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Mini-Review

Chemokine Regulation of Cell Trafficking in Lung Cancer Metastasis

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ABSTRACT

Chemokine regulation of cell trafficking has been identified as a critical pathway in metastatic progression. This review focuses on the role of the CXCR4-CXCL12-CXCR7 axis in regulating lung cancer invasion and metastasis. All of these factors have been identified as overexpressed in lung cancer specimens and in most cases are associated with poorer outcome. Preclinical studies have demonstrated an important role for CXCR4 in the steps of invasion, cell migration, and stromal cell adhesion and have suggested that CXCR4 is associated with "stem-like" cells that may be critical for initiating metastases. These data, together with emerging preclinical data on the efficacy of CXCR4 inhibitors suggests that CXCR4 should be explored as a clinical target for blocking metastatic progression in lung cancer.

INTRODUCTION

Tumor cells usurp a variety of normal signalling pathways in the body for their own malevolent growth, including those regulating cell trafficking in development, angiogenesis, inflammation, and injury. A growing body of data has demonstrated chemokine regulation of cell trafficking as an important feature of solid tumor metastasis.

Metastatic progression remains the single most important cause of death in most tumor types. Lung cancer is the most common cause of cancer death among both men and women with 5 year survival rates of less than 20% overall. This dismal outcome is primarily a result of metastatic disease progression, which is common in both non-small cell and small cell lung cancer.

Non-small cell lung cancer (NSCLC) represents about 85% of all lung cancer. Even

among early stage patients, relapse rates are significant. The relapse rate even among stage I patients is 35-54% [1], and nearly all patients who relapse go on to die of their lung cancer. The rates of metastatic progression are even higher in small cell lung cancer (SCLC), which still comprises 12-15% of all lung cancer. Among limited-stage patients with localized disease, the relapse rate is at least 80% and among extensive-stage patients, the relapse rate is 95-98% [2]. Among patients who relapse, median survival is frequently less than six months [3]. As the primary reason for shortened survival is widespread progression of metastatic disease, understanding the factors that promote this process and identifying targets for intervention could favorably impact survival rates.

Chemokines, or chemotactic cytokines, are differentially expressed in a tissue-specific manner to direct the "homing" of cells during development, inflammation, or injury. Leukocyte trafficking, for example, is a process that involves interaction with the endothelium during circulation, activation of integrins by chemokine receptor interaction with particular chemokines, and trans-endothelial migration of the cells out of the circulation into tissues that retain these cells via chemokine gradients. This process is strikingly similar to the process of tumor cell extravasion into the bloodstream from a primary tumor, migration of cancer cells to distant metastatic sites, invasion into tissues, and the outgrowth of macrometastases.

There are approximately 50 identified chemokines and close to 20 known chemokine receptors [4]. Chemokines are grouped into four families based on the positions of 4 conserved cysteine residues (C, CC, CXC, and CX3C where X denotes a non-conserved amino acid) and chemokine receptors are named correspondingly based on their preferred ligands [5]. These receptors are primarily seven-transmembrane-domain proteins that belong to the G-protein-coupled receptor family.

Chemokines are expressed in a tissue-specific manner and, concomitant with the most frequent areas of metastasis in lung cancer, CXCL12 is most highly expressed in bone marrow, liver, lung, and brain [6]. The axis of CXCL12 and its known receptors CXCR4 and CXCR7 is thought to play a primary role in metastatic progression of both NSCLC and SCLC.

