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Research Article

CXCR4 and CXCL12 are inversely expressed in colorectal cancer cells and modulate cancer cell migration, invasion and MMP-9 activation

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Abstract

Colorectal cancer (CRC) is characterized by a distinct metastatic pattern resembling chemokine-induced leukocyte trafficking. This prompted us to investigate expression, signal transduction and specific functions of the chemokine receptor CXCR4 in CRC cells and metastases. Using RT-PCR analysis and Western blotting, we demonstrated CXCR4 and CXCL12 expression in CRC and CRC metastases. Cell differentiation increases CXCL12 mRNA levels. Moreover, CXCR4 and its ligand are inversely expressed in CRC cell lines with high CXCR4 and low or not detectable CXCL12 expression. CXCL12 activates ERK-1/2, SAPK/JNK kinases, Akt and matrix metalloproteinase-9. These CXCL12-induced signals mediate reorganization of the actin cytoskeleton resulting in increased cancer cell migration and invasion. Moreover, CXCL12 increases vascular endothelial growth factor (VEGF) expression and cell proliferation but has no effect on CRC apoptosis. Therefore, the CXCL12/CXCR4 system is an important mediator of invasion and metastasis of CXCR4 expressing CRC cells.

Introduction

Colorectal carcinoma (CRC) is the second leading cause of death due to cancer in the United States, accounting for more than 50,000 deaths annually [1]. The use of all currently available therapies has only modest impact on overall survival of patients with advanced-stage, metastatic disease. Although a number of molecules have been implicated in the metastasis of cancer cells, the precise mechanisms determining the directional migration and invasion of CRC cells into specific organs remain to be established. New evidence indicates that chemokines play a major role in this process of organ-selective metastasis [2].

Chemokine receptors are coupled to heterotrimeric G proteins and induce cell movement towards a concentration gradient of the cognate chemokine ligand [3]. Human stromal-cell derived factor-1 (SDF-1), also known as CXCL12 according to a new classification system [4] is thought to be the primordial chemokine. It binds and signals solely through the chemokine receptor CXCR4. Interestingly, it is the only chemokine that is essential for survival [5] as shown in mice genetically deficient in the chemokine receptor CXCR4 or its ligand CXCL12 which die perinatally with major defects in the vascular development (particularly in the gastrointestinal tract), hematopoi-

Abbreviations: CRC, colorectal cancer; ECM, extracellular matrix; EMSA, electrophoretic mobility shift assay; ERK, extracellular signal-regulated kinase; FCS, fetal calf serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; mAb, monoclonal antibody; MAP-kinase, mitogen-activated protein-kinase; MEK, mitogen-activated protein kinase kinase; MMP, matrix metalloproteinase; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium; PI, phosphatidylinositol; RT-PCR, reverse transcriptase polymerase chain reaction; SAPK/JNK, stress-activated protein kinase/c-Jun-N-terminal kinase.

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esis and cardiogenesis [5,6]. Moreover, CXCR4 is essential in stem cell localization [6], serves as a chemoattractant for T cells and thymocytes in vitro and in vivo [7,8] and functions as a co-receptor for HIV-1 [9].

A recent study demonstrated that CXCR4 signaling plays a crucial role in metastasis of breast cancer by inducing chemotactic and invasive responses [2]. Metastasis was found predominantly in organs with abundant CXCL12 expression. Neutralization of CXCL12/CXCR4 interactions leads to a marked inhibition of lymph node and lung metastases [2]. The involvement of CXCR4 in metastasis is not limited to breast cancer, as CXCR4 is expressed in several other tumor cell lines [10–14] that also respond to CXCL12. Therefore, chemokine receptors expressed on tumor cells represent potential targets for therapeutic interventions.

