) is a proinflammatory CXC chemokine associated with the promotion of neutrophil chemotaxis and degranulation. This chemokine activates multiple intracellular signaling pathways downstream of two cell-surface, G protein – coupled receptors (CXCR1 and CXCR2). Increased expression of IL-8 and/or its receptors has been characterized in cancer cells, endothelial cells, infiltrating neutrophils, and tumor-associated macrophages, suggesting that IL-8 may function as a significant regulatory factor within the tumor microenvironment. The induction of IL-8 signaling activates multiple upstream signaling pathways that (*a*) impinge on gene expression via regulation of numerous transcription factor activities, (*b*) modulate the cellular proteome at the level of translation, and/or (*c*) effect the organization of the cell cytoskeleton through post-translational regulation of regulatory proteins. As a consequence of the diversity of effectors and downstream targets, IL-8 signaling promotes angiogenic responses in endothelial cells, increases proliferation and survival of endothelial and cancer cells, and potentiates the migration of cancer cells, endothelial cells, and infiltrating neutrophils at the tumor site. Accordingly, IL-8 expression correlates with the angiogenesis, tumorigenicity, and metastasis of tumors in numerous xenograft and orthotopic *in vivo* models. Recently, IL-8 signaling has been implicated in regulating the transcriptional activity of the androgen receptor, underpinning the transition to an androgen-independent proliferation of prostate cancer cells. In addition, stress and drug-induced IL-8 signaling has been shown to confer chemotherapeutic resistance in cancer cells. Therefore, inhibiting the effects of IL-8 signaling may be a significant therapeutic intervention in targeting the tumor microenvironment.

Background

Interleukin-8 (IL-8), alternatively known as CXCL8, is a proinflammatory CXC chemokine. Transcription of the IL-8 gene encodes for a protein of 99 amino acids that is subsequently processed to yield a signaling competent protein of either 77 amino acids in nonimmune cells or 72 amino acids in monocytes and macrophages. Expression of IL-8 is primarily regulated by activator protein and/or nuclear factor-κB – mediated transcriptional activity, although additional hormone response elements and NF-IL-6 consensus sites have been characterized on the IL-8 gene promoter. Accordingly, expression of IL-8 has been shown to be regulated by a number of different stimuli including inflammatory signals (e.g., tumor necrosis factor α, IL-1β), chemical and environmental stresses (e.g., exposure to chemotherapy agents and hypoxia), and steroid hormones (e.g., androgens, estrogens, and dexamethasone; reviewed in ref. 1).

The biological effects of IL-8 are mediated through the binding of IL-8 to two cell-surface G protein – coupled receptors, termed CXCR1 and CXCR2 (2, 3). These receptors share considerable structural similarity suggesting that these genes arose through gene duplication. Signals are transmitted across the membrane through ligand-induced conformational changes, exposing epitopes on the intracellular loops and carboxy-terminal tail of the receptor that promote coupling to functional heterotrimeric G proteins. Classically, the chemotactic response induced in response to CXC-chemokines is attenuated in the presence of pertussis-toxin, suggesting that Gαi is the predominant G protein coupled to this family of receptors (4, 5). Certain IL-8 – promoted responses are insensitive to pertussis-toxin, however, suggesting that these receptors may couple to and activate other as yet uncharacterized Gα proteins (6). This promiscuity of G protein coupling may be dictated by differential cell-specific expression of the Gα proteins that influence the affinity-based equilibrium established between the receptors and the intracellular pool of G proteins. Furthermore, the diversity of signaling pathways activated by CXCR1 and CXCR2 can also be understood on the basis of the increasingly recognized importance of the Gβγ subunits in signaling to primary effectors, in addition to the discovery that GPCRs can signal through additional non – G protein – dependent pathways.

CXCR1 and CXCR2 also exhibit a markedly distinct ligand-binding pharmacology. CXCR1 receptors are activated only in response to binding of IL-8 and granulocyte chemotactic protein-2. Alternatively, CXCR2 is activated by multiple CXC-chemokines including growth-related oncogenes (GROα, β, and γ), neutrophil-activating peptide, and granulocyte chemotactic protein-2 (reviewed in ref. 1). The differential pharmacology

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and potential redundancy of these chemokine receptors is a significant consideration in designing therapeutic strategies to attenuate the effects of CXC-chemokine signaling within the tumor microenvironment.