EXPRESSION PATTERNS AND OUTCOME

CXCR4 is a chemokine receptor first identified as a coreceptor for HIV entry into dendritic cells [7, 8]. Expression has been seen in multiple tumor types, including both subtypes of lung cancer. Where it has been examined, CXCR4 expression is ubiquitous in SCLC cell lines and tumors [9, 10]. In NSCLC, expression appears more variable as are associations with outcome. Both nuclear and cytoplasmic expressions of CXCR4 have been observed in tumor tissue, but not in normal bronchial epithelium. Some studies suggest a negative association of CXCR4 expression and outcome [11-13] while others suggest a positive association with outcome [14, 15]. One retrospective study of stage I NSCLC (61 patient samples) suggested a lower rate of metastases and a significantly longer duration of survival with strong nuclear staining [15]. Another study of 154 patient samples suggested that while nuclear staining was associated with improved disease-free survival in adenocarcinoma, cytomembranous staining was associated with distant metastasis and poorer disease-free survival [12], suggesting that cell surface expression of CXCR4 drives interactions with CXCL12 leading to increased metastatic progression, while receptor internalization and nuclear staining leads to improved outcomes by virtue of less chemokine interaction. Notably, this study showed a significantly higher level of CXCR4 staining in adenocarcinoma compared with squamous cell carcinoma (43% vs. 15% overall). Finally, evaluation of a population of circulating cells positive for pan-cytokeratin and CXCR4 in a small group of NSCLC patients demonstrated significantly improved survival among those with lower levels of double-positive cells [16].

Previous studies of NSCLC cells grown in severe-combined immunodeficiency (SCID) mice have demonstrated increased expression of CXCR4 in metastases as compared with the primary tumor site [17]. Elevated CXCR4 expression has also been associated with higher microvascular density and CXCR7 expression. CXCR7, a more recently identified receptor for CXC12 [18], is also frequently expressed in NSCLC tumor samples and has been associated with poor prognosis [19]. CXCR7 is also expressed on tumor-associated endothelium but not on normal vasculature [20].

CXCL12 (also known as SDF-1), the sole ligand for CXCR4 and one of two ligands for CXCR7, is also frequently seen at high levels in both NSCLC and SCLC cell lines [21]. CXCL12 expression in tumors has been associated with increased nodal metastases [12] and higher disease stage [22]. Methylation of CXCL12 is associated with expression and aberrant methylation of CXCL12 was associated with poor prognosis in NSCLC tumor samples [23].

Notably, in NSCLC tumor samples, CXCL12 was also significantly higher in females, adenocarcinoma patients, and non-smokers [21]. Other studies have suggested a particular link between high CXCR4 expression in females and outcome [24]. EGFR mutations were found to be significantly more frequent in patients with high CXCR7 expression [19] and EGF activation of EGFR also was shown to increase CXCR4 expression in NSCLC cell lines [25]. Together, these findings suggest that the CXCR4-CXCL12-CXCR7 axis may be particularly important to further explore in patients with activating EGFR mutations (more commonly found in females, non-smokers, and in adenocarcinoma).

CXCL12 expression has not only been observed intra-tumorally, but also in carcinoma-associated fibroblasts (CAFs) [22]. Interestingly, in a study of primary NSCLC, high-CXCL12 CAFs were found in association with CXCL12-negative tumor cells; CXCL12-positive tumor cells had no CXCL12 in the surrounding stroma. Other studies that have demonstrated significant CXCR4 expression in primary NSCLC tumors have also failed to demonstrate CXCL12 expression within the tumor [16, 26] suggesting that they may have mutually exclusive roles within tumors. Although multiple studies have demonstrated that tumor expression of CXCR4 allows homing to distant sites of future metastases where CXCL12 is expressed, the role of intratumoral or tumoral-associated CXCL12 is less clear.

FUNCTIONAL INTERACTIONS OF CXCR4, CXCL12, AND CXCR7

CXCL12 interaction with CXCR4 in SCLC cell lines has been demonstrated to induce actin polymerization, enhance migration and stromal cell adhesion, and increase $\alpha4\beta1$integrin expression on tumor cells [9] consistent with promotion of invasion and migration of tumor cells as part of metastatic progression. Stromal cell adhesion is thought to be a factor in chemotherapy resistance in SCLC and in vitro, this resistance can be abrogated by CXCR4 inhibition [27, 28]. Some studies have also shown increased increased Akt and P70 S6 kinase phosphorylation and increased tumor cell proliferation resulting from CXCL12 interaction with CXCR4 [29], consistent with direct effects on tumor growth.

Hypoxia has been demonstrated to upregulate CXCR4 expression [25, 30] with HIF genes and the PI3K/AKT/mTOR signaling pathway mediating this upregulation. A seminal study in 2003 demonstrated that CXCR4 is downregulated by the pVHL by targeting HIF for degradation under normoxic conditions; hypoxia allows for HIF-mediated activation of CXCR4 [31]. HIF genes were also shown to be essential in regulating CXCL12-mediated adhesion, migration, and invasion of NSCLC lines [32]. Global gene expression profiling of a mouse model of NSCLC generated by conditional activation of HIF2α and Kras mutation also demonstrated an increase in CXCL12 expression in response to HIF2α activation [33]. Matrix metalloprotease 9 (MMP-9), which serves as an effector of tumor cell invasion and is upregulated by HIF1a in other settings, is upregulated in lung NSCLC cell lines by CXCL12 [34].

Hypoxic environments within the bone marrow are also thought to be responsible for the upregulation of CXCL12 expression there and the creation of a CXLC12 gradient. This gradient normally serves to signal homing of CXCR4 expressing hematopoietic progenitor cells [35] but may also signal CXCR4-expressing tumor cells to "home" to bone marrow. Using heterotopic or orthotopic models of human lung cancer made by injecting SCID mice with the A549 human NSCLC cell line, Phillips and colleagues demonstrated high levels of CXCL12 expression in adrenal glands, lung, liver, and bone marrow [17]. Depletion of CXCL12 with a neutralizing antibody inhibited metastasis formation in this model.

The role of CXCR7 in this homing process is less clear. CXCR7, CXCR7, which binds to CXCL11 and CXCL12, does not appear to activate G-protein mediated signaling. However, it does forms heterodimers with CXCR4 and the complex recruits β-arrestin to activate alternate signaling pathways,

including ERK 1/2, MAPK, and SAPK [36]. In other contexts (using lymphoma cell lines), CXCR7 was shown to interact with β-arrestin 2 to mediate transendothelial migration of CXCR4+ CXCR7+ cells [37]. β-arrestin 2 was also independently shown to be an important factor for CXCR4-CXCL12 mediated chemotaxis [38]. CXCR7 and CXCL12 both colocalize with high frequency to tumor cells and tumor-associated endothelium in brain metastases from patients with NSCLC (with concomitant expression of CXCR4 seen in the nucleus of tumor cells) suggesting an important role for CXCR7 in the exravasation of tumor cells homed by CXCL12 gradients to a new site for metastatic outgrowth of disease.

CXCR4 EXPRESSION AND "STEM-LIKE" CELLS IN LUNG CANCER

Cancer stem cells are defined variably but frequently as progenitor cells of tumors which, like traditional stem cells, are capable of continuous self-renewal and propagation. Cancer stem cells are thought to be relatively insensitive to the effects of chemotherapy and radiation and may be able repopulate or propagate tumors following treatment. They may also be the cells most important for metastatic progression as some groups have demonstrated that only selected cells within tumors have tumor-propagating potential [39, 40].

Bertolini and colleagues used CD133, a known marker of hematopoietic progenitor cells, to identify epithelial cells within primary lung cancer specimens that may be "stem-like" cells [40]. Using xenograft models, they demonstrated that isolated CD133+ cells had higher tumorigenic potential in serial transplantation assays in SCID mice. Double staining with CD133 and CXCR4 identified a subpopulation that increased post-cisplatin resistance and demonstrated higher invasive and mobility features. Reckamp and colleagues demonstrated that only a subset of pan-keratin expressing circulating cells in NSCLC also expressed CXCR4, but these were the cells that were associated with survival outcome suggesting that these are the cells associated with metastatic progression [16]. Phillips and colleagues used an orthotopic model to show that 99% of NSCLC cells identified in metastatic sites were CXCR4+, whereas only 35% of tumors in the primary tumor were CXCR4+ [17]. Together these studies suggest

that CXCR4 is found on a subpopulation of "stem-like" cells in NSCLC and these cells are associated with metastatic progression and poorer survival.

