

DANCE ROUND

WE DANCE ROUND IN A RING AND SUPPOSE,
BUT THE SECRET SITS IN THE MIDDLE AND KNOWS.
ROBERT FROST

CHEMOKINE-CHEMOKINE RECEPTOR PATHWAY AS A TUMOR THERAPEUTIC TARGET: THE SIGNIFICANCE OF SDF-1-CXCR4 PAIR

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Chemokines, a large family of chemotactic cytokines, are the major regulators of immune cell trafficking. The chemokine stromal cell-derived factor-1 (SDF-1, or CXCL12) and its cognate receptor, CXCR4 (CD184), are an important ligand-receptor pair, which play a crucial role in numerous biological processes including hematopoiesis, inflammation, angiogenesis, and cell proliferation. Moreover, accumulating evidence indicates that SDF-1-CXCR4 pair plays important roles in regulating processes essential for tumor biology. Molecular strategies aimed at inhibiting the SDF-1-CXCR4 pathway, such as small peptide CXCR4-specific antagonists, anti-CXCR4 antibodies, and small interfering RNA might therefore prevent tumor progression and metastasis. In the present Dance Round, we focus on (i) the role of the SDF-1-CXCR4 signaling in the regulation of tumor spread, growth, and vascularization, and (ii) the significance of this ligand-receptor pair as a novel therapeutic target for neoplastic disease. Biomed Rev 2005; 16: 77-81.

Key words: cancer, CXCL12, metastasis, therapy

INTRODUCTION

The chemokines (from chemotactic cytokines) comprise a large family of small proteins (8-14 kD molecular weights) that play a pivotal role in the regulation of immune cell trafficking (1,2). Recently these signaling molecules were also implicated in tumor cell biology (3-5). Based on the position of the first two cysteines (C) adjacent to the aminoterminal, chemokines are classified into four groups abbreviated as CXC, CC, C, and CX3C (1-5). The biological effects of chemokines are mediated through their interaction with a family of heptahelical G-protein-coupled receptors. Based on their specific chemokine ligands, the receptor proteins are categorized as

CC chemokine receptors (CCR), CXC chemokine receptors (CXCR), C chemokine receptor (CR), and CX3C chemokine receptor (CX3CR) (1-5). Until now nearly 20 chemokine receptors and more than 50 chemokines have been identified (<http://cytokine.medic.kumamoto-u.ac.jp>).

Stromal cell-derived signals have a profound influence on the maintenance, progression and metastatic spread of hematopoietic and epithelial cancers. Mesenchymal or marrow-derived stromal cells, which constitute a large proportion of the non-neoplastic cells within the tumor microenvironment, constitutively secrete the chemokine stromal cell-derived

Received 2 October 2005 and accepted 15 December 2005.

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factor-1 (SDF-1/CXCL12). SDF-1 attracts cancer cells and vascular endothelial cells, acting through its receptor, CXCR4 (CD184), the latter also being co-receptor for HIV entry. Thus, in a paracrine way, CXCR4-mediated signals stimulate tumor growth and tumor vascularization, respectively (reviewed in 5).

Here we summarize the role of SDF-1-CXCR4 signaling pathway in tumor biology including metastasis, growth, and angiogenesis. And, emphasize the significance of SDF-1-CXCR4 pair as a novel molecular target for the treatment of neoplastic disease.

SDF-1-CXCR4 PAIR IN TUMOR METASTASIS

Functions of SDF-1 and CXCR4 in cancer first were described in metastatic breast cancer (6-9), and more recent studies have identified the roles of this signaling pathway in lung cancer, prostate cancer, colorectal cancer, pancreatic cancer, and ovarian cancer (10-15). CXCR4 and CCR7 are highly expressed in human breast cancer cells, and their respective ligands exhibit peak levels of expression in organs representing the first destination of metastasis (6). These authors' findings indicate that chemokines and their receptors play a critical role in determining the metastatic destination of tumor cells. Accordingly, Lepteva *et al* (7), using small interfering RNA (siRNA) against CXCR4, demonstrated downregulation in CXCR4 expression in human MDA-MB-231 breast cancer cells, which resulted in a significant decrease of cancer cell invasion, and decreased *in vitro* and *in vivo* cancer cell growth. Likewise, the necessity of CXCR4 in breast cancer metastasis by silencing of CXCR4 of breast cancer cells has been confirmed (8). And, an inhibitory effect of CXCR4 specific antagonists in breast cancer was recently reported (9). Further, non-small cell lung cancer (NSCLC) specimens resected from patients as well as NSCLC cell lines expressed CXCR4 but not SDF-1, while no change in proliferation or apoptosis in response to SDF-1 has been demonstrated (10). Organs which are the preferred destination of human NSCLC elaborate high levels of SDF-1, and neutralizing antibodies can abrogate organ specific metastasis in animal model, suggestive of critical role played by SDF-1-CXCR4 pair in the NSCLC metastasis (10). High CXCR4 expression in tumor specimens from colorectal cancer patients was also reported, and this associated with increased risk for local recurrence and/or distant metastasis (11). In line with this, multivariate analysis revealed that mRNA^{CXCR4} expression level was significant variable for overall survival, event-free survival, and metastasis-free survival in patients with osteosarcoma

(12). In contrast, it was reported that CXCR4 is located in the nucleus and/or in the cytoplasm of tumor cells in stage I NSCLC, and patients whose tumors had CXCR4-positive nuclear staining had a significantly longer duration of survival than patients whose tumors had no nuclear expression (13). Moreover, these authors found the 5-year metastasis rates were lower in CXCR4-positive than negative patients. As for other tumors, Mori *et al* (14) demonstrated that SDF-1-CXCR4 axis was involved in pancreatic cancer metastasis.

SDF-1-CXCR4 PAIR IN TUMOR GROWTH

There is considerable evidence that some chemokines are involved in tumor and normal cell survival and growth. Lapteva *et al* (7) reported breast cancer cells lacking CXCR4 expression proliferated at a much slower rate than control cells *in vitro*. Surprisingly, tumor cells lacking CXCR4 expression failed to grow in SCID mice in repeated experiments. They provided the first direct evidence for the essential role of CXCR4 in breast cancer growth *in vivo*. Hartmann *et al* (15) found that SDF-1 induced integrin activation, which resulted in an increased adhesion of small cell lung cancer (SCLC) cells to fibronectin and collagen. They further revealed this was mediated by $\alpha 2$, $\alpha 4$, $\alpha 5$, and $\beta 1$ integrins along with CXCR4 activation, which could be inhibited by CXCR4 antagonists. Stromal cells protected SCLC cells from chemotherapy-induced apoptosis, and CXCR4 inhibitors can antagonize this protection. Their findings suggest that activation of integrins and CXCR4 chemokine receptors co-operate in mediating adhesion and survival signals from the tumor microenvironment to SCLC cells (15). It is therefore evident that signaling mediated by the SDF-1-CXCR4 pathway can lead to increased survival and proliferation of tumor cells. As to the molecular mechanism upon SDF-1-CXCR4 in cell proliferation, studies from other groups show that SDF-1 can activate the ERK1/2 and Akt kinases that play an important role in regulating cancer cell proliferation. Barbero *et al* (16) demonstrated that exogenous SDF-1 α could induce glioblastoma cells proliferation in a dose-dependent manner. These authors confirmed that SDF-1 α -dependent proliferation is correlated with phosphorylation and activation of both ERK 1/2 and Akt and that these kinases are independently involved in glioblastoma cell proliferation. Evidence that CXCR4 and SDF-1 are expressed in ovarian cancer cell lines was also presented (17). SDF-1 α induces a dose-dependent proliferation in ovarian cancer cells by the specific interaction with CXCR4 and a biphasic activation of ERK1/2 and Akt kinases. These findings suggest that a cross talk between SDF-1-CXCR4 and EGFR intracellular pathways may link signals of cell proliferation in ovarian cancer.

SDF-1-CXCR4 AXIS IN TUMOR VASCULARIZATION

SDF-1-CXCR4 pair is required for both normal cardiovascular development and postnatal vascular remodeling. Salvucci *et al* (18) showed that SDF-1 is expressed by the vascular endothelium from selected healthy as well as tumor tissues. Primary endothelial cells constitutively express SDF-1 *in vitro*. Vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) both increase the endothelial cell SDF-1 expression. Using pertussis toxin and antibodies to inhibit SDF-1-CXCR-4 pathway resulted in disrupted extracellular matrix-dependent endothelial cell tube formation. *In vivo* study by pertussis toxin and neutralizing antibodies directed at SDF-1 inhibit growth factor-dependent neovascularization (18). Their results indicate that SDF-1-CXCR4 identifies VEGF- and bFGF-regulated autocrine signaling systems that are essential regulators of endothelial cell proliferation and angiogenesis. Likewise, ovarian tumors strongly express SDF-1, which interacts with VEGF to synergistically induce neoangiogenesis in human ovarian cancers (19). Moreover, SDF-1 secreted by carcinoma-associated fibroblasts (CAF) promoted angiogenesis by recruiting endothelial progenitor cells into breast cancer (20). These authors also found that CAF-secreted SDF-1 could stimulate tumor growth directly through CXCR4 expressed by carcinoma cells. Furthermore, SDF-1 secreted by tumor tissue can promote the migration of CD34⁺ hematopoietic cells and then promote tumor vascularization. Collectively, SDF-1-CXCR4 pair is related to metastasis, survival/growth, and vascularization of tumors (also see references 37-42 in Yoshioka's review on pages 35-41 of this volume).

SDF-1-CXCR4 PAIR AS A TUMOR THERAPEUTIC TARGET

Increasing evidence supports the hypothesis that the inhibition of CXCR4-mediated tumorigenic signals may exert therapeutic effects in various cancers. Examples include small peptide CXCR4-specific antagonists and CXCR4 antibodies (Table). Using RNA interference technology silence the CXCR4 in breast cancer resulted in impaired invasion of breast cancer cells in matrigel invasion assay and inhibited breast cancer metastasis in an animal model (8). Also, targeting SDF-1-CXCR4 pair can attenuate *in vivo* tumor growth by inhibiting angiogenesis in a VEGF-independent manner (25).

CONCLUSION

Recent studies have demonstrated that chemokine-chemokine receptor interactions, in particular those relating to SDF-1-CXCR4, can impact at any stage of tumor biology from tumor development to metastasis. Molecular strategies

Table. Selected list of CXCR4 antagonists

T134 (23)
T140 (23,24,28,30)
T22 (30)
TC14012 (23,28)
TN14003 (14,28)
Kisspeptin (29)
AMD3100 (22,23)
AMD3465 (31)
Anti-CXCR4 monoclonal antibody 12G5 (21,31)
Rabbit anti-rat CXCR4-neutralizing antibody (25)
CXCR4 mAb (Clone mAB1371) (26)
Antibody that can distinguish CXCR4 phosphorylated on serine 339 (27)

References are indicated in parentheses.

aimed at inhibiting the CXCR4-mediated tumorigenic pathways, such as small peptide CXCR4-specific antagonists, anti-CXCR4 antibodies, and siRNA might therefore have therapeutic activity in patients with various malignancies. As such, CXCR4 antagonists, although initially developed for treatment of AIDS (30,31), actually may become effective agents for the therapy of neoplastic disease.

ACKNOWLEDGMENTS

This work is supported from National Natural Science Foundation of China (NSFC 30572120).

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