The expression of CXCR4 in intestinal epithelial cells has been shown previously [15,16] and preliminary experiments in animal models indicate a role for CXCR4 in CRC metastasis particularly in triggering the outgrowth of micrometastases [17]. However, the mechanisms of CXCR4 signaling and its functional role in the migration and metastasis of human CRC cells are unsolved questions in the analysis of colorectal cancerogenesis. Here, we demonstrate that CXCR4 and CXCL12 are expressed in human CRC cells and sites of metastasis. Signal transduction experiments indicate that CXCR4 is functional in human CRC cells mediating specific functions such as CRC migration, invasion and proliferation.

Materials and methods

Reagents

Polyclonal antibodies to extracellular signal-regulated kinase (ERK)-1/2 (phosphorylated at Thr183/Tyr185 and total), stress-activated protein kinase (c-Jun N-terminal kinase) SAPK/JNK (phosphorylated at Thr183/Tyr185 and total), p38 (phosphorylated at Thr180/Tyr182 and total) and Akt (phosphorylated at Ser473 and total) were purchased from Cell Signaling (Beverly, MA). Horseradish peroxidase linked anti-rabbit secondary antibody was purchased from Amersham (Arlington Heights, IL). Recombinant human CXCL12 and monoclonal antibodies to human CXCR4 and CXCL12 were from R&D Systems (Minneapolis, MN). MEK-1 inhibitor PD98059, SAPK/JNK inhibitor SP600125, p38 inhibitor SB203580 and phosphatidylinositol3- (PI3) kinase inhibitor wortmannin were from Tocris Cookson (Bristol, U.K.).

Cell culture

The human CRC cell lines T84, SW480, Caco-2, HT-29, HCT116, the lymph node metastasis derived cell line SW620 and the transformed rat intestinal cell line IEC-6, were obtained from American Type Culture Collection (Rockville, MD). While T84 cells were grown in Dulbecco's modified Eagle medium/F-12 (GIBCO BRL/Life Technologies, Gaithersburg, MD), the other cell lines were grown in Dulbecco's modified Eagle medium (GIBCO) with 100 IU/mL penicillin, 100 μg/mL streptomycin and 10% heat-inactivated fetal calf serum (PAA, Pasching, Austria) in a humidified 5% CO_2 atmosphere at 37°C. For signal transduction experiments with CXCL12, cells were starved overnight in serum-free medium.

Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated using Trizol reagent (GIBCO BRL/Life Technologies, Gaithersburg, MD) and treated with ribonuclease (RNase)-free deoxyribonuclease (DNA-free™-Kit, Ambion) to remove potential genomic DNA contaminants. Three micrograms of total RNA was reverse transcribed using Roche first strand cDNA synthesis kit. The following conditions were used for semiquantitative PCRs: 25 or 35 cycles (depending on the specific PCR) of denaturing at 95°C for 45 s, annealing at 58°C for 45 s, extension at 72°C for 45 s using previously published primers for CXCR4 [16], VEGF [18] and GAPDH [19]. The primers used for CXCL12 were forward 5′-AGAGCCAACGTCAAGCATCT-3′ and reverse 5′-CGTCTTTGCCCTTTCATCTC-3′. All PCR reactions included GAPDH primers to quantify PCR products. To control for genomic contamination, an identical parallel PCR reaction was performed containing starting material that had not been reverse transcribed.

Gel electrophoresis and immunoblotting

Cellular proteins, cytosolic and membrane fractions were extracted as described previously [20,21]. The protein concentration of each sample was quantified by the Bradford method. Immunoblotting was performed as previously described [22].

Enzyme-linked immunosorbent assay (ELISA)

For the quantification of IL-8 release, BD OptEIA Human IL-8 ELISA Kit II (BD Biosciences, Bedford, MA) and for quantification of VEGF and CXCL12, Quantikine ELISA Kits from R&D Systems (Minneapolis, MN) were used according to the manufacturer's instructions.

Evaluation of MMP-2 and MMP-9 activity by gelatin zymography

HT-29 and T84 cells were cultured in DMEM containing 0.1% FCS in the presence or absence of human CXCL12 for 24 and 48 h, respectively. Conditioned medium was