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SDF-1/CXCR4 promotes epithelial–mesenchymal transition and progression of colorectal cancer by activation of the Wnt/β-catenin signaling pathway

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ABSTRACT

Stromal cell-derived factor 1 (SDF-1) and its receptor, CXCR4, play an important role in angiogenesis and are associated with tumor progression. This study aimed to investigate the role of SDF-1/CXCR4-mediated epithelial–mesenchymal transition (EMT) and the progression of colorectal cancer (CRC) as well as the underlying mechanisms. The data showed that expression of CXCR4 and β-catenin mRNA and protein was significantly higher in CRC tissues than in distant normal tissues. CXCR4 expression was associated with β-catenin expression in CRC tissues, whereas high CXCR4 expression was strongly associated with low E-cadherin, high N-cadherin, and high vimentin expression, suggesting a cross talk between the SDF-1/CXCR4 axis and Wnt/β-catenin signaling pathway in CRC. *In vitro*, SDF-1 induced CXCR4-positive colorectal cancer cell invasion and EMT by activation of the Wnt/β-catenin signaling pathway. In contrast, SDF-1/CXCR4 axis activation-induced colorectal cancer invasion and EMT was effectively inhibited by the Wnt signaling pathway inhibitor Dickkopf-1. In conclusion, CXCR4-promoted CRC progression and EMT were regulated by the Wnt/β-catenin signaling pathway. Thus, targeting of the SDF-1/CXCR4 axis could have clinical applications in suppressing CRC progression.

Introduction

Colorectal cancer (CRC) is a significant health problem [1]. Although the 5-year survival rate reaches 90% in patients with local CRC, it decreases to 12% in patients with a distant metastasized disease [1]. To date, there has been no significant breakthrough in control of CRC, once lymph node metastasis occurs [2,3]. Therefore, it is of great importance to identify the factors involved in CRC progression, possibly leading to novel targets for antitumor strategies.

According to the "homing" theory, chemokines have the ability to chemoattract tumor cells to target tissues [4], and chemokines and their receptors may promote cancer metastasis [5]. CXCR4, a seven-transmembrane G protein-coupled receptor (GPCR), is the receptor for stromal cell-derived factor (SDF-1) and plays an important role in the human body, including angiogenesis and cancer metastasis [6,7] [8–13]. In CRC, CXCR4 is essential for directional tumor metastasis [14–16]. Nevertheless, the exact mechanism underlying the downstream modulation of SDF-1/CXCR4 function on CRC progression is poorly understood.

Wingless related proteins (Wnt) are important regulators of cell proliferation, differentiation, and adhesion [17–20]. Constitutive activation of the Wnt/β-catenin signaling pathway is common in colon cancer, especially in sporadic cases [21]. Aberrant secretion of Wnt factors or an adenomatous polyposis coli (APC) mutation can cause abnormal β-catenin activation, leading to activation of Wnt signaling and epithelial–mesenchymal transition (EMT) [22,23]. EMT is an essential developmental process through which cells of epithelial origin lose cell–cell contacts and cell polarity, and acquire a mesenchymal phenotype. EMT increases motility, invasiveness and metastasis of tumor cells [24–26].

SDF-1/CXCR4 promotes tumor progression by activating the canonical Wnt signaling pathway [27,28]. Thus, CXCR4 may possess an important function in CRC progression by activating the Wnt/β-catenin/LEF1 pathway. Since CXCR4 is the most common chemokine receptor expressed in CRC and aberrant activation of the Wnt signaling pathway is common in colon cancer, this study aimed

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to examine the role of Wnt signaling pathway in CXCR4-promoted EMT and CRC progression.

Materials and methods

Patients and tissue samples

This study was approved by the Ethics Committee of Clinical Research of The First Affiliated Hospital, Medical College, Xi'an Jiaotong University (Shannxi, China). All patients provided written informed consent.

Tissue specimens were obtained from 122 patients who underwent surgical resection of CRC lesions at The First Affiliated Hospital, College of Medicine of Xi'an Jiaotong University between January 2007 and January 2008. None of the patients had received prior radiotherapy or chemotherapy. All patients were diagnosed histologically (Table 1). Paraffin tissue blocks were retrospectively retrieved, which contained both cancerous and distant non-cancerous tissues. Fresh tissues were obtained during surgery, immediately snap-frozen in liquid nitrogen, and stored at –80 °C until use.

Immunohistochemistry

Immunohistochemistry was performed according to a previous study [29]. Antibodies included a rabbit polyclonal anti-CXCR4 antibody at a dilution of 1:50, anti-β-catenin antibody at a dilution of 1:200, anti-E-cadherin antibody at a dilution of 1:100, anti-N-cadherin antibody at a dilution of 1:100, and anti-vimentin antibody at a dilution of 1:100 (Beijing Biosynthesis Biotechnology, China). These antibodies were specific for immunohistochemistry. The negative control sections were incubated with phosphate buffered saline (PBS) to replace the primary antibody.