IL-8 Signaling Pathways and Cellular Responses

This review will focus on providing an overview of several well-characterized signaling pathways that are induced downstream of IL-8 receptors and which underpin the significance of this chemokine in promoting the malignant progression of cancer. Many studies have shown overexpression of IL-8 by tumor cells, often induced in response to chemotherapeutic interventions or environmental stresses such as hypoxia. The increased synthesis and secretion of IL-8 from tumor cells has wider significance to the tumor microenvironment given the characterized expression of CXCR1 and CXCR2 receptors on cancer cells, endothelial cells, and neutrophils/tumor-associated macrophages. In this review, we summarize the key signaling pathways that are activated in these cell-types in response to IL-8 (Fig. 1) and then discuss the potential significance of IL-8 signaling in modulating communication between the different cell types present within the tumor microenvironment (Fig. 2). The final aspect of this review then summarizes the potential significance and emerging data regarding IL-8 and IL-8 receptors as therapeutic targets in cancer.

Activation of serine/threonine kinases. Phosphatidylinositol-3 kinase is one of the principal targets of heterotrimeric Gαi and βγ subunits. This lipid/protein kinase was identified as one of principal effectors of IL-8–promoted chemotaxis of

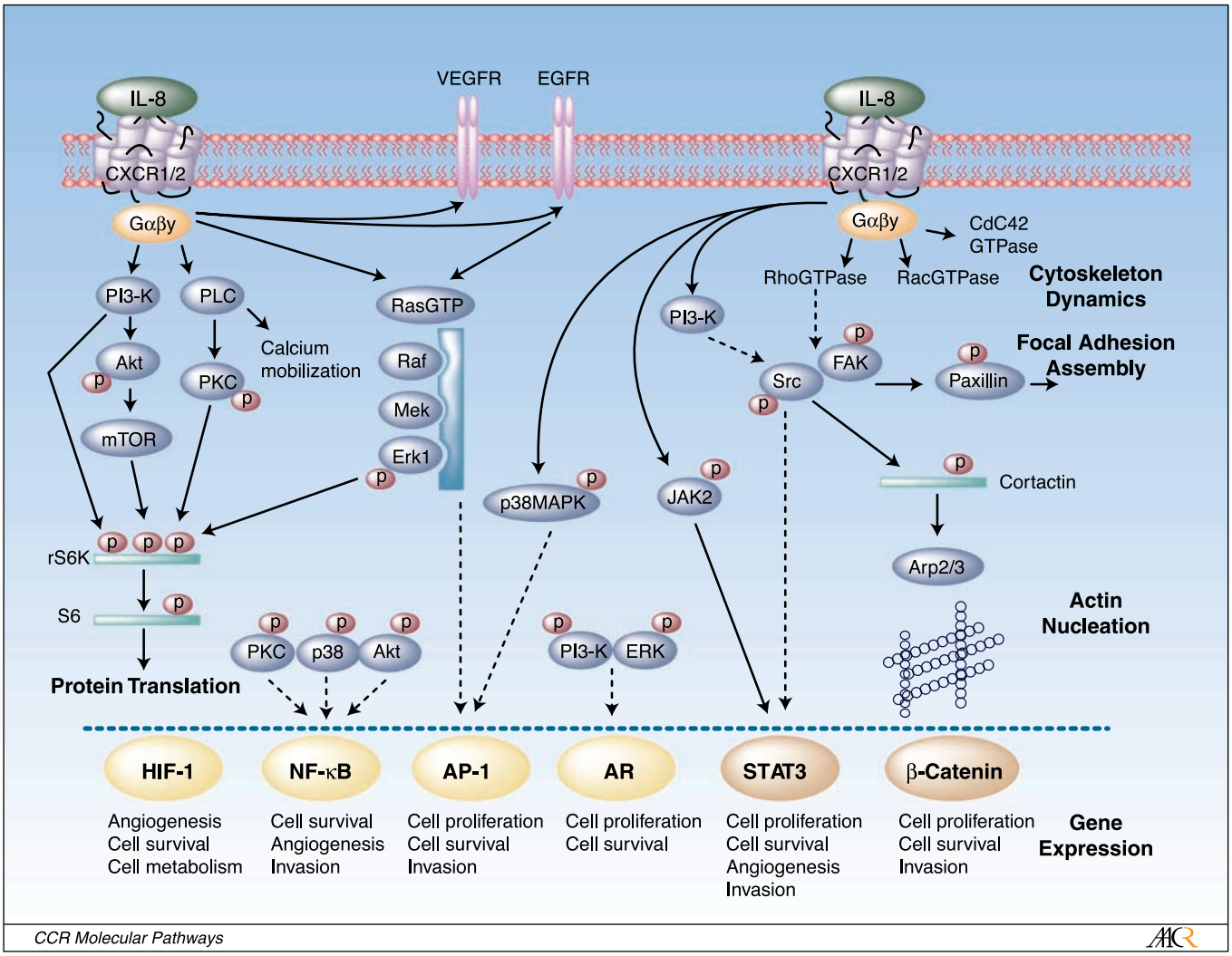

Fig. 1. Characterized IL-8 signaling pathways. A schematic diagram illustrating the range of signaling pathways that are activated after stimulation of CXCR1 and/or CXCR2 receptors with IL-8. After activation of heterotrimeric small G proteins, IL-8 signaling promotes activation of the primary effectors phosphatidyl-inositol-3-kinase or phospholipase C, promoting the activation of Akt, PKC, calcium mobilization and/or MAPK signaling cascades. These signaling pathways have been shown to promote protein translation (*left*) and regulate the activity of a range of transcription factors (*bottom*). *Solid gold lines,* transcription factors whose activity has been shown to be positively regulated by IL-8 signaling using various reporter assays. In the case of signal transducers and activators of transcription 3 (*STAT3*) and β-catenin, IL-8 signaling has been shown to promote nuclear translocation of these factors; however, transcriptional activation of either factor remains to be shown. *Dashed lines,* the putative pathways through which IL-8 signaling regulates transcription factor activity. In addition, IL-8 signaling activates members of the RhoGTPase family and activates a number of nonreceptor tyrosine kinases (e.g., Src family kinases and FAK) that regulate the architecture of the cell cytoskeleton and its interaction with the surrounding extracellular environment (*right*).

