doi: 10.22100/ijhs.v1i3.77
Original Article

IJHS 2015;1(3):33-34
ijhs.shmu.ac.ir

IJHS
International Journal of Health Studies

Exploring the Correlation between Expression of Emt Transcription Factors and CXCR4 Expression

Naghmeh Ahmadiankia[1*]
[1] Cancer Prevention Research Center, Shahroud University of Medical Sciences, Shahroud, Iran.

Received: 5 October 2015
Accepted: 1 November 2015

Abstract

Background: *Metastasis is one the most leading cause of death from cancer. The chemokine receptor of CXCR4 has an important role in cell migration and cancer metastasis. Additionally, metastasis is always associated with the process of epithelial-mesenchyme transition (EMT). In this study the correlation between expression of CXCR4 and EMT-TFs has been examined.*
Methods: *The expression of CXCR4 in knocked out SUM159 cell line for EMT-TFs of slug, snail, twist and ZEB1 were examined.*
Results: *The results revealed that the expression of CXCR4 decreased significantly in twist and ZEB1 knocked out cells, however in other groups no change was observed. Decreased expression of CXCR4 indicated that ZEB1 and twist may be one of regulators of CXCR4 expression.*
Conclusions: *ChIP assay should be performed in future experiments to see the definite role of ZEB1 and twist as transcription factors for CXCR4.*

Keywords: CXCR4, Cancer, Metastasis, Epithelial mesenchymal transition.
[*]**Corresponding to:** N Ahmadiankia, **Email:** ahmadiankia@ gmail.com
Please cite this paper as: Ahmadiankia N. Exploring the correlation between expression of emt transcription factors and CXCR4 expression. Int J Health Stud 2015;1(3):33-34.

Introduction

Cancer is the most common cause of death in the world with high emotional, economical, and social impacts on a patients' life. In many cases, metastasis is the most common cause of mortality.[1] Hence, the identification of mechanisms governing this process is critical for therapeutic purposes. Chemokine receptors play important roles in the process of tumor metastasis.[2,3] CXCR4 is a chemokine receptor that plays a critical role in cell migration and cancer metastasis.[4-6] In addition, it has been demonstrated that tumor metastasis is closely associated with the program termed EMT. During passage through an EMT, epithelial cancer cells lose their epithelial characteristics including cell–cell adhesion and instead acquire the traits of mesenchymal cells that give them migratory and invasive characteristics.[7]

Considering the close association of EMT with cancer metastasis and also the important role of CXCR4 in cancer metastasis, the question is whether there is any correlation between the expression of CXCR4 and EMT-TFs. Among different transcription factors, Slug, Snail, Twist, and Zinc finger E-box binding homeobox 1 (ZEB1) are important mediators of EMT. These proteins are responsible for orchestrating the gene expression programs that activate effectors of the EMT phenotype through the repression of epithelial genes and the activation of mesenchyme genes.[6]

In the present study, we examined for any correlation between the expression of EMT-TFs of Slug, Snail, Twist, and ZEB1 and the chemokine receptor of CXCR4. It was assumed that the results may help us to understand the probable role of EMT-TFs in controlling the expression of CXCR4 and also the basis and mechanisms involved in metastasis.

Materials and Methods

The SUM159 cell line was maintained at 37°C in DMEM, supplemented with 10% fetal bovine serum (FBS) and 0.01% penicillin/streptomycin, under a humidified atmosphere containing 5% CO2.

Different EMT-TFs of Slug, Snail, Twist, and ZEB1 in SUM159 subline were knocked out by using the CRISPR/Cas9 technique. These were provided by Yun Zhang from Weinberg Laboratory.

RNA preparation and cDNA synthesis were performed using a RNeasy Mini Kit (QIAGEN) and a high-capacity cDNA Reverse Transcription kit (Applied biosystems), respectively, both as per the manufacturer's protocol. A cDNA sample prepared from 1 µg total RNA was used for each PCR. The PCR reactions using SYBR Green Mix I (Roche Diagnostics), data collection, and data analysis were performed on the LightCycler 480 System (Roche diagnostics). The thermal cycling parameters for the PCR were as follows: 95°C for 5 min, followed by 35 cycles of 95°C for 10 s, 60°C for 7 s, and 72°C for 25 s. The relative mRNA quantity was normalized against the relative quantity of GUSB mRNA in the same sample. The primers used are indicated in Table 1.

Data are expressed as mean ± SEM and the student's t-test (unpaired) was used for comparison between the two groups; $P<0.05$ being considered statistically significant.

Table 1. Description of the designed primers

Genes	Primer sequences
CXCR4	Forward 5′ CTCCAAGCTGTCACACTCCA 3′
	Reverse 5′ TCGATGCTGATCCCAATGTA 3′
GUSB	Forward 5′ CTCATTTGGAATTTTGCCGATT 3′
	Reverse 5′ CCGAGTGAAGATCCCCTTTTTA 3′

Results

QRT-PCR revealed that the expression of CXCR4 decreased significantly after the knockout of Twist and ZEB1 using CRISPR/Cas9 in SUM159. However, the knockout of Slug and Snail did not affect the expression of CXCR4 (Figure 1).

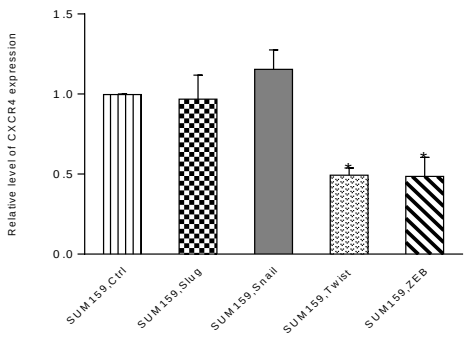

Figure 1. relative expression of CXCR4 in SUM159 after knockout of EMT-TFs. Slug and Snail knockout didn't affect the expression of CXCR4 however CXCR4 expression decreased significantly after Twist and ZEB1 knockout. *P<0.05 was considered significant as compared to control.

