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Original Article

Thioredoxin-1 promotes colorectal cancer invasion and metastasis through crosstalk with S100P

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ABSTRACT

Thioredoxin-1 (Trx-1) is a small redox-regulating protein, which plays an important role in several cellular functions. Despite recent advances in understanding the biology of Trx-1, the role of Trx-1 and its underlying signaling mechanism in colorectal cancer (CRC) metastasis have not been extensively studied. In this study, we observed that Trx-1 expression is increased in CRC tissues compared to the paired non-cancerous tissues and is significantly correlated with clinical staging, lymph node metastasis and poor survival. Overexpression of Trx-1 enhanced CRC cell invasion and metastasis *in vitro* and *in vivo*. Conversely, suppression of Trx-1 expression decreased cell invasion and metastasis *in vitro* and *in vivo*. Moreover, Trx-1 activates S100P gene transcription. S100P, in turn, promotes Trx-1 expression and nuclear localization by upregulating p-ERK1/2 and downregulating TXNIP expression. Our finding provides new insight into the mechanism of Trx-1/S100P axis in the promotion of CRC metastasis, and suggests that the Trx-1/S100P axis and their related signaling pathways could be novel targets for the treatment of metastatic CRC.

Introduction

Colorectal cancer (CRC) is a major cause of morbidity and mortality throughout the world. In recent years, CRC incidence rates are rapidly increasing in developing countries that previously showed a decreased risk of CRC [1]. Despite great advances in diagnosis and treatment during the last few decades, the overall five-year survival rate for CRC remains 40 to 45 percent [2]. Metastasis is the leading reason for the resultant mortality of CRC patients, but the underlying molecular mechanisms of CRC metastasis are still not fully understood.

Oxidative stress has been demonstrated to be involved in the development and progression of several cancers, including CRC [3]. The thioredoxin (Trx) system is a major antioxidant system and plays an important role in various cellular functions, including maintaining cellular redox homeostasis and mediating growth, survival, and chemoresistance [4,5]. It contains thioredoxin-1 (Trx-1, TXN), a small 12-kDa protein with redox-activation present in the cytoplasm [6]. Overexpression of Trx-1 is associated with altered cellular redox status, growth promotion of cancer cells, anti-apoptotic and inflammation modulation by means of reducing equivalents and a transcriptional regulator [7–10]. Trx-1 expression was increased in several human tumor tissues, including liver [11], lung [12], pancreas [13], colorectal [14], uterine cervix [15], and gastric carcinoma [16]. Increased Trx-1 expression is associated with inhibition of apoptosis, enhanced proliferation of tumor cells, aggressive tumor growth, high reactive oxygen species generation and a poor survival rate [10]. Trx-1 overexpression increases both hypoxia-induced factor 1α (HIF-1α) levels and HIF-1 transactivation in cancer cells, which in turn enhances tumor angiogenesis by increasing vascular endothelial growth factor (VEGF) production

Abbreviations: ChIP, chromatin immunoprecipitation; CRC, colorectal cancer; DAPI, 4′-6-Diamidino-2-phenylindole; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IF, immunofluorescence; IHC, immunohistochemistry; PCR, polymerase chain reaction; ROC, receive operating characteristic; RT, reverse transcription; TXNIP, thioredoxin interacting protein; Trx-1, thioredoxin-1.

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Table 1
Trx-1 expression and clinicopathological parameters in colorectal cancer specimens.

	All cases	Trx-1 protein		
		Normal expression	Overexpression	*P* value
Sex				0.039*
Male	47	19	28	
Female	63	14	49	
Histologic grade(WHO)				0.762
Low	95	28	67	
High	15	5	10	
Clinical stage				0.007*
I-II	73	28	45	
III-IV	37	5	32	
pN status				0.041*
N0	57	22	35	
N1–N2	53	11	42	
Recurrence				0.183
No	56	20	36	
Yes	54	13	41	

Statistical analyses were performed by χ^2 test. *$P < 0.05$.

[17]. In addition, Trx-1 has been shown to activate several transcription factors such as NF-κB, AP-1, and SP-1, all of which seem to regulate cell growth and survival [17,18]. However, the role of Trx-1 and its underlying signaling mechanism in the CRC metastasis have not been extensively studied.

S100P is a member of the S100 family of proteins containing 2 EF hand calcium-binding motifs that is involved in the regulation of a number of cellular processes through its calcium-binding ability [19–21]. S100P expression is upregulated in various cancers and is associated with poor clinical outcomes [20,22–25]. Most studies have indicated that overexpression of S100P correlates with tumor growth and metastasis [22,23,26]. We previously reported that S100P expression is upregulated in CRC and correlates with clinical staging and lymph node metastasis and recurrence [22]. We have also demonstrated that overexpression of S100P promotes the migration and invasion of CRC cells, whereas silencing S100P expression by lentiviral vector mediated shRNA inhibits CRC invasion and metastasis *in vitro* and *in vivo*, suggesting that S100P plays an important role in CRC metastasis [22,27].

