http://pmjournal.ir Original Article
Summer 2019, Volume 4, Issue 14 (1-3)

EMT-TF Expression in Colorectal Cancer in Tehran Population

Mohammad Ali Saremi [1], Najme Shojaee [1]

[1] Personalized Medicine Research Center of AmitisGen, Tehran, Iran

** Corresponding author: Mohammad Ali Saremi, Personalized Medicine Research Center of AmitisGen. E-mail: masaremi@yahoo.com*

DOI: 10.21859/pmj01021

Submitted: 2019/04/26
Accepted: 2019/05/24

Keywords:
Epithelial-Mesenchymal Transcription Factors
Colorectal Cancer
Targeted Therapy

Abstract

Epithelial to mesenchymal transition (EMT) is a process by which epithelial cells lose their epithelial characteristics such as cell polarity and cell–cell contact, and gain mesenchymal properties, such as increased motility. In colorectal cancer (CRC), EMT is associated with an invasive or metastatic phenotype. EMT induced by special transcription factors such as EMT-TF. The ZEB and Twist proteins were defined as EMT-TFs. All of these factors are expressed in embryogenesis and are capable of regulating developmental programs. In the current study, we evaluated Zeb1 and Twist1 expression levels in 30 CRC patients and 30 adjacent tissue from same patient. Our findings revealed a significant association of Zeb1 and Twist1 expression levels with CRC incidence, suggesting their potential for targeted therapy of disease.

INTRODUCTION

Colorectal cancer (CRC) is one of the most common cancers in the world. This cancer is the second common cancer and the fourth leading cause of cancer death worldwide. The mortality rate for CRC cancer is also high worldwide, and 394,000 cases annually die from the cancer [1]. Although CRC is commonly occurred in both sexes, but its incidence has been found to be higher in men as compared to women, and the standardized incidence of CRC has been estimated to be 20.6% in men and 14.6% in women /100000 population. CRC is one of the most common cancers among Iranian population [2]. Despite the development of treatment regimens, there is no effective therapy for advanced CRC with metastatic phenotype [3]. Understanding the molecular mechanisms underlying these transitions, especially how CRC cells are capable of acquiring invasive and metastatic properties, is important for the development of optimized strategies to treat patients suffering from CRC [4]. Epithelial to mesenchymal transition (EMT) is a reversible process that promotes epithelial cells to acquire a mesenchymal phenotype. A growing body of evidence indicates EMT plays a vital role in cancer progression and metastasis in many types of malignancies such as CRC [5]. EMT has been found to be orchestrated by EMT-activating transcription factors (EMT-TFs) such as SNAIL, TWIST and ZEB families. EMT-TFs play important roles in all stages of cancer progression, e.g., initiation, primary tumor growth, invasion, dissemination and metastasis [6]. One of the main targets of EMT-inducing transcription factors is E-cadherin, that is implicated in suppression of cell migration. It is noteworthy, that loss of E-cadherin seems to be necessary for epithelial cell movement [7]. EMT-TF repressed transcription by binding directly to the E-box sequences of the E-cadherin promoter, leading to suppression of E-cadherin expression [8]. The ZEB family of transcription factors consists of two members including ZEB1 and ZEB2, which are capable of binding regulatory gene sequences at E-boxes. These factors function as transcriptional repressors and activators, thereby repressing epithelial genes and activating mesenchymal genes, respectively. ZEBs primarily mediate transcriptional repression by recruiting the co-repressor C-terminal-binding protein (CTBP) to E-boxes [9]. Basic helix-loop-helix (bHLH) transcription factors are key players in a wide array of developmental processes, in this family, TWIST1 and TWIST2 are important regulators of EMT. In cancer, TWIST1 was found to be capable not only of repressing E-cadherin, but also of inducing the expression of mesenchymal markers, such as fibronectin and N-cadherin, during EMT [10]. In this study, we investigated the expression levels of these two genes in tumor specimens of patients with CRC.

METHODS

Thirty invasive colorectal carcinomas and 30 normal adjacent tissues from same patient were obtained from the Imam Khomeini Hospital tumor bank. The age range of the patients was between 35 and 77. RNA was extracted from tumor and normal samples with the RiboEX (Gene all, southern Korea), followed by

chloroform extraction according to the manufacturer's protocol. Furthermore, extracted RNA was quantified with a NanoDrop and quality were determined by 1.5% agarose gel electrophoresis. cDNA was prepared from 1 µg RNA using reverse transcriptase (Roche) and a oligo (dT primer [Invitrogen]). Real-time PCR was performed in a rotorgene Q (Qiagen, Germany), using the Ampliqon SYBR Green Master mix (Ampliqon, Denmark). GAPDH gene was used as reference gene. Primer set sequences were indicated in Table 1. Relative genes expression levels were calculated by 2^-DDct.

RESULTS

We analyzed the levels of mRNA expression in 30 CRC samples in comparison with normal colorectal mucosa using quantitative real-time PCR. GAPDH gene was applied as internal control and the analysis of gene expression was also performed using 2^-DDCt method, where the relative reference was each matched normal mucosa. The melt curve was analyzed for specificity of reaction (Fig 1). Gene expression patterns of Twist1 and ZEB1 were found to be statistically different among normal and tumoral tissues (P = 0.001; P = 0.021) (Fig 2).