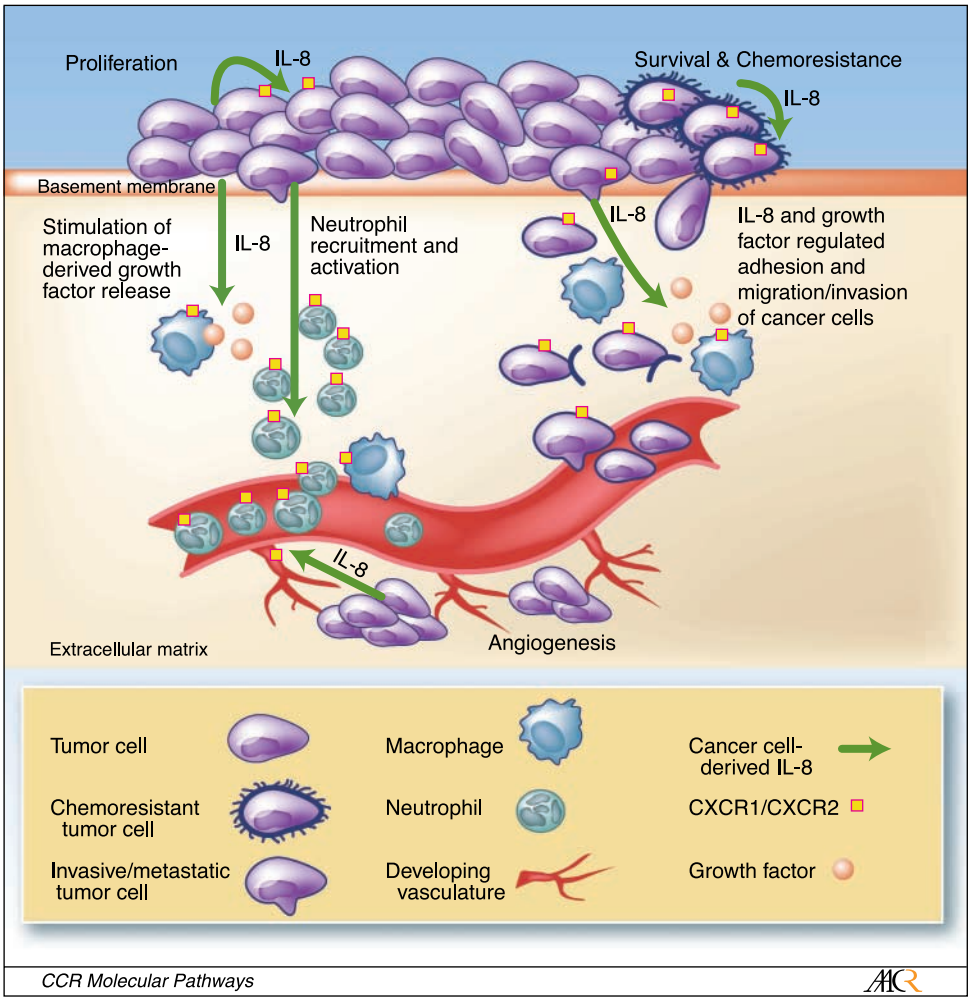

Fig. 2. The role of IL-8 signaling in the tumor microenvironment. Tumor-derived IL-8 has the capacity to exert profound effects on the tumor microenvironment. For example, secretion of IL-8 from cancer cells can enhance the proliferation and survival of cancer cells through autocrine signaling pathways. In addition, tumor-derived IL-8 will activate endothelial cells in the tumor vasculature to promote angiogenesis and induce a chemotactic infiltration of neutrophils into the tumor site. Although IL-8 can promote cell invasion and migration, the capacity of IL-8 to induce tumor-associated macrophages to secrete additional growth factors will further increase the rate of cell proliferation and cancer cell invasion at the tumor site. The multiple effects of IL-8 signaling upon different cell types present within the tumor microenvironment suggests that targeting of CXC-chemokine signaling (including but not limited to IL-8) may have important implications to halt disease progression and assist in sensitizing tumors to chemotherapeutic and biological agents.

neutrophils, resulting in increased phosphorylation of its substrate serine/threonine kinase, PKB/Akt (7). Increased Akt expression and activity have been detected in multiple forms of cancer and its role in modulating cell survival, angiogenesis, and cell migration have established this kinase as an important therapeutic target in cancer (reviewed in ref. 8). Activation of Akt by IL-8 signaling has been shown in a number of cancer cell lines. Studies conducted by our laboratory suggest that IL-8 signaling not only induces activation of Akt but also increases the expression of Akt in androgen-independent prostate cancer cell lines (9).

IL-8 signaling also regulates the activity of the mitogen-activated protein kinase (MAPK) signaling cascade that constitutes a number of serine/threonine kinases that are colocalized via their interaction with scaffolding proteins in close proximity to cell-surface receptors. The substrate specificity of these kinases results in the activation of distinct signaling cascades, the best characterized of which is the Raf-1/MAP/ERK kinase 1/Erk cascade. IL-8 signaling has been shown to induce the activation of this classic MAPK signaling cascade, with downstream phosphorylation of Erk1/2 detected in both neutrophils (10) and cancer cells (9, 11, 12). In neutrophils, phosphatidylinositol-3 kinase activity has been identified as a key intermediate in coupling IL-8 receptors to MAPK signaling (10), whereas studies conducted in ovarian and lung cancer cell lines show that IL-8 signaling transactivates the epidermal growth factor receptor, promoting the downstream activation of MAPK signaling, mediated through growth factor receptor binding protein 2/SOS-promoted activation of the monomeric small G protein, Ras-GTPase (11, 12). Activation of MAPK signaling is consistent with the cell proliferation– and cell survival–promoting effects of IL-8 that have been reported in neutrophils (13), and endothelial (14) and cancer cell lines (9, 11, 12, 15–17). Activation of the Erk-MAPK signaling ultimately describes a putative pathway linking IL-8 signaling to the activation of E2F and activator protein transcription factors, whose function is to primarily regulate the transcription of many genes implicated in cell proliferation. Furthermore, IL-8 signaling activates the p38 MAPK signaling cascade (18); however, the functional importance of this MAPK to IL-8–induced responses remains to be determined.