Three pathologists reviewed the immunostained sections under a light microscope and scored them in 10 randomly selected ×20 power fields. The staining intensity was graded as: 0, no staining; 1, weak; 2, moderate; and 3, strong. The percentage of positive cells was scored as: 1, < 25%; 2, 26–50%; 3, 51–75%; and 4, > 76%. These two scores were added together, and each tissue sample was categorized into four groups: 0, ≤ 5% cells were stained; 1–3, weak expression; 4–5, moderate expression; and 6–7, strong expression. Finally, the number of cells with low-to-weak expression and the number of cells with moderate-to-strong expression were compared statistically.

Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR)

qRT-PCR was performed according to a previous study [29]. The primers were designed and synthesized by Takara (Dalian, China) (Supplementary Table S1), and GAPDH was used as the internal control. Each measurement was performed in triplicate and repeated twice. Expression levels of *CXCR4, CTNNB1, LEF1, MYC, CCND1, MMP-7, CD44*, E-cadherin, N-cadherin, vimentin, and *GAPDH* mRNA were evaluated using a relative quantification approach ($2^{-\Delta\Delta Ct}$ method) against GAPDH levels.

Cell lines, culture and treatment

CRC cell lines (Colo205, SW480, SW620, CaCO2, RKO, LoVo, and HT29) were obtained from American Type Culture Collection (ATCC; Manassas, VA, USA) and cultured in specific culture medium according to ATCC. Cells were cultured in the absence of serum overnight prior to treatment with SDF-1 (100 ng/ml; PeproTech Inc. Rocky Hill, CT, USA) for the indicated period of time. Dickkopf-related protein-1 (DKK-1; 200 ng/ml, PeproTech, Inc.) was added in the cell culture medium to suppress the activation of the canonical Wnt signaling pathway.

Plasmid construction

pGPU6/GFP/Neo-shCXCR4 carrying CXCR4 shRNA and negative control pGPU6/GFP/Neo-shNC were a kind gift from Dr. Zheng Wang (Xi'an Jiaotong University, Shannxi, China) as reported previously [25]. We also constructed a plasmid pIRES2/EGFP/Neo-CXCR4 by cloning the CXCR4 transcript using PCR of a human lymph node cDNA library. The primers used were 5′-TATCTCGAGGCCACC ATGTCCATTCCTTTGCCTCTTTG-3′ and 5′-CGCGTCGACTTAGCTGGAGTGAAAA CTTGAAGACTC-3′. Plasmid sequences were confirmed by DNA sequencing before use.

Generation of stable CXCR4-knockdown or expressing CRC cell lines

CRC cell lines (Lovo and RKO) were seeded into 6-well culture plates and transfected with pGPU6/GFP/Neo-shCXCR4, pIRES2/EGFP/Neo-CXCR4, or control vectors using TurboFect *in vitro* Transfection Reagent (Fermentas, Lithuania) according to the manufacturer's instructions. Forty-eight hours later, G418 (Invitrogen) was added to the cell culture at a final concentration of 800 μg/ml (LoVo) or 600 μg/ml (RKO). The medium was changed once every three days. After 3 weeks, G418-resistant colonies were isolated and cultured in 96-well culture plates for further experiments.

Table 1
Association between CXCR4 and β-catenin expressions and clinicopathological features from CRC patients.

		CXCR4			β-catenin		
	n (%)	Positive	Negative	*P*	Positive	Negative	*P*
Age (yrs.)							
< 60	56 (45.9)	35	21	0.402	36	20	0.450
≥ 60	66 (54.1)	46	20		38	28	
Gender							
Male	60 (49.2)	41	19	0.655	37	23	0.822
Female	62 (50.8)	40	22		37	25	
Tumor location							
Colon	81 (66.4)	58	23	0.087	51	30	0.463
Rectum	41 (33.6)	23	18		23	18	
CEA							
< 3.4 ng/ml	47 (38.5)	29	18	0.385	24	23	0.086
≥ 3.4 ng/ml	75 (61.5)	52	23		50	25	
Tumor size							
< 5 cm	45 (36.9)	24	21	0.020*	20	25	0.005**
≥ 5 cm	77 (63.1)	57	20		54	23	
Histological grade							
Well/moderate	50 (41.0)	28	22	0.043*	23	27	0.006**
Poor	72 (59.0)	53	19		51	21	
UICC							
I + II	49 (40.2)	26	23	0.011*	22	27	0.004**
III + IV	73 (59.8)	55	18		52	21	
Lymph node metastasis							
Positive	80 (65.6)	64	16	<0.001**	60	20	<0.001**
Negative	42 (34.4)	17	25		14	28	
Distant metastasis							
Positive	31 (25.4)	28	3	0.001**	26	5	0.002**
Negative	91 (74.6)	53	38		48	43	

* $P < 0.05$.
** $P < 0.01$.