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SDF−1/CXCR4 expression in bladder cancer tissue and the correlation with negative costimulatory molecule PD−L1, cell apoptosis and invasion

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ABSTRACT

Objective: To study the SDF-1/CXCR4 expression in bladder cancer tissue and the correlation with negative costimulatory molecule PD-L1, cell apoptosis and invasion. **Methods:** A total of 118 cases of bladder cancer tissue and para-carcinoma tissue surgically removed in our hospital between May 2014 and May 2016 were selected as the research samples, the RNA was extracted and then reverse-transcribed into cDNA, and the expression levels of SDF-1/CXCR4, PD-L1/PD-1, cell apoptosis-related molecules and cell invasion-related molecules were detected. **Results:** SDF-1 and CXCR4 mRNA expression in bladder cancer tissue were significantly higher than those in para-carcinoma tissue; PD-L1, PD-1, Rec1, Survivin, MRPS5, Nanog, BCAPP2Ac, TRPM8, TRPV2, ILK, β-catenin and GUGBP1 mRNA expression in bladder cancer tissue were significantly higher than those in para-carcinoma tissue and positively correlated with SDF-1 and CXCR4 mRNA expression. **Conclusion:** Highly expressed SDF-1/CXCR4 in bladder cancer tissue are closely related to the high expression of negative costimulatory molecule PD-L1, pro-proliferation molecules and pro-invasion molecules, and SDF-1/CXCR4 can promote the immune escape, proliferation and invasion of bladder cancer cells.

1. Introduction

Bladder cancer is one of the most common malignant tumors in urinary system, it accounts for more than 90% of the malignant urinary system tumors and the incidence is rising in recent years[1]. Transurethral resection and bladder perfusion chemotherapy are the major means of clinical treatment of bladder cancer, but because of the stronger cancer cell proliferation and invasion ability, the occurrence of long-term recurrence and intracavity implantation and metastasis of bladder cancer are extremely high[2,3]. The biological axis formed by stromal cell derived factor 1 (SDF-1) and its receptor chemokine receptor 4 (CXCR4) is important to adjust cell growth and invasion. In vitro study has shown that targeted knockdown of CXCR4 expression in bladder cancer cells can inhibit cell proliferation and invasion process[4]. However, the SDF-1/CXCR4 biological axis expression in bladder cancer tissues and the function change are still not clear at present. In the following study, the SDF-1/CXCR4 expression in bladder cancer tissue and the correlation with negative costimulatory molecule PD-L1, cell apoptosis and invasion were analyzed.

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2.Materials and methods

2.1Experimental samples and materials

A total of 118 cases of bladder cancer tissue and para-carcinoma tissue surgically removed in our hospital between May 2014 and May 2016 were selected as the research samples, the bladder cancer tissue and para-carcinoma tissue were washed with normal saline, put in the cryopreserved tubes, shortly frozen in liquid nitrogen for 30 min, then taken out and preserved in a -80 ℃ cryogenic refrigerator for a long time. RNA extraction, the cDNA first strand synthesis and fluorescence quantitative PCR kits were purchased from Beijing ComWin Biotech Company.

2.2Experimental methods

Bladder cancer tissue and para-carcinoma tissue were collected, proper amount of tissue was weighed, added in total RNA extracting solution from the RNA extraction kit, fully homogenized and then centrifuged at 4 ℃ for 5 min, the tissue residue was removed, the supernatant was kept, the RNA extraction kit instructions were followed to separate RNA. The cDNA first strand synthesis kits were used to reverse-transcribe the RNA into cDNA. The cDNA samples were collected, the fluorescence quantitative PCR kit instructions were followed to configure the reaction system 3, PCR apparatus was used to amplify stromal cell-derived factor 1 (SDF-1), chemokine receptor-4 (CXCR4), programmed cell death-1 (PD-1), programmed cell death-ligand 1 (PD-L1), Rec1, Survivin, mitochondrial ribosomal protein S5 (MRPS5), Nanog, transient receptor potential melastatin 8 (TRPM8), transient receptor potential cation channel subfamily V member 2 (TRPV2), integrin linked kinase (ILK), CUG-binding protein 1 (CUGBP1) and reference gene β-actin respectively, the primer sequences and annealing temperature were shown in Table 1, and the mRNA expression was calculated according to the amplification curve.

2.3Statistical methods

Statistical software SPSS 20.0 was used to input and analyze data, measurement data analysis between two groups was by t test, correlation analysis between two measurement data was by Pearson test and $P<0.05$ indicated statistical significance in differences.

3.Results

3.1SDF-1, CXCR4, PD-L1 and PD-1 expression in bladder cancer tissue

Analysis of the SDF-1 and CXCR4 expression in bladder cancer tissue and para-carcinoma tissue was shown in Table 2: SDF-1 and CXCR4 mRNA expression in bladder cancer tissue were significantly higher than those in para-carcinoma tissue; analysis of PD-L1 and PD-1 expression in bladder cancer tissue and para-carcinoma tissue was shown in Table 2: PD-L1 and PD-1 mRNA expression in bladder cancer tissue were significantly higher than those in para-carcinoma tissue. Differences in SDF-1, CXCR4, PD-L1 and PD-1 mRNA expression were statistically significant in bladder cancer tissue and para-carcinoma tissue ($P<0.05$).

3.2Cell apoptosis-related molecule expression in bladder cancer tissue

Analysis of Rec1, Survivin, MRPS5, Nanog and BCAPP2Ac expression in bladder cancer tissue and para-carcinoma tissue was as follows: Rec1, Survivin, MRPS5, Nanog and BCAPP2Ac mRNA expression in bladder cancer tissue were significantly higher than those in para-carcinoma tissue. Differences in Rec1, Survivin, MRPS5, Nanog and BCAPP2Ac mRNA expression were statistically significant in bladder cancer tissue and para-carcinoma tissue ($P<0.05$).

Table 1.

PCR primer sequences and annealing temperature.

Genes	Forward primer (5′ 3′)	Reverse primer (5′ 3′)	Annealing temperature (℃)
SDF-1	AGCATCTGCAGTCAGTCA	TAGCTAGGTACGTTAGCA	60.0
CXCR4	TCGATCGACTAGGCGTAGCC	CGATGCTAGGCAGTCATG	58.0
PD-L1	TAGCTAGCTAGTCGATAT	GCTAGTAGCTAGCTAGCT	58.0
PD-1	GCTAGCTGGCTAGCTAGCTA	GCTAGCTAGCTGATCGAGG	56.0
Rec1	AGCTGATCGACGTACGTAAG	CGTAGCATGCTAGATTACG	62.0
Survivin	CTAGTCGCTGATGTCATCT	TTCCAAGTCGATTTGTCATC	60.0
MRPS5	AGGTACAGCAGGCGAATG	CGTAGCTAGTGCTAGCAA	58.0
Nanog	TGTCAACGTACGGGATTGC	ATGCTAGCAAGTCATTTCAG	58.0
TRPM8	TAGCATGAAACGTCATAA	TCGATTTAGCGTACGACGT	60.0
TRPV2	CGTTAGTCGATTGCTACGA	TCTAAGTCGCGTAGCTAGCG	58.0
ILK	GATGTCTAGTCATGAGGA	TGCATGCATGCATGCATG	58.0
GUGBP1	CATGAATGCATGGGATAA	ACTAGGCGTAGCCGGACC	56.0

Table 2.

SDF-1, CXCR4, PD-L1 and PD-1 expression in bladder cancer tissue.

Tissue origin	*n*	SDF-1	CXCR4	PD-L1	PD-1
Bladder cancer	118	2.37±0.35	2.14±0.28	1.89±0.23	2.03±0.27
Para-carcinoma tissue	118	1.06±0.14	1.09±0.17	0.97±0.12	1.04±0.12
T		13.589	10.372	9.383	9.227
P		<0.05	<0.05	<0.05	<0.05

3.3Cell invasion-related molecule expression in bladder cancer tissue

Analysis of TRPM8, TRPV2, ILK, β-catenin and GUGBP1 expression in bladder cancer tissue and para-carcinoma tissue was as follows: TRPM8, TRPV2, ILK, β-catenin and GUGBP1 mRNA expression in bladder cancer tissue were significantly higher than those in para-carcinoma tissue. Differences in TRPM8, TRPV2, ILK, β-catenin and GUGBP11 mRNA expression were statistically significant in bladder cancer tissue and para-carcinoma tissue (P<0.05).

3.4Correlation of SDF-1/CXCR4 with immune, proliferation and invasion molecules

Pearson correlation analysis showed that SDF-1 and CXCR4 mRNA expression in bladder cancer tissue were positively correlated with PD-L1, PD-1, Rec1, Survivin, MRPS5, Nanog, BCAPP2Ac, TRPM8, TRPV2, ILK, β-catenin and GUGBP1 mRNA expression.

4. Discussion

Cancer cell proliferation and invasion are the main biological behaviors that cause bladder cancer recurrence and intracavity implantation and metastasis, and the regulation mechanism of bladder cancer cell proliferation and invasion is unclear at present. SDF-1/CXCR4 biological axis can regulate cell growth, and the combination of the two in the form of ligand-receptor can regulate a variety of biological behaviors of cells[5,6]. CXCR4 is a typical G-protein-coupled receptor, and the combination of SDF-1 and CXCR4 can induce intracellular actin polymerization and cellular pseudopod formation, enhance cell movement ability and promote the cells to break through the limit of basement membrane and invasively grow[7,8]. In recent years, the relationship of SDF-1/CXCR4 biological axis with the occurrence and development of malignant tumors has received more and more attention[9,10], *in vitro* study of domestic YANG De-lin[4] has shown that targeted knockdown of CXCR4 expression in bladder cancer cells can inhibit cell proliferation and invasion. It indicates that the SDF-1/CXCR4 biological axis is involved in regulating the biological behaviors of bladder cancer cells. In order to further clarify the role of SDF-1/CXCR4 biological axis in the occurrence and development of bladder cancer, the SDF-1/CXCR4 expression in bladder cancer tissue was analyzed in the study at first, and the results showed that SDF-1 and CXCR4 mRNA expression in bladder cancer tissue were significantly higher than those in para-carcinoma tissue. This means that the high expression of SDF-1/CXCR4 is closely related to the occurrence and development of bladder cancer. The immune escape of cancer cells is the important pathologic basis that causes the occurrence and development of bladder cancer, and the immune cell apoptosis mediated by PD-L1 and PD-1 in local lesions will inhibit anti-tumor immune response and cause immune escape of cancer cells[11]. In the study, analysis of the correlation between SDF-1/CXCR4 biological axis and the immune escape of bladder cancer cells showed that PD-L1 and PD-1 mRNA expression in bladder cancer tissue were significantly higher than those in para-carcinoma tissue and positively correlated with SDF-1 and CXCR4 mRNA expression. This means that the highly expressed SDF-1 and CXCR4 in bladder cancer tissue could increase the PD-L1 and PD-1 expression and cause the immune escape of cancer cells.

Cancer cell proliferation is an important biological behavior that causes bladder cancer recurrence after surgery and chemotherapy, and the bladder cancer cell proliferation process is regulated by Rec1, Survivin, MRPS5, Nanog and other proliferation and apoptosis-related molecules. Rec1 is the intracellular proteolytic enzyme across the endoplasmic reticulum, and it can participate in -CaaX protein modification and promote cell proliferation; Survivin is the molecule with significant anti-apoptotic effect, and it can inhibit apoptosis process[12]; MRPS5 is a member of the mitochondrial ribosomal protein family, and it can regulate mitochondrial energy metabolism to promote cell proliferation[13]; Nanog is the marker gene of cancer stem cells, and its encoded protein can maintain the stem cell characteristics and continuous proliferation state of the cancer cells[14]. The related in vitro research results have confirmed that the SDF-1/CXCR4 biological axis can regulate and promote bladder cancer cell proliferation. In order to

Table 3.

Cell apoptosis-related molecule expression in bladder cancer tissue.

Tissue origin	n	Rec1	Survivin	MRPS5	Nanog
Bladder cancer	118	2.41±0.32	1.74±0.23	2.25±0.28	1.97±0.23
Para-carcinoma tissue	118	1.07±0.14	0.97±0.12	1.03±0.16	0.92±0.11
T		15.472	8.498	11.741	12.582
P		<0.05	<0.05	<0.05	<0.05

Table 4.