In addition to activating phosphatidylinositol-3 kinase, the coupling of chemokine receptors to Gαi-proteins links IL-8 signaling to the activation of phospholipase C (ref. 19). Phospholipase C signaling promotes the conversion of membrane-associated lipids to diacylglycerol and inositol triphosphate, resulting in calcium mobilization and activation of typical, novel, and atyptical isoforms of protein kinase C (PKC). Administration of IL-8 to neutrophils induces the phosphorylation of multiple PKC isoforms, including PKCα,

PKCβI, and PKCβII, underpinning the secretory function, respiratory burst activity and Mac-1–mediated adhesion of these cells (20, 21). Furthermore, activation of CXCR1, but not CXCR2, signaling in reconstituted cells was shown to activate the novel PKC isoform, PKCε, a response implicated in mediating a CXCR1-induced densensitization of CXCR2 signaling (21). Activation of a phospholipase C–dependent PKC signaling pathway has also been characterized in cancer cells after stimulation with IL-8 (22). Activation of PKC, concurrent with the modulation of intracellular Ca^{2+} signaling, has been shown to regulate IL-8–promoted chemotaxis of human bladder cancer cells, a response that is proposed to result from PKC-mediated regulation of the actin cytoskeleton. In addition, we have shown IL-8–promoted activation of a further atypical isoform, PKCζ, and shown this to regulate protein expression at the level of translation in androgen-independent prostate cancer cells (9).

Activation of protein tyrosine kinases. Protein tyrosine kinases are a further downstream target of IL-8 signaling responses in cancer and endothelial cells. IL-8 signaling has been shown to promote the transactivation of the epidermal growth factor receptor in ovarian cancer (10) and vascular endothelial cells (23), promoting downstream activation of MAPK signaling. More recently, IL-8 signaling has been shown to induce the phosphorylation of the vascular endothelial growth factor receptor (VEGFR-2) in endothelial cells, regulating the permeability of the endothelial barrier (24). Nonreceptor tyrosine kinases are also key intermediates in IL-8–induced signaling cascades. Independent studies conducted in CXCR1- and CXCR2-reconstituted cell lines, HEK293 and RBL, confirm that members of the Src family of kinases and p125 focal adhesion kinase (FAK) are upstream signaling substrates of IL-8 (25–27). Increased phosphorylation of Src-kinases and FAK have also been detected in cancer cells after stimulation with IL-8 (28). We have correlated IL-8 expression and signaling with increased autophosphorylation of FAK in malignant cells of prostate cancer biopsy tissue, and immunoblotting experiments have characterized that IL-8 induces a FAK-Src-cortactin signaling pathway in prostate cancer cell lines (29). Activation of FAK and Src signaling kinases have been correlated with increased cell proliferation, cell survival, and chemoresistance, in addition to regulating cell spreading, motility, and invasion (reviewed in refs. 30, 31).

Activation of Rho-GTPases. Together with the activation of nonreceptor tyrosine kinases (e.g., Src-induced cortactin modulation of the Arp2/3 complex), small monomeric GTPases of the Rho-family are key regulators of the actin cytoskeleton. Consistent with their chemotactic function, CXCR1 and CXCR2 receptors have been shown to differentially activate two members of the Rho-family of GTPases in endothelial cells, with CXCR1 promoting a rapid induction of RhoGTPase activity, whereas CXCR2 signaling resulted in a delayed onset of RacGTPase activity (6). Consequently, IL-8 signaling promotes the polymerization of the actin cytoskeleton and latterly, effects a retraction of the cell cytoskeleton. We have also shown dynamic, time-dependent regulation of RhoGTPase, RacGTPase, and Cdc42GTPase activity after administration of IL-8 to prostate cancer cells,[1] suggesting that activation of GTPase and nonreceptor tyrosine kinase signaling pathways are important mediators of IL-8–promoted cancer cell motility and invasion.

