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RESEARCH ARTICLE

Relationship between Expression of EMT Transcription Factor of *ZEB1* and *CXCR4* Chemokine Receptor

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ABSTRACT

Metastasis is one the most leading cause of death from cancer and the chemokine receptor of CXCR4 has a critical role in cancer metastasis. Moreover, metastasis is always correlated with epithelial-mesenchymal transition (EMT). In this study, the correlation between expression of EMT-TF of ZEB1 and CXCR4 has been examined. The results revealed that in ZEB1 knocked out cells, the expression of CXCR4 decreased significantly. This indicated that ZEB1 might be one of the regulators of CXCR4 expression.

Key words: CXCR4, Cancer, Metastasis, Epithelial Mesenchymal Transition

Introduction

Metastasis is the most common cause of death following cancer [1] and identification of mechanisms controlling this process is critical for cancer therapy. It has been declared that chemokine receptors have important roles in the process of cancer metastasis [2, 3]. CXCR4 is one of chemokine receptors that its critical role in cell migration and cancer metastasis has been revealed [4-6]. Moreover, it has been determined that metastasis is intimately associated with the program described EMT. During the passage through EMT, epithelial cancer cells lose their epithelial characteristics rather acquiring the behaviors of mesenchymal cells including migratory characteristics [7]. Considering the intimate correlation of EMT with tumor metastasis and also the important role of CXCR4 in cancer metastasis, we studied the correlation between expression of CXCR4 and EMT-TF of ZEB1 (Zinc finger E-box binding homeobox 1). Hopefully, the results will help

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us to find out the role of ZEB1 in controlling the expression of CXCR4 and also the mechanisms involved in metastasis.

Materials and methods

Cell culture

The SUM159 cell line was cultured in DMEM, supplemented with 10% FBS and 0.01% penicillin/streptomycine.

ZEB1 knockout in MDA-MB-231 cell line

The EMT transcription factor of ZEB1 was knocked out by using CRISPR/Cas9 technique in SUM159 cell line. This knockout cell line was provided by Yun Zhang from Weinberg Lab.

RNA preparation and qRT-PCR analysis

RNA preparation and cDNA synthesis were performed using RNeasy Mini Kit (QIAGEN) and high-capacity cDNA Reverse Transcription kit (Applied Biosystems), respectively, both based on the manufacturer's protocol. The qRT-PCR reactions were performed using SYBR Green Mix I (Roche Diagnostics) on the LightCycler 480 System (Roche Diagnostics). The thermal cycling parameters were as follows: 95°C for 5 min, 35 cycles of 95°C for 10 sec, 60°C for 7 sec, and 72°C for 25 sec. Designed primers are described in Table 1.

Table 1: Description of the designed primers.

Gene	Primer sequences
CXCR4	**F: 5'-CTCCAAGCTGTCACACTCCA-3'**
	R: 5'-TCGATGCTGATCCCAATGTA-3'
GUSB	**F: 5'-CTCATTTGGAATTTTGCCGAT-3'**
	R: 5'-CCGAGTGAAGATCCCCTTTTTA-3'

Statistical analysis

Data are given as mean ± SEM. Student's t test (unpaired) was used to compare two groups ($p < 0.05$ being considered significant).

Results

The interrelationship between expression of ZEB1 and CXCR4

QRT-PCR analysis revealed that in SUM159, expression of chemokine receptor of CXCR4 decreased significantly after ZEB1 knockout (Figure 1).

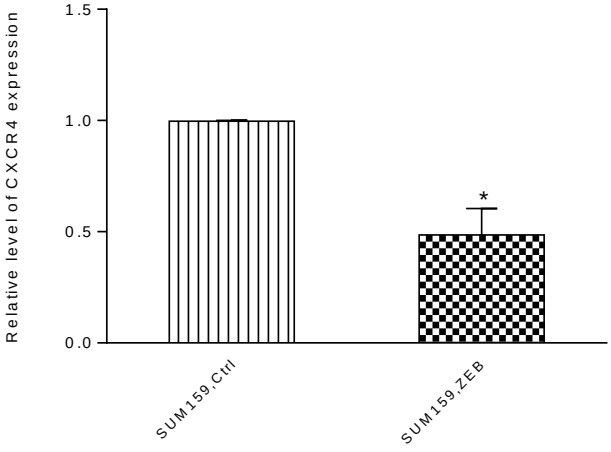

Figure 1: CXCR4 expression in SUM159 after ZEB1 knockout. Expression of CXCR4 decreased after ZEB1 knockout. *P<0.05 was considered significant as compared to control.

Discussion

CXCR4 is one of members of the superfamily of G protein-coupled receptors, and is involved in the process of cell migration and tumor metastasis [4, 5]. Moreover, it was confirmed that metastasis has an intimate correlation with EMT [7]. Different transcription factors like Snail, Slug, Twist and ZEB1 have been identified to control EMT. Among them, ZEB1 has been identified as critical one in the process of EMT. Additionally it was revealed that ZEB1 induces EMT and maintains the mesenchymal characteristics [8]. ZEB1 knockdown induced MDA-MB-231 to express epithelial markers [9]. ZEB1 knockdown also reduced the invasive ability of colorectal cancer cells [10]. Considering the intimate correlation of metastasis and CXCR4 expression and

also association of metastasis and EMT, we studied the relationship between expression of CXCR4 and ZEB1. The result of our study revealed that knockout of ZEB1 decreased expression of CXCR4. It can be assumed that ZEB1 might be a transcription factor for CXCR4. Increased metastasis following EMT, might be the effect of ZEB1 as a transcription factor on expression of CXCR4. Bioinformatic analysis has been predicted ZEB1 as a transcription factor for CXCR4. This should be confirmed by chip assay.

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References:

[1] Mehlen P, Puisieux A. Metastasis: a question of life or death. *Nat Rev Cancer* 2006 Jun;6(6):449-58. PMID: 16723991. DOI: 10.1038/nrc1886.

[2] Naderi-Meshkin H, Bahrami AR, Bidkhori HR, Mirahmadi M, Ahmadiankia N. Strategies to improve homing of mesenchymal stem cells for greater efficacy in stem cell therapy. *Cell Biol Int.* 2015 Jan;39(1):23-34. PMID: 25231104. DOI: 10.1002/cbin.10378.

[3] Mishan MA, Heirani-Tabasi A, Mokhberian N, Hassanzade M, Kalalian Moghaddam H, Bahrami AR, Ahmadiankia N. Analysis of Chemokine Receptor Gene Expression in Esophageal Cancer Cells Compared with Breast Cancer with Insights into Metastasis. *Iranian J Public Health.* 2015 Oct;44(10):1353-8. PMID: 26576348.

[4] Naderi-Meshkin H, Matin MM, Heirani-Tabasi A, Mirahmadi M, Irfan-Maqsood M, Edalatmanesh MA, Shahriyari M, Ahmadiankia N, Moussavi NS, Bidkhori HR, Bahrami AR. Injectable hydrogel delivery plus preconditioning of mesenchymal stem cells: exploitation of SDF-1/CXCR4 axis towards enhancing the efficacy of stem cells' homing. *Cell Biol Int.* 2015 Mar 31. PMID: 25825165. DOI: 10.1002/cbin.10474.

[5] Mishan MA, Ahmadiankia N, Matin MM, Heirani-Tabasi A, Shahriyari M, Bidkhori HR, Naderi-Meshkin H, Bahrami AR. Role of Berberine on molecular markers involved in migration of esophageal cancer cells. *Cell Mol Biol.* (Noisy-le-Grand, France). 2015;61(8):37-43. PMID: 26667771.

[6] Ahmadian Kia N, Bahrami AR, Ebrahimi M, Matin MM, Neshati Z, Almohaddesin MR, Aghdami N, Bidkhori HR. Comparative analysis of chemokine receptor's expression in mesenchymal stem cells derived from human bone marrow and adipose tissue. *J. Mol. Neurosci.* : MN. 2011 Jul;44(3):178-85. PMID: 20938756. DOI: 10.1007/s12031-010-9446-6.

[7] Ye X, Weinberg RA. Epithelial-Mesenchymal Plasticity: A Central Regulator of Cancer Progression. *Trends Cell Biol.* 2015 Nov;25(11):675-86. PMID: 26437589. DOI: 10.1016/j.tcb.2015.07.012.

[8] Pattabiraman DR, Weinberg RA. Tackling the cancer stem cells - what challenges do they pose? *Nat Rev Drug Discov.* 2014 Jul;13(7):497-512. PMID: 24981363. DOI: 10.1038/nrd4253.

[9] Aigner K, Dampier B, Descovich L, Mikula M, Sultan A, Schreiber M, Mikulits W, Brabletz T, Strand D, Obrist P, Sommergruber W, Schweifer N, Wernitznig A, Beug H, Foisner R, Eger A. The transcription factor ZEB1 (deltaEF1) promotes tumour cell dedifferentiation by repressing master regulators of epithelial polarity. Oncogene. 2007 Oct 25;26(49):6979-88. PMID: 17486063. DOI: 10.1038/sj.onc.1210508.

[10] Spaderna S, Schmalhofer O, Wahlbuhl M, Dimmler A, Bauer K, Sultan A, Hlubek F, Jung A, Strand D, Eger A, Kirchner T, Behrens J, Brabletz T. The transcriptional repressor ZEB1 promotes metastasis and loss of cell polarity in cancer. *Cancer Res.* 2008 Jan 15;68(2):537-44. PMID: 18199550. DOI: 10.1158/0008-5472.can-07-5682.