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Original Article

Association of treRNA with lymphatic metastasis and poor prognosis in colorectal cancer

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Abstract: Background: There is an emerging concept that long noncoding RNAs (lncRNAs) are involved in tumorigenesis and could be used as biomarkers. However, the clinical significance of human translational regulatory lncRNA (treRNA) in CRC is largely unknown. The purpose of the study was to examine the value of treRNA as a biomarker in colorectal cancer patients. Methods: treRNA expression was studied in 78 tumors and adjacent tissues in colorectal cancer patients using quantitative real-time PCR. Results: treRNA was found to be highly expressed in colorectal cancer tissue in contrast to adjacent tissue ($P<0.05$). Moreover, positive correlation was found between high treRNA expression and lymph node metastasis ($P<0.05$). Patients with high treRNA expression were found with compromised overall survival (OS) compared with the low treRNA expression group, according to Kaplan-Meier analysis. Moreover, Cox regression model analysis suggested high expression of treRNA as an independent poor prognostic factor for CRC patients. Conclusions: Overexpression of treRNA could be associated with lymphatic metastasis and compromised survival of CRC. treRNA has potential to be used as a new biomarker for CRC lymphatic metastasis and survival.

Keywords: treRNA, colorectal cancer, lymphatic metastasis, survival, lncRNA

Introduction

Colorectal cancer is a common lethal malignancy and one of the leading causes of mortality worldwide [1]. Surgery remains the cornerstone of curative treatment [2]. Unfortunately, some patients suffer local recurrences and metastases after removal of the primary tumor and prognosis for these patients remains unsatisfactory [2]. Identifying biomarkers for prognostic stratification and selection of adjuvant treatments has become important nowadays [3].

Long non-coding RNAs (lncRNAs) are long endogenous cellular RNAs (>200 nt) that could not code protein. There is an emerging concept that lncRNAs play important roles in cancer and could be used as biomarkers [4, 5].

Human translation regulatory long non-coding RNA (treRNA), located adjacent to SNAI1 on chromosome 20q13, has been shown to exert an enhancer-like function in cis [6]. Studies have reported the positive role of treRNA in metastasis [7-9] *in vitro* and *in vivo*. Gumireddy et al. demonstrated that treRNA was overexpressed in lymph node metastasis compared to matching primary lesions of breast tumors [10]. Knockdown of treRNA in A549 lung cancer cells suppressed cell migration and invasion but did not affect proliferation [10]. A key mechanism for promotion of metastasis is based on treRNA-mediated suppression of translation of E-cadherin [7, 8], a well-known of epithelial marker [11, 12]. Recently, treRNA was shown to be associated with resistance to chemotherapy in chronic lymphocytic leukemia through enhanced resistance to cytotoxic mediated DNA damage [13]. However, the value of treRNA as a biomarker in CRC patients remains unclear.

In our current study, treRNA expression in colorectal cancer tumors and adjacent tissue was measured. Associations between treRNA and clinical and pathological features and prognosis in colorectal cancer patients was further analyzed.

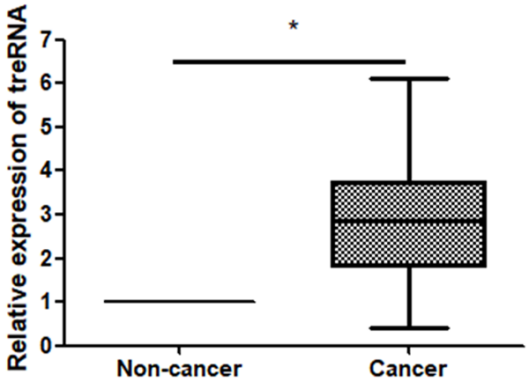

Figure 1. treRNA expression levels in cancer tissue and adjacent non-cancer tissues of CRC patients. treRNA expression levels were normalized to β-actin. Data are mean ± SD, **P*<0.05.

Materials and methods

Tissue specimens

Seventy-eight pairs of tumor and adjacent tissues were obtained from CRC patients that underwent surgery for colorectal cancer without radiotherapy or chemotherapy between 2015 and 2016 at the Second Affiliated Hospital of Xi'an Jiaotong University, China. Diagnosis of all patients was confirmed pathologically by experienced pathologists. This study was conducted with permission from the Ethics Committee of Xi'an Jiaotong University. Informed consent was obtained from all participants.

Real-time quantitative reverse transcription PCR (qRT-PCR)

Expression levels of treRNA were measured by SYBR-Green real-time PCR (Takara) and normalized using β-actin. The primers used in the study were as follows: treRNA F: 5'-CTCCACTCCGCCTGGAAATC-3'; treRNA R: 5'-CAGGACTGGCTGAGGTTTGT-3'; β-actin F: 5'-ATCGTGCGTGACATTAAGGAGAAG-3' and β-actin R: 5'-AGGAAGGAAGGCTGGAAGAGTG-3'.

Statistics

SPSS version 13.0 software was used for all statistical analyses. Expressions were studied by t-value test. Association between treRNA expression and clinical and pathological features were analyzed by Chi-square test. Survival curves were plotted by Kaplan-Meier method and differences between survival curves were analyzed by log-rank test. Factors that affect survival were identified by Cox proportional hazards model. All experiments were repeated and data are presented as mean ± SD. Statistically significance was defined as *P* values lower than 0.05.

Results

Upregulation of treRNA expression in CRC

We detected treRNA expression in 78 pairs of CRC and adjacent tissues. qRT-PCR analysis showed that treRNA expression was significantly higher in tumors than in adjacent normal tissues (P<0.05, **Figure 1**).

Correlation between treRNA expression and clinicopathological characteristics in CRC patients

Association between treRNA and clinicopathological features was further explored in colorectal cancer patients. The median expression of treRNA in tumors was 2.85 which was set as the threshold for our current study. Patients were divided into two groups according to their treRNA expression: high treRNA group (≥2.85; 39) and low treRNA group (<2.85; 39). Association between treRNA expression and clinical and pathologic features of patients is listed in **Table 1**. Our results showed that treRNA expression was associated with histologic grading, TNM staging, and lymph node metastasis (*P*<0.05). However, no association was found between its expression and clinical features such as age, gender, size of tumor, and local infiltration (*P*>0.05). Our findings suggest that treRNA expression might promote progression in colorectal cancer.

Prognostic values of treRNA expression in CRC

Correlation of treRNA expression with overall survival of CRC patients was further explored using Kaplan-Meier analyses. Our results showed that overall survival time and disease-free survival of low treRNA expression group were significantly longer than those of high treRNA group (P<0.05, **Figure 2**). Moreover, we

Table 1. Correlation between treRNA expression and clinicopathological characteristics in CRC patients

Parameter/group	Group	Total	treRNA expression		*P* value
			High	Low	
Age (years)	≤60	36	15	21	0.173
	>60	42	24	18	
Gender	Male	38	18	20	0.651
	Female	40	19	21	
Tumor location	Left colon	24	11	13	0.755
	Right colon	25	14	11	
	Anorect.	29	14	15	
Tumor size	≤4 cm	36	15	21	0.173
	>4 cm	42	24	18	
Differentiation	Well or moderate	40	23	17	0.174
	Poor	38	16	22	
T stage	T1 or T2	29	15	14	0.101
	T3 or T4	49	28	21	
Lymphatic Metastasis	Positive	18	13	5	0.032
	Negative	60	26	34	

found by multivariate Cox analysis that treRNA expression was an independent prognostic factor for overall survival (HR=2.249, 95% CI, 1.020-4.462; P=0.045) and disease-free survival (HR=2.016, 95% CI, 1.028-3.951; P= 0.041) in CRC patients (**Table 2**).

Discussion

There is an emerging concept that lncRNAs are involved in tumorigenesis and could be used as biomarkers [14]. For example, Wang and his colleagues found CCAT2 could be a new biomarker for metastasis and prognosis in various cancers [15]. Su and his colleagues found lncRNA ZEB1-AS1 regulates expression of ZEB1 and downstream molecules in prostate cancer [16]. Yang and his colleagues found that reduced expression of MT1JP in gastric cancer tissues was associated with tumor diameter, differentiation, and TNM staging [17].

Human Translation regulatory long non-coding RNA (treRNA), located adjacent to SNAI1 on chromosome 20q13, have been shown to exert an enhancer-like function in cis [6]. Transcription factor SNAI1 is a well-known cancer invasion and metastasis promoter which induces epithelial-mesenchymal-transition (EMT) by regulating ZEB1, MUC1, and E-cadherin [18]. treRNA inhibits E-cadherin translation and protein expression through forming treRNA-RNP complex and binding to 3'-UTR of E-cadherin mRNA [7, 8]. E-cadherin is a well-known cancer promoter positively relating to metastasis and invasion in a series of cancers such as gastric, breast, colorectal, and bladder cancers [11, 12].

To our knowledge, our current study is the first to report associa-tion between treRNA and clinicopathological characteristics and prognosis in CRC patients. In this study, treRNA was overexpressed in CRC tissues and closely correlated with lymph node metastasis. Moreover, overexpression of treRNA could be an independent poor prognostic biomarker in CRC patients.

In conclusion, this study provides evidence that overexpression of treRNA may be associated with lymphatic metastasis and poor prognosis of CRC. However, further mechanical research and additional well-designed studies with large samples in different ethnic groups are necessary to confirm and extend our findings.

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Disclosure of conflict of interest

None.

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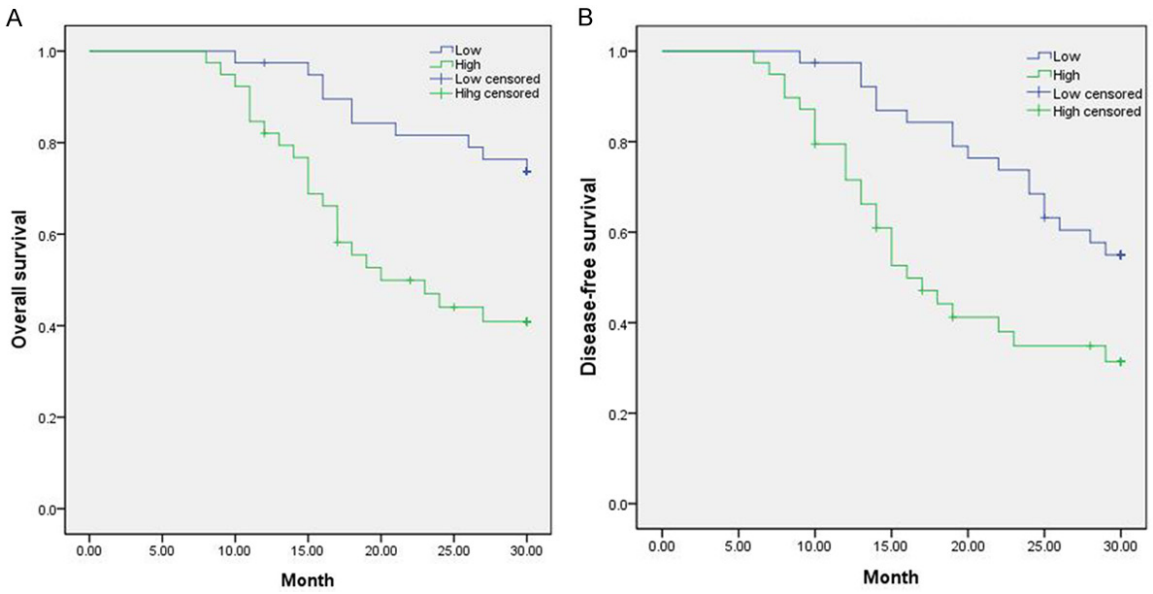

Figure 2. Correlation between treRNA expression and overall survival (A) or disease-free survival (B) of colorectal cancer patients.

Table 2. Cox regression analyses of overall survival and disease-free survival in CRC patients

	Overall survival			Disease-free survival		
	Hazard ratio	95% CI	P	Hazard ratio	95% CI	P
Univariate analyses						
Age	1.285	0.639-2.585	0.482	1.323	0.713-2.453	0.375
Gender	0.593	0.293-1.201	0.147	0.745	0.403-1.378	0.348
Tumor location	0.710	0.467-1.080	0.110	0.901	0.625-1.299	0.577
Tumor size	1.080	0.996-1.172	0.062	1.044	0.974-1.119	0.221
Differentiation	1.096	1.011-1.189	0.027	1.037	1.968-1.110	0.298
T stage	2.192	1.095-4.388	0.027	1.971	1.065-3.646	0.031
Lymphatic Metastasis	2.866	1.393-5.896	0.004	2.530	1.299-4.924	0.006
treRNA expression	3.144	1.482-6.672	0.003	2.506	1.329-4.724	0.005
Multivariate analyses						
Differentiation	1.107	1.018-1.205	0.018	1.039	0.968-1.115	0.085
T stage	2.338	1.161-4.711	0.017	2.124	1.139-3.962	0.018
Lymphatic Metastasis	2.801	1.290-6.079	0.009	2.290	1.116-4.702	0.024
treRNA expression	2.249	1.020-4.962	0.045	2.016	1.028-3.951	0.041

References

[1] Siegel RL, Miller KD, Fedewa SA, Ahnen DJ, Meester RGS, Barzi A and Jemal A. Colorectal cancer statistics, 2017. CA Cancer J Clin 2017; 67: 177-193.

[2] Meyerhardt JA and Mayer RJ. Systemic therapy for colorectal cancer. N Engl J Med 2005; 352: 476-487.

[3] Saltz L. Systemic therapy for metastatic colorectal cancer. J Natl Compr Canc Netw 2013; 11 Suppl: 649-652.

[4] Bolha L, Ravnik-Glavac M and Glavac D. Long noncoding RNAs as biomarkers in cancer. Dis Markers 2017; 2017: 7243968.

[5] Du Z, Fei T, Verhaak RGW, Su Z, Zhang Y, Brown M, Chen YW and Liu XS. Integrative genomic analyses reveal clinically relevant long noncoding RNAs in human cancer. Nat Struct Mol Biol 2013; 20: 908-13.

[6] Orom UA, Derrien T, Beringer M, Gumireