

Chemokine expression in tumor-to-tumor metastasis

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Abstract. Chemokines play an important role in cancer metastasis by modulating the directional cell movement and migration of tumor cells. The most commonly overexpressed chemokine receptor in human cancer is CXCR4. Once activated by its ligand CXCL12 (stromal cell-derived factor-1 ligand/SDF1), CXCR4 stimulates several key migratory, proliferative and survival signaling cellular pathways. CXCR4 is expressed in small-cell lung carcinoma (SCLC) cells and other tumors. To further characterize the role of chemokines in tumor-to-tumor metastasis, we analyzed the tissue expression of CXCR4 and CXCL12 in the surgical specimen of a patient with this phenomenon. We performed immunohistochemical analysis for the expression of CXCR4 and CXCL12 in metastatic tumor tissue of a 69-year-old Caucasian male with extensive SCLC metastatic to a renal oncocytoma. The oncocytoma tissue harboring SCLC showed CXCL12 expression, but not CXCR4. A high expression of the two molecules was found in a normal renal parenchymal control. Our results suggest that CXCR4 and CXCL12 plays a role in this condition, but their expression may be affected by the microenvironment of the harboring malignancy. Further characterization of these phenomena is needed to shed light on the biological mechanisms of tumor metastasis.

Introduction

Chemokines were originally discovered and studied from the perspective of inflammation. However, their role in modulating directional cell movement and the migration of cancer cells has been demonstrated and is considered critical (1,2).

The human chemokine system includes almost 50 chemokines and 14 receptors (2). The most commonly overexpressed chemokine in human cancer is CXCR4 (stromal cell-derived factor-1 receptor; fusin) and its ligand CXCL12 (stromal cell-derived factor-1 ligand; SDF1 α). CXCR4 activation by

CXCL12 stimulates several key migratory, proliferative and survival signaling cellular pathways (3).

We hypothesized that CXCR4 and CXCL12, avidly expressed by small-cell lung carcinoma (SCLC) cells and documented to play important roles in the pathophysiology of metastasis, play a prominent role in the dissemination of tumoral cells and their subsequent invasion to a second malignancy.

Case report

A 69-year-old Caucasian male with a history of 60 packs/year tobacco consumption and coronary artery disease was admitted to the hospital with a history of malaise, a 2-month 20-pound weight loss and a left kidney mass. The serum sodium level was 133 meq/l and hemoglobin, 15.5 g/dl. Laparoscopic partial nephrectomy was performed. A CT scan of the thorax was performed on the 5th post-operative day. The scan showed a mass-like right lobe opacification, bilateral pleural effusions and extensive mediastinal and right hilar adenopathy. Three days after discharge the patient was readmitted with acute dyspnea and atrial flutter. Chest X-rays confirmed a large right pleural effusion. The serum sodium level of 128 meq/l was due to a syndrome of inappropriate anti-diuretic hormone secretion. A right thoracentesis was performed, and approximately 2 l of fluid was removed. A bronchoscopy with transbronchial biopsy showed tumor invasion within the right main stem bronchus and nests of small-cell carcinoma with immunohistochemical stains positive for pankeratin, synaptophysin, CD56 and TTF1.

The patient was discharged from the hospital on demeclocycline for syndrome of inappropriate anti-diuretic hormone secretion, and cytotoxic chemotherapy was commenced with a combination of carboplatin and etoposide.

Materials and methods

Morphologic and immunohistochemical analysis. A left partial nephrectomy specimen was obtained measuring 4.5x3.7x3.2 cm. Sectioning of the tissue revealed a single tan, focally hemorrhagic, well-circumscribed tumor, 2x2x2 cm, confined within the renal parenchyma. A histological examination showed that this tumor mass was composed of a primary renal oncocytoma containing multiple small islands of small-cell carcinoma.

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These foci of metastatic small-cell carcinoma were confined to the oncocytoma. No metastatic small-cell carcinoma was identified in the renal parenchyma surrounding the oncocytoma.

The oncocytoma itself exhibited classic histological features. The tumor was composed of homogeneous cells with abundant acidophilic granular cytoplasm and central to eccentrically located round nuclei with even chromatin (Fig. 1A). These cells formed solid compact nests (alveolar pattern) and areas of variably sized tubules set in the background of a loose edematous or hyalinized stroma. Mitotic activity was not appreciable. Immunohistochemical stains showed positive staining for EMA and negative staining for vimentin and colloidal iron.

The foci of the small-cell carcinoma present within the oncocytoma also exhibited classic features. The small foci were composed of solid sheets of small cells with extremely scant cytoplasm and nuclei with finely granular chromatin, absent or inconspicuous nucleoli and prominent nuclear moulding. Mitotic activity was rapid (Fig. 1B). Immunohistochemical stains confirmed the diagnosis with the cells showing positive staining for AE1/AE3, chromogranin, synaptophysin, CD56 and TTF-1.

Diagnosis was consistent with tumor-to-tumor metastasis with a primary SCLC as the site of origin and a renal oncocytoma as the harboring malignancy.

Chemokine analysis

Processing of the surgical specimens. Formalin-fixed and paraffin-embedded slides of normal renal parenchyma and tumor tissues for each antibody were hydrated. Antigens were retrieved in citrate buffer for 20 min and cooled at room temperature.

The slides were blocked for peroxidase activity in 3% hydrogen peroxide (H₂O₂) for 5 min and rinsed in Tris buffer. The slides were also blocked for possible non-specific background staining with casein in PBS for 15 min.

Staining for CXCL12 (SDF1 α , mAb clone 7801B, IG1 class; R&D Systems, Minneapolis, MN) and CXCR4 (fusin, goat polyclonal AB, directed to the amino terminus of SDF1 α sc-6729; Santa Cruz Biotechnology, Santa Cruz, CA) was carried out at room temperature for 30 min using a 1:50 dilution. Slides were rinsed in Tris buffer.

For CXCL12, the secondary antibody, an anti-mouse IgG-HRP labeled polymer was applied for 30 min. For CXCR4, an anti-goat IgG-HRP labeled polymer was applied for 10 min. Slides were rinsed in Tris buffer twice. The chromogen, DAB, was applied for 5 min at room temperature and slides were rinsed in di H₂O and counterstained in hematoxylin.

Results

CXCL12 antibody staining of the renal specimen showed an avid expression on the endothelial cell lining of vascular channels, glomeruli and tubules within the histological sections of the patient's oncocytoma and kidney parenchyma (Fig. 1C-F). CXCL12 also stained the SCLC cells within the oncocytoma (Fig. 1C-F). We then explored the CXCL12 and CXCR4 expression in a normal kidney control and confirmed their expression as well (data not shown). CXCR4 staining was

not noted in the oncocytoma specimen nor were the small-cell carcinoma cells nested within the tumor.

Discussion

Several hypotheses have been proposed to explain the biology of tumor-to-tumor metastasis. In 1889, Paget in his 'seed and soil theory' mentioned that gross tumor development is a consequence of the provision of a fertile environment (the soil), in which compatible tumor cells (the seeds) proliferate. A mechanical theory was proposed by Ewing in 1928, describing site specificity as a direct consequence of the anatomical location of a primary tumor (4). High lipid and glycogen content in the kidney were also proposed as a potential mechanism to attract metastatic cells (4,5). However, few malignancies metastasize to the kidneys.

More recent evidence links chemokines with the pathogenesis of metastasis in more than 23 human cancer cells including SCLC (6).

In vivo data showed that certain chemokines serve as tissue-specific attractant molecules for tumor cells, promoting tumor cell migration to a particular site through direct action of the chemokine ligands on chemokine receptors via the activation of heterotrimeric G proteins. The G protein subunits then stimulate multiple signal transduction pathways, involving the phosphatidylinositide 3 kinase (PIK-3)/Akt pathway and various Src family kinases (6).

SCLC cells use the CXCR4 receptor to migrate to the bone marrow tumor microenvironment, which is rich in CXCL12. This migration is due to the activation of integrins after CXCR4/CXCL12 involvement, allowing the cells to interact with extracellular matrix components (7).

The CXCR4/CXCL12 axis has been found to be up-regulated in renal cell carcinoma and other malignancies such as chronic lymphocytic leukemia, breast carcinoma, multiple myeloma, melanoma and ovarian carcinoma and may constitute a novel therapeutic target (1,7).

The development of tumor metastasis from a second primary malignancy is uncommon and remains biologically puzzling. Its low incidence has made its full biological characterization evasive.

Initially described by Campbell, the event must meet the following criteria to be considered tumor-to-tumor metastasis: i) the presence of more than one primary tumor; ii) the recipient tumor has to be a true neoplasm; iii) there must be evidence of true metastasis from the second neoplasm; iv) the second malignancy must grow or invade the tissues of the hosting tumor; v) the metastatic growth must not be due to contiguous growth or embolism of tumor cells, and vi) tumors that metastasize to the lymphatic system where a lymphatic malignancy already exists are not considered tumor-to-tumor metastasis (4,8-10).

Renal cell carcinoma and meningioma are the most common malignant and benign recipients, respectively, whereas the lung is the most common metastatic donor in both settings (10).

Though inconclusive, our results suggest that the high tissue expression of CXCL12 observed in the tumor tissues is also present in normal kidney parenchyma (control sample). Contrary to our hypothesis, CXCR4 expression was not noted

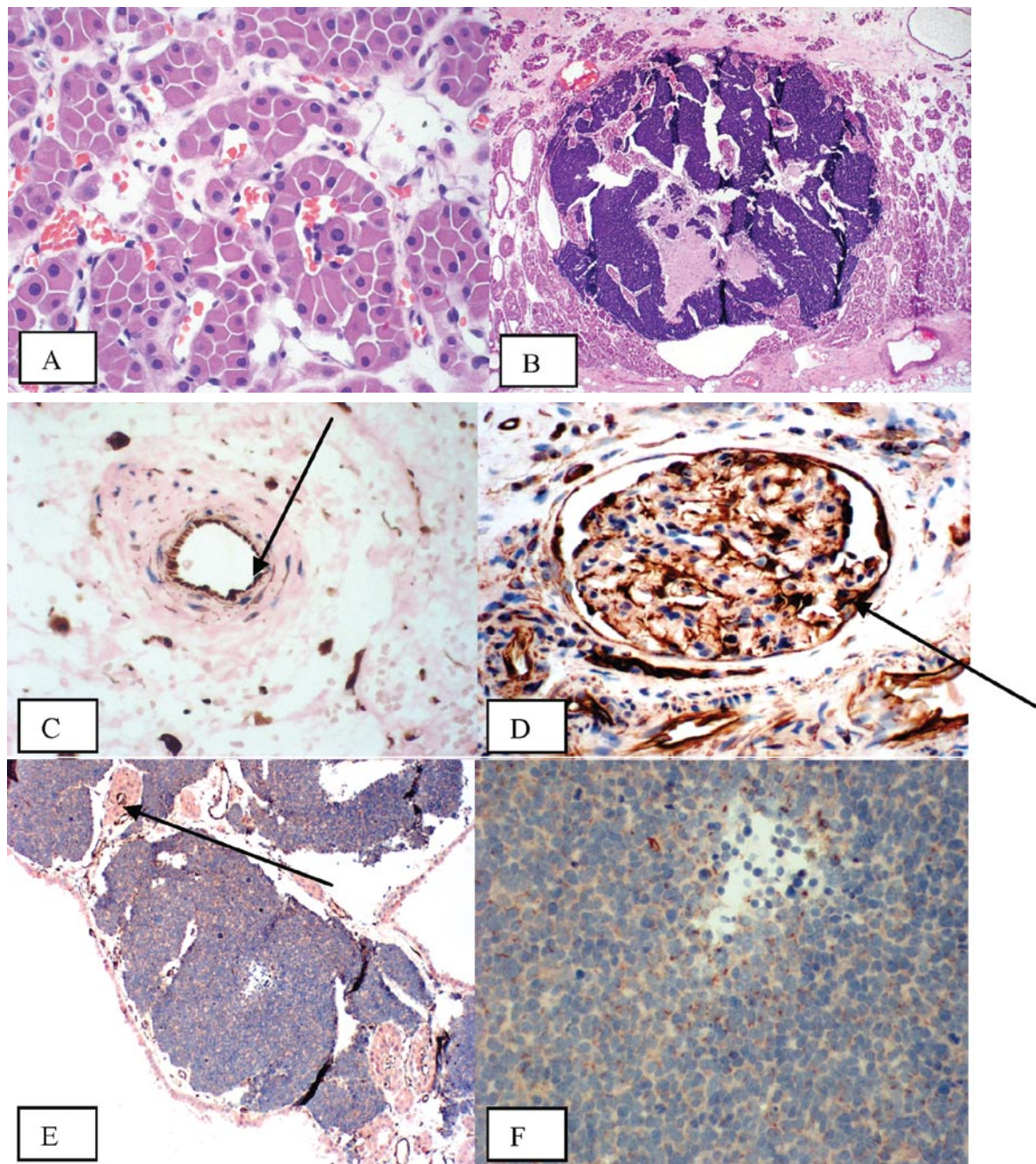


Figure 1. (A) Higher power field H&E staining of the oncocytoma showing homogeneous cells with abundant acidophilic granular cytoplasm and central to eccentrically located round nuclei. (B) Lower power field of small-cell carcinoma nests present within the oncocytoma. (C) CXCL12 staining in the oncocytoma showing vascular channels and (D) glomeruli. CXCL12 staining in small-cell carcinoma cells within the oncocytoma; (E) lower power field and (F) higher power field.

in the SCLC metastasis in our case specimen. However, the lack of expression is perhaps related to the interaction of the oncocytoma's stromal microenvironment and the metastatic nests of SCLC cells. In this particular event the host for SCLC was indeed a renal neoplasm. Unlike oncocytomas, renal cell carcinoma cells induce CXCR4 transcription via hypoxia inducible factor (HIF1). Moreover, the up-regulation of prolyl and asparaginyl hydroxylase have been identified in oncocytomas (11). These enzymes decrease the production of HIF1- α via proteasome degradation by down-regulating

the transcription of CXCR4 (11,12). This partially explains the absence of CXCR4 receptors in our specimen and likely plays a role in the low metastatic potential that oncocytomas exhibit.

The molecular events between CXCR4 and its ligand CXCL12 have yet to be elucidated in tumor-to-tumor metastasis. In this regard, the question remains as to whether the mechanism of the propagation of cells is related to the chemokine axis, the oncocytoma's microtumoral environment or, more likely, a combination of both. This preliminary

observation warrants further investigation with functional molecular studies to characterize the environment surrounding chemokines in tumor-to-tumor metastasis.

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