Cell invasion-related molecule expression in bladder cancer tissue.

Tissue origin	n	TRPM8	TRPV2	ILK	β-catenin	GUGBP1
Bladder cancer	118	1.87±0.23	2.25±0.28	1.93±0.22	2.18±0.27	2.35±0.34
Para-carcinoma tissue	118	0.95±0.11	1.07±0.13	1.02±0.11	1.06±0.13	1.08±0.15
T		11.264	11.883	9.190	11.039	12.783
P		<0.05	<0.05	<0.05	<0.05	<0.05

validate the regulating effect of SDF-1/CXCR4 on cell proliferation in histological level, the apoptosis-related molecules Rec1, Survivin, MRPS5 and Nanog expression in bladder cancer tissue and their correlation with SDF-1/CXCR4 were analyzed in the study, and the results showed that Rec1, Survivin, MRPS5, Nanog and BCAPP2Ac mRNA expression in bladder cancer tissue were significantly higher than those in para-carcinoma tissue and positively correlated with SDF-1 and CXCR4 mRNA expression. It indicates that the highly expressed SDF-1 and CXCR4 in bladder cancer tissue are closely related to the high expression of a variety of pro-proliferation genes, and the SDF-1 and CXCR4 can increase the pro-proliferation gene expression to promote the bladder cancer cell proliferation.

Invasive cancer cell growth is an important biological behavior that causes the intracavity implantation and metastasis of bladder cancer, and the bladder cancer cell proliferation is regulated by TRPM8, TRPV2, ILK, β-catenin, GUGBP1 and other invasion-related molecules. Both TRPM8 and TRVP2 are the important cation channels that can regulate the calcium influx to activate the calcium-dependent signaling pathways and thus promote cell invasion[15,16]; ILK is a kind of intracellular signaling protein that can interact with integrin β1 and connect actin cytoskeleton, and then activate downstream β-catenin and other signaling molecules to promote cell invasion[17]; CUGBP1 is a member of the RNA connexin CELF family, and it regulates epithelial-mesenchymal transition and promote cell invasion through C/EBP pathway[18]. In order to validate the regulating effect of SDF-1/CXCR4 on bladder cancer cell invasion in histological level, the apoptosis-related molecules TRPM8, TRPV2, ILK, β-catenin and GUGBP1 expression in bladder cancer tissue and their correlation with SDF-1/CXCR4 were analyzed in the study, and the results showed that TRPM8, TRPV2, ILK, β-catenin and GUGBP1 mRNA expression in bladder cancer tissue were significantly higher than those in para-carcinoma tissue and positively correlated with SDF-1 and CXCR4 mRNA expression. It indicates that the highly expressed SDF-1 and CXCR4 in bladder cancer tissue are closely related to the high expression of a variety of pro-invasion genes, and the SDF-1 and CXCR4 can increase the pro-invasion gene expression to promote bladder cancer cell invasion.

Based on the above discussion and analysis, it is concluded as follows: the SDF-1/CXCR4 are highly expressed in bladder cancer tissue and closely related to the highly expressed negative costimulatory molecule PD-L1, pro-proliferation molecules and pro-invasion molecules, and SDF-1/CXCR4 can promote the immune escape, proliferation and invasion of bladder cancer cells.

References

[1] Longo TA, Gopalakrishna A, Tsivian M, Van Noord M, Rasch CR, Inman BA, et al. A systematic review of regional hyperthermia therapy in bladder cancer. *Int J Hyperthermia* 2016; **32**(4): 381-389.

[2] Salehiniya H, Ghobadi Dashdebi S, Rafiemanesh H, Mohammadian-Hafshejani A, Enayatrad M. Time trend analysis of cancer ↑ incidence in caspian sea, 2004-2009: a population-based cancer registries study (northern Iran). *Caspian J Intern Med* 2016; **7**(1): 25-30.

[3] Wang Yi-qin, Yao Jun, Wang Zhao-fan, Ye Ming, Yang Chao, Zhang Ai-jun. Assessment of malignant degree of tumor and degree of trauma after HOLBT and TURBT treatment of superficial bladder cancer. *J Hainan Med Univ* 2016; **22**(13): 1406-1409, 1413.