Discussion

The chemokine receptor CXCR4 belongs to the large superfamily of G protein-coupled receptors and is directly involved in numerous biological processes, including cell migration and cancer metastasis.[4,5] In addition, it was revealed that metastasis has a close association with EMT.[7] Different transcription factors have been identified that control EMT, which includes members of the SNAIL, TWIST, and ZEB family. Among different EMT-TFs, Twist and ZEB1 have fundamental roles in EMT. ZEB1 was sufficient to induce EMT and was necessary for maintaining the mesenchyme phenotype.[8] ZEB1 knockdown could cause mesenchyme MDA-MB-231 cells to express epithelial markers.[9] ZEB1 knockdown also reduced the metastasis-forming ability of the HCT116 colon colorectal cancer cell line.[10] The down regulation of tumor suppressing STF cDNA 3 promotes the epithelial–mesenchyme transition and tumor metastasis of osteosarcoma by the Wnt/GSK-3β/β-catenin/Snail signaling pathway.[11] MicroRNA-30a increases tight junction protein expression to suppress the epithelial–mesenchyme transition and metastasis by targeting Slug in breast cancer.[12] TGFβ1-Smad3-Jagged1-Notch1-Slug signaling pathway participates in tumor genesis and the progress of tongue squamous cell carcinoma.[13]

Considering the close association of metastasis with CXCR4 expression and also the association of metastasis with EMT, we studied the correlation between the expression of CXCR4 and EMT-TFs. We wonder if there is any correlation between these transcription factors, which are central mediators of EMT and the expression of CXCR4.

The result of our study showed that the expression of CXCR4 has decreased following a lowered expression of Twist and ZEB1. In conclusion, Twist and ZEB1 may be transcription factors controlling the expression of CXCR4. Therefore, it was concluded that increased metastasis during EMT may be the consequence of the effect of Twist and ZEB1 as transcription factors on the expression of CXCR4. ZEB1 has been predicted as the transcription factor for CXCR4 by bioinformatics analysis. These results should be confirmed by the chip assay.

Acknowledgement

The author thanks Prof. Robert Weinberg and members of the Weinberg lab for their advices and technical support. This work was supported by a grant from Whitehead Institute.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Mehlen P, Puisieux A. Metastasis: a question of life or death. Nat Rev Cancer 2006;6:449-58. 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;39:23-34. doi: 10.1002/cbin.10378

3. Mishan MA, Heirani-Tabasi A, Mokhberian N, Hassanzade M, Kalalian Moghaddam H, Bahrami AR, et al. Analysis of chemokine receptor gene expression in esophageal cancer cells compared with breast cancer with insights into metastasis. Iran J Public Health 2015;44:1353-8.

4. Naderi-Meshkin H, Naderi-Meshkin HT, Matin MM, Heirani-Tabasi A, Mirahmadi M, Irfan-Maqsood M, et al. 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. doi: 10.1002/cbin.10474

5. Mishan MA, Ahmadiankia N, Matin MM, Heirani-Tabasi A, Shahriyari M, Bidkhori HR, et al. Role of Berberine on molecular markers involved in migration of esophageal cancer cells. Cell Mol Biol (Noisy-le-grand) 2015; 61:37-43.

6. Ahmadian Kia N, Bahrami AR, Ebrahimi M, Matin M, Neshati Z, Rais Almohaddesin M, et al. Comparative analysis of chemokine receptor's expression in mesenchymal stem cells derived from human bone marrow and adipose tissue. J Mol Neurosci 2011;44: 178-85. 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;25:675-86. 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;13:497-512. doi: 10.1038/nrd4253

9. Aigner K, Dampier B, Descovich L, Mihula M, Sultan A, Schreiber M, et al. The transcription factor ZEB1 (deltaEF1) promotes tumour cell dedifferentiation by repressing master regulators of epithelial polarity. Oncogene 2007;26:6979-88. doi: 10.1038/sj.onc.1210508

10. Spaderna S, Schmalhofer O, Wahlbuhl M, Dimmler A, Bauer K, Sultan A, et al. The transcriptional repressor ZEB1 promotes metastasis and loss of cell polarity in cancer. Cancer Res 2008;68:537-44. doi: 10.1158/0008-5472.CAN-07-5682

11. Yang-fan L, Huanzi D, Guang-ning Y, Gang M, Xi Zh, Qiao-nan G. Downregulation of tumor suppressing STF cDNA 3 promotes epithelial-mesenchymal transition and tumor metastasis of osteosarcoma by the Wnt/GSK-3beta/beta-catenin/Snail signaling pathway. Cancer Lett 2016;373:164-73. doi:10.1016/j.canlet.2016.01.046

12. Chia-Wei C, Jyh-Cherng Y, Yi-Hsien H, Chung-Chin Y, Jui-I C, Po-Ming C, et al. MicroRNA-30a increases tight junction protein expression to suppress the epithelial-mesenchymal transition and metastasis by targeting Slug in breast cancer. Oncotarget 2016. doi: 10.18632/oncotarget.7656

13. Tonghan Z, Lizhong L, Xiaoling L, Ji-nan W, Jueyao C, Kui S, et al. TGFbeta1-Smad3-Jagged1-Notch1-Slug signaling pathway takes part in tumorigenesis and progress of tongue squamous cell carcinoma. J Oral Pathol Med 2016. doi: 10.1111/jop.12406