While the existence and role of cancer stem cells in lung cancer (and other solid tumors) is not clearly defined, it is an especially important idea for SCLC. SCLC is clinically defined by high response rates to chemotherapy and radiotherapy which "debulk" the tumor, but also by high rates of chemotherapy resistance and metastatic progression. Given the functional involvement of CXCR4 and CXCL12 in mediating stromal cell interactions, invasivenesss, and mobility of SCLC cell lines as well as their potential role in establishing distant metastases, blockade of the CXCR4-CXLC12 axis may be especially important in this subset of lung cancer.

INHIBITION OF THE CXCR4-CXCL12-CXCR7 AXIS AS A THERAPEUTIC TARGET IN LUNG CANCER

Since its identification as a co-receptor for HIV entry, several inhibitors of CXCR4 have been developed and tested. To date, in cancer, these inhibitors have been primarily studied in stem cell mobilization for autologous transplantation in hematologic malignancies, where the effect of CXCR4 inhibition results in release of CXCR4-expressing hematopoietic progenitors from the bone marrow into the circulation.

Plerixafor (Mozobil, AMD3100) (Genzyme, Cambridge, MA) was approved by the FDA in December 2008 for this indication. Perixafor is a bicyclam derivative first investigated as an anti-HIV drug [41] which acts as a partial agonist to CXCR4. Unexpected leukocytosis was noted in initial trials and later studies demonstrated that the drug reduced AKT phosphorylation by CXCR4 resulting in a disruption of the interaction of multiple myeloma cells with bone marrow stromal cells (as reflecting by an increase in circulating cells) and enhancement of their sensitivity to anti-myeloma therapy [42]. Randomized, placebo-controlled trials in non-Hodgkin's lymphoma and multiple myeloma showed decreased time to adequate cell count for stem cell collection prior to autologous transplantation [43, 44].

Studies of this drug or other CXCR4 antagonists in solid tumors are more limited but

preclinical studies of AMD3100 have shown reduction in dissemination of tumor or metastatic spread in mouse models of ovarian cancer, melanoma, and oral squamous cell cancer among others, and in vitro inhibition of migration or invasion has been demonstrated in several contexts as well [45-47]. However, there are no ongoing clinical trials of AMD3100 in solid tumors. BMS-936564 (MDX-1338) is a novel CXCR4 antibody also under clinical evaluation in multiple myeloma and acute myeloid leukemia. Other CXCR4 inhibitors that are not yet in clinical evaluation in any tumor type include the peptide antagonists T22 and TN10043 [48]. Nox-A12 is a CXCL12 antagonist also under clinical evaluation for stem cell mobilization.

CTCE-9908 (British Canadian Biosciences Corp) is a peptide CXCR4 antagonist which has undergone early clinical development for use in solid tumors. This compound is derived from the N-terminal sequence of CXCL12 and demonstrates anti-metastatic as well as direct anti-tumor effects in many in vitro and in vivo models, including in orthotopic xenografts of hepatocellular carcinoma, prostate cancer, and breast cancer [48-52]. This compound has recently completed a phase I trial in solid tumors with no dose-limiting toxicities observed [53] and some disease stabilization seen with reduction in tumor burden in an ovarian cancer patient.

Notably, even in pre-clinical studies, the effects of both AMD3100 and CTCE-9908 as single agents were small compared to combinations with chemotherapy [45, 54]. The short-lived duration of stable disease in the CTCE-9908 study also suggests that single agent activity in the metastatic setting may be limited. Inhibition of CXCR4-CXCL12 interaction alone may not be enough given the potential contribution of CXCR7 to CXCL12-mediated tumor metastasis [55]. The bulk of preclinical data suggest that the real utility of inhibiting the CXCR4-CXCL12-CXCR7 axis may also be much earlier in the process of metastatic progression—i.e. before it occurs [56]. This makes trial design in lung cancer more challenging given the propensity for early metastatic progression of both SCLC and NSCLC. With the right trial design, however, the potential impact of blocking or delaying metastatic progression is enormous. As single agent safety data has already been gathered from healthy volunteer studies and stem cell mobilization studies, some of the compounds already in clinical development in hematologic malignancies are ripe for study in lung cancer using chemotherapy combinations in the neoadjuvant or adjuvant setting in NSCLC and in combination studies in 1st-line treatment of SCLC.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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