Regulation of cellular gene and protein expression profiles. As a result of promoting numerous upstream signaling cascades, the activity of many transcription factors has been shown to be induced in response in IL-8 signaling. The capacity to activate activator protein and E2F transcriptional activity through modulation of MAPK signaling has already been discussed. In addition, IL-8 (and GROα) signaling has been shown to increase the transcriptional activity of nuclear factor-κB in melanoma (32) and prostate cancer cell lines (33), a response that may be mediated through the capacity of IL-8 signaling to activate p38 stress-activated protein kinase/MAPK, Akt-promoted IκK phosphorylation, or alternatively through increased PKCζ activity. Other studies conducted in NIH3T3 fibroblasts have indicated that activation of the CXCR2 receptor by IL-8 leads to a Janus-activated kinase 2–dependent phosphorylation of a member of the signal transducers and activators of transcription family, signal transducers and activators of transcription 3 (34). Although, transcriptional activation has yet to be shown, phosphorylation of signal transducers and activators of transcription 3 is associated with nuclear translocation and transcriptional activity of these proteins. Recently, we have shown that IL-8 signaling increases the transcriptional activity of the androgen receptor in prostate cancer cell lines, suggesting a potential role of this chemokine in modulating the transition of prostate cancer to an androgen-independent state (35). We have also shown that IL-8 signaling induces nuclear translocation and transcriptional activity of β-catenin and hypoxia-inducible factor-1 in prostate cancer cells.[2] In the context of prostate cancer, activation of signal transducers and activators of transcription 3 and β-catenin is not only significant from the perspective of their independent transcriptional activity, but their capacity to act as coactivators of the androgen receptor (36). As a consequence of activating this range of transcription factors, each independently associated with cancer progression, IL-8 signaling is likely to induce the transcription of multiple genes involved in angiogenesis, cell cycle regulation, migration and invasion, and the evasion of apoptosis (Fig. 1).

In addition to regulating the proteome through modulation of gene transcription, we have recently shown that IL-8 signaling can effect rapid changes in protein expression through regulating the function of proteins associated with translation. Specifically, we have shown that IL-8 signaling promotes multisite phosphorylation of ribosomal S6 kinase, as a consequence of activating Akt-mammalian target of rapamycin, Erk, and PKCζ signaling (9). In concert with increasing the phosphorylation and activation of the downstream substrate protein, ribosomal S6, IL-8 signaling was shown to induce mammalian target of rapamycin–dependent phosphorylation of the translation inhibitory protein, 4E-BP1, releasing its inhibitory action and thus promoting the assembly of the eiF4F-translational complex. Consequently, we have characterized a rapid increase in cyclin D1 expression resulting from IL-8 stimulation of prostate cancer cells, consistent with IL-8 signaling promoting transition of cells from the G_1 to S phase of the cell cycle.

[1] S. McFarlane and D. Waugh, unpublished observations.

[2] J. Pettigrew, P. Maxwell and D. Waugh, unpublished observations.

Translational Opportunities

The expression of IL-8 receptors on cancer cells, endothelial cells, neutrophils, and tumor-associated macrophages suggests that the secretion of IL-8 from cancer cells may have a profound effect on the tumor microenvironment (Fig. 2). As a consequence of inducing many of the signaling pathways described above, activation of IL-8 receptors on endothelial cells is known to promote an angiogenic response, inducing the proliferation, survival, and migration of vascular endothelial cells (1, 14). Furthermore, intratumoral IL-8 expression is proposed to be a key regulator of infiltrating neutrophil recruitment into the tumor microenvironment, the potential consequence of which on the promotion of metastasis has been expertly reviewed elsewhere (37). The expression of CXCR1 and CXCR2 on cancer cell lines and cancer cells of tumor biopsy tissue also suggests that cancer cells are subject to the effects of autocrine/paracrine IL-8 signaling, which has been associated with stimulating cell proliferation (15–17), migration, and invasion (22, 38, 39), and more recently, has received attention in assisting cancer cells to evade stress-induced apoptosis (40). Consistent with the preponderance of effects indicated from *in vitro* studies, the expression of IL-8 has been shown to correlate with the angiogenesis, tumorigenicity, and metastatic potential of many solid cancers in xenograft and orthotopic *in vivo* models (41–46). In addition, other studies indicate that intratumoral IL-8 levels may also enhance the colonization of metastatic lesions. For example, tumor-derived IL-8 has been shown to induce the differentiation and activation of osteoclasts, underpinning the characteristic osteolytic metastasis of breast cancer cells that have disseminated to the bone (47). Accordingly, inhibiting these pronounced effects of IL-8 signaling within the tumor microenvironment may have significant therapeutic potential in modulating disease progression.