In the present study, we observed that the expression of Trx-1 is increased in human CRC tissues compared to the paired non-cancerous tissues and high Trx-1 expression is associated with advanced clinical stages, lymph node metastasis and poor clinical outcomes. We also found a novel positive feedback loop between Trx-1 and S100P, which promotes CRC invasion and metastasis. Trx-1 regulates S100P gene transcription through interaction with SP1. S100P in turn promotes Trx-1 expression and nuclear localization by upregulating p-ERK1/2 and downregulating Thioredoxin Interacting Protein (TXNIP) expression. Our finding provides new insight into the mechanism of Trx-1/S100P axis in the promotion of CRC metastasis, and suggests that the crosstalk between Trx-1 and S100P and their related signaling pathways could be significant potential therapeutic targets for the treatment of CRC metastasis.

Materials and methods

Cell culture and chemicals

Human CRC cell lines SW480, SW620 and DLD-1, and HEK293T cells were purchased from the Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China. SW480 cells were maintained in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA). SW620, DLD-1 and HEK293T cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS. Cells were cultured in a humidified 37 °C incubator with 5% CO_2. PD98059 (Cat. #S1177) was purchased from Selleck Chemicals (Houston, TX, USA).

Lentiviral vector construction and transduction

See Supplementary Materials and Methods for details.

Cell proliferation, migration and invasion assays, real-time Polymerase Chain Reaction (PCR) and Western blot analysis

See Supplementary Materials and Methods for details.

siRNA transfection

siRNAs were purchased from GenePharma (GenePharma Co., Ltd., Shanghai, China). The sequence of selected regions to be targeted by siRNAs for TXNIP was 5′-CAU CCU UCG AGU UGA AUA UTT -3′. Cells were transfected with 50 nM scramble siRNA (Negative control, NC), or TXNIP-siRNA by Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol.

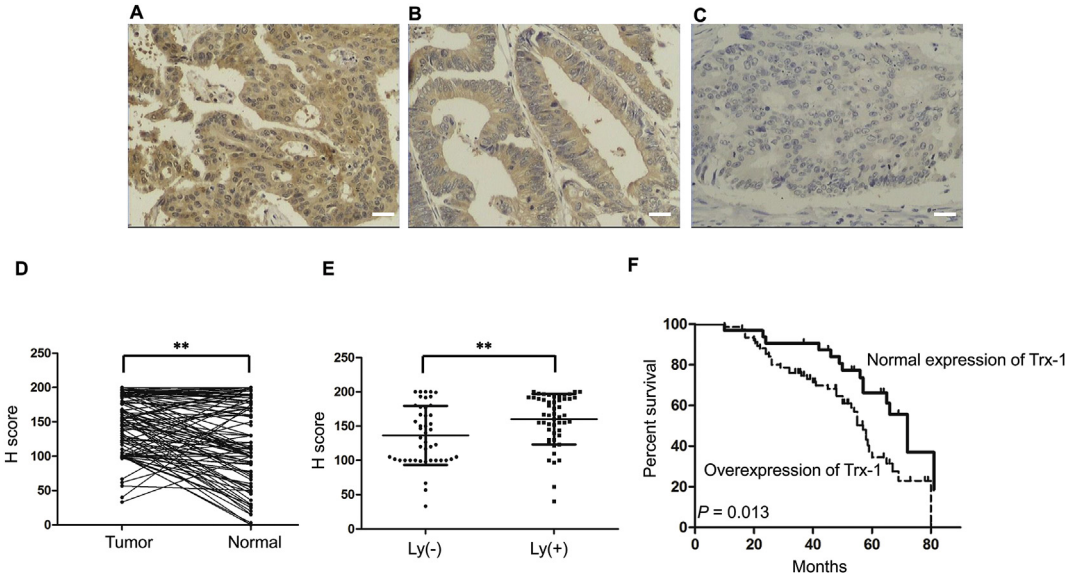

Fig. 1. Expression of Trx-1 in colorectal carcinoma tissues and its prognostic significance in colorectal cancer patients. (A) Strong immunopositive staining of cancerous tissue, H score = 200; (B) Moderate immunopositive staining of cancerous tissue, H score = 120; (C) Negative staining of cancerous tissue, H score = 0; Scale bar = 20 μm. (D) Trx-1 expression of colorectal cancer tissue was significantly higher than that of the matched adjacent normal tissues as indicated by immunohistochemical (IHC) staining. **$P < 0.01$. (E) Significant upregulation of Trx-1 protein expression was revealed in CRC with lymph node metastases, relative to CRC without lymph node metastasis. **$P < 0.01$. (F) Kaplan–Meier survival analysis according to Trx-1 expression in patients with colorectal cancer (log-rank test, $P = 0.013$).