Table 1. Primer Sequence

Gene	Primer sequence
Twist1	F- CATGTCCGCGTCCCACTAG
	R- TGTCCATTTTCTCCTTCTCTGG
ZEB1	F- GCACCTGAAGAGGACCAGAG
	R- TGCATCTGGTGTTCCATTTT
GAPDH	F- CCATGTTCGTCATGGGTGTG
	R- CAGGGGTGCTAAGCAGTTGG

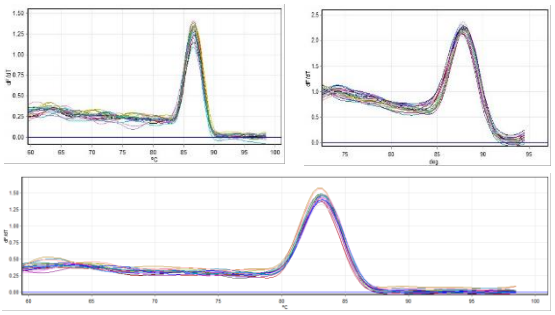

Figure 1: C: ZEB1 Melt Curve, B: Twist1 Melt Curve

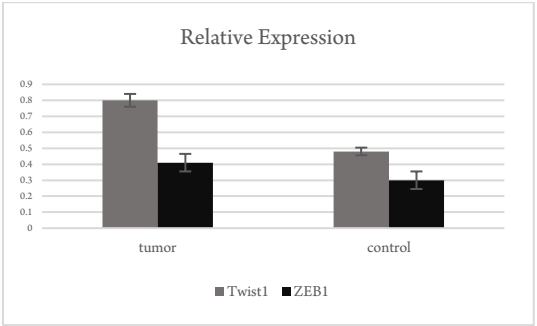

Figure 2: ZEB1 & Twist1 relative gene expression

DISCUSSION

CRC is one of the most common cancers in Iran. This cancer is the third most common cancer in Iranian men and the fourth most common cancer in women [2]. Most deaths from this cancer are due to metastasis and invasion of the primary tumor. EMT is considered as a complicated cellular process, by which epithelial cells acquire a mesenchymal phenotype [11]. This process plays a key role in the development of an invasive phenotype in tumor cells. The activation of this transdifferentiation program depends on contextual microenvironmental signals and is orchestrated by a network of EMT-inducing transcription factors (EMT-TFs) [12]. In the present study, ZEB1 and Twsit1 expression levels as two key EMT transcription factors were evaluated in CRC tissues and control samples. The results demonstrated a significant relationship between tumor and healthy groups in terms of Twist1 (P = 0.001) and ZEB1 (P = 0.021). As expected, increased expression of these genes was found to be capable of inhibiting E-cadherin gene expression and in turn induced N-cadherin expression [7]. Our results suggest that TWIST1and ZEB1 are possible tumor markers for poor outcomes in CRC. However, further investigation would be required to study the involvement of TWIST1 and ZEB1 in distant metastasis and patient survival. Valdes et al. reported TWIST1 mRNA levels were higher in patients with nodal invasion in primary CRC [13]. Overexpression of TWIST1 and ZEB1 could be explored as a potential predictive factor for poor prognosis in different tumor types, therefore, these genes can be used as predictive markers to identify metastases.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424. doi: 10.3322/caac.21492 pmid: 30207593
2. Safaee A, Fatemi SR, Ashtari S, Vahedi M, Moghimi-Dehkordi B, Zali MR. Four years incidence rate of colorectal cancer in Iran: a survey of national cancer registry data - implications for screening. Asian Pac J Cancer Prev. 2012;13(6):2695-8. doi: 10.7314/apjcp.2012.13.6.2695 pmid: 22938443
3. Drewes JL, Housseau F, Sears CL. Sporadic colorectal cancer: microbial contributors to disease prevention, development and therapy. Br J Cancer. 2016;115(3):273-80. doi: 10.1038/bjc.2016.189 pmid: 27380134
4. Markowitz SD, Bertagnolli MM. Molecular origins of cancer: Molecular basis of colorectal cancer. N Engl J Med. 2009;361(25):2449-60. doi: 10.1056/NEJMra0804588 pmid: 20018966
5. Bates RC, Mercurio AM. The epithelial-mesenchymal transition (EMT) and colorectal cancer progression. Cancer Biol Ther. 2005;4(4):365-70. doi: 10.4161/cbt.4.4.1655 pmid: 15846061
6. Puisieux A, Brabletz T, Caramel J. Oncogenic roles of EMT-inducing transcription factors. Nat Cell Biol. 2014;16(6):488-94. doi: 10.1038/ncb2976 pmid: 24875735
7. Gheldof A, Berx G. Cadherins and epithelial-to-mesenchymal transition. Prog Mol Biol Transl Sci. 2013;116:317-36. doi: 10.1016/B978-0-12-394311-8.00014-5 pmid: 23481201

8. Vu T, Datta PK. Regulation of EMT in Colorectal Cancer: A Culprit in Metastasis. Cancers (Basel). 2017;9(12). doi: 10.3390/cancers9120171 pmid: 29258163
9. Vandewalle C, Van Roy F, Berx G. The role of the ZEB family of transcription factors in development and disease. Cell Mol Life Sci. 2009;66(5):773-87. doi: 10.1007/s00018-008-8465-8 pmid: 19011757
10. Peinado H, Olmeda D, Cano A. Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? Nat Rev Cancer. 2007;7(6):415-28. doi: 10.1038/nrc2131 pmid: 17508028
11. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Invest. 2009;119(6):1420-8. doi: 10.1172/JCI39104 pmid: 19487818
12. Jing Y, Han Z, Zhang S, Liu Y, Wei L. Epithelial-Mesenchymal Transition in tumor microenvironment. Cell Biosci. 2011;1:29. doi: 10.1186/2045-3701-1-29 pmid: 21880137
13. Valdes-Mora F, Gomez del Pulgar T, Bandres E, Cejas P, Ramirez de Molina A, Perez-Palacios R, et al. TWIST1 overexpression is associated with nodal invasion and male sex in primary colorectal cancer. Ann Surg Oncol. 2009;16(1):78-87. doi: 10.1245/s10434-008-0166-x pmid: 19002529