Targeting IL-8 signaling within the cancer cell compartment may also assist in sensitizing cancer cells to conventional chemotherapy and novel treatment strategies. Exposure to numerous chemotherapy agents (e.g., 5-fluorouracil, Adriamycin, dacarbazine, paclitaxel) has been shown to induce IL-8 expression and secretion in cancer cells (48–50). More recently, we have shown that chemotherapy agents also induce transcriptional regulation of both the IL-8 and IL-8 receptor genes, thus increasing the level of autocrine/paracrine IL-8 signaling experienced by the cell (33). As a consequence of IL-8 signaling being coupled to the transcriptional regulation of Bcl-2 and IAP-family genes, and that of the native caspase-8 inhibitory protein, c-FLIP, we have shown that inhibiting drug-induced IL-8 signaling sensitizes prostate cancer cell lines to DNA damage agents such as oxaliplatin (33) and death receptor agonists such as TRAIL (51). Furthermore, we have characterized the potentiation of autocrine/paracrine IL-8 signaling in prostate cancer cells on exposure to hypoxia (40). Inhibition of hypoxia-induced IL-8 signaling was shown to diminish antiapoptotic gene expression and restore the sensitivity of these hypoxic cancer cells to etoposide. Accordingly, the induction of IL-8 signaling seems to be an adaptive response of cancer cells that is used to withstand environmental or chemical stresses. The sensitization of cancer cells to undergo apoptosis on inhibiting IL-8 signaling at the level of the IL-8 receptor or at key points in the downstream IL-8 signaling pathway suggests the therapeutic relevance of targeting IL-8 signaling to modulate the overall tumor response to conventional and novel therapies.

The development of humanized monoclonal antibodies against IL-8 (e.g., ABX-IL-8) has enabled several investigations to determine the effects of suppressing IL-8 signaling on tumor progression and development. Administration of ABX-IL-8 has been shown to attenuate the growth of bladder cancer xenograft models (45) and decrease the tumorigenic and metastatic potential of A375SM and TXM-13 melanoma xenograft models (41). Coadministration of ABX-IL-8 was also observed to potentiate the sensitivity of melanoma xenografts to dacarbazine, consistent with the role of this chemokine in facilitating chemoresistance in *in vitro* studies. More recently, a strategy using liposome-encapsulated small interfering RNA has been exploited to suppress IL-8 expression within ovarian tumor xenografts (52). As a result, tumors treated with the IL-8–targeted small interfering RNA strategy exhibited growth retardation, reduced microvessel density, and importantly, an increased response to the taxane, docetaxel. Therefore, the suppression of IL-8 signaling within the tumor microenvironment has a favorable outcome with regard to halting tumor progression and increasing sensitivity to clinically useful chemotherapy agents in several solid tumors.

Targeting IL-8 expression, either through antibodies or through small interfering RNA strategies, however, fails to account for the signaling effect of other CXC-chemokines in the tumor microenvironment. For example, we have shown that chemotherapy agents also induce increased expression of GROα. Similar to IL-8, GROα is a proangiogenic chemokine and has been shown to induce antiapoptotic protein expression in cancer cell lines.[3] Strategies targeting IL-8 will not account for the signaling effects of GROα (or other CXC-chemokines) within the tumor microenvironment. Therefore, receptor-targeted strategies that eliminate the redundant function of chemokine signaling may have greater utility than agents that solely dampen the effects of IL-8. Many small molecule inhibitors of CXCR1/2 signaling with appropriate pharmacokinetic properties to permit application in preclinical animal models are now emerging in the literature (53–58). Because these agents exhibit a range of receptor selectivity, their use will also assist in comparing the relative therapeutic benefit of targeting the CXCR2 receptor as opposed to dual targeting of the CXCR1 and CXCR2 receptor on cells within the tumor microenvironment. Furthermore, it will be important for these preclinical studies to determine the potential side effects and toxicities of these chemokine receptor antagonists given the key role of CXCR1 and CXCR2 signaling in modulating neutrophil function.

In conclusion, there is significant support for targeting IL-8 signaling (and that of its associated proangiogenic CXC-chemokines) in numerous solid tumors (e.g., gastric, pancreatic, melanoma, ovarian, bladder, and prostate). Multiple small molecule antagonists and humanized monoclonal

[3] C. Wilson and D. Waugh, unpublished observations.

antibodies are now emerging from development programs that will permit extensive preclinical investigation of how attenuating chemokine signaling may influence disease progression and modulate the response to combination chemotherapy. Furthermore, because the majority of clinical studies confirm overexpression of this chemokine in the most advanced stages of disease, this suggests that suppressing the effects of IL-8 or its associated CXC-chemokines may have important implications for the systemic treatment of aggressive and metastatic disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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