[4] Yang De-lin, Huo Qian, Wang Yi-shui, Yang Xu-sheng, Wang Kai, WANG Jian-song, et al. Effect of chemokine stromal cell-derived factor-1 and its receptor CXCR4 on invasion capacity and intralu-minal implantation of human bladder cancer cells. *J Med Postgraduates* 2014; **27**(10): 1028-1032.

[5] Pozzobon T, Goldoni G, Viola A, Molon B. CXCR4 signaling in health and disease. *Immunol Lett* 2016; **177**: 6-15.

[6] Broussas M, Boute N, Akla B, Berger S, Beau-Larvor C, Champion T, et al. A new Anti-CXCR4 antibody that blocks the CXCR4/SDF-1 axis and mobilizes effector cells. *Mol Cancer Ther* 2016; **15**(8): 1890-1899.

[7] Huang WS, Hsieh MC, Huang CY, Kuo YH, Tung SY, Shen CH, et al. The association of CXC receptor 4 mediated signaling pathway with oxaliplatin-resistant human colorectal cancer cells. *PLoS One* 2016; **11**(9): e0159927.

[8] Liu MH, Bian BS, Cui X, Liu LT, Liu H, Huang B, et al. Mesenchymal stem cells regulate mechanical properties of human degenerated nucleus pulposus cells through SDF-1/CXCR4/AKT axis. *Biochim Biophys Acta* 2016; **1863**(8): 1961-1968.

[9] Wang Y, Xie Y, Oupick D. Potential of CXCR4/CXCL12 Chemokine Axis in Cancer Drug Delivery. *Curr Pharmacol Rep* 2016; **2**(1): 1-10.

[10] Wang Z, Sun J, Feng Y, Tian X, Wang B, Zhou Y. Oncogenic roles and drug target of CXCR4/CXCL12 axis in lung cancer and cancer stem cell. *Tumour Biol* 2016; **37**(7): 8515-8528.

[11] Liu Zhi-hua, Ye Yun-lin, Bian Jun, Lai De-hui, Zheng Fu-Fu, Dai Yu-ping. Effect of Negative Costimulatory Molecule PD-L1 on Intravesical Instillation Therapy in Non-muscle Invasive Bladder Cancer after Surgery. *J Sun Yat-sen Univ (Med Sci)* 2015; **36**(2): 221-224.

[12] Senol S, Yildirim A, Ceyran B, Uruc F, Zemheri E, Ozkanli S, et al. Prognostic significance of survivin, β-catenin and p53 expression in urothelial carcinoma. *Bosn J Basic Med Sci* 2015; **15**(4): 7-14.

[13] Liu Qiliang, Liu Limei, Liu Chungang, Qian Cheng, Huang Qingyuan. Effect of MRPS5 on proliferation and stemness of bladder cancer cells. *J Third Mil Med Univ* 2015; **37**(6): 558-562.

[14] Gawlik-Rzemieniewska N, Galilejczyk A, Krawczyk M, Bednarek I. Silencing expression of the NANOG gene and changes in migration and metastasis of urinary bladder cancer cells. *Arch Med Sci* 2016; **12**(4): 889-897.

[15] Zeng Ge-wa, Zhou Xing, Jiang Lei-ming, Xiao Ning, Wang Dong-hui, XIE Wen-qi, et al. Effect of TRPM8 overexpression on bladder cancer T24 cell proliferation and invasion. *Guangdong Med J* 2015; **36**(3): 356-358.

[16] Stone L. Bladder cancer: Two molecular subtypes identified. *Nat Rev Urol* 2016; **13**(10): 566.

[17] Zhuang X, Lv M, Zhong Z, Zhang L, Jiang R, Chen J. Interplay between intergrin-linked kinase and ribonuclease inhibitor affects growth and metastasis of bladder cancer through signaling ILK pathways. *J Exp Clin Cancer Res* 2016; **35**(1): 130.

[18] Duan Kun, Li Yan-yan, Zhao Yan, Xia Wei, Wu Fan, Ge Yong-chao. CUG-binding protein 1 expression in bladder cancer and its effect on BIU-87 cell line proliferation and invasive. *J Pract Med* 2016; **32**(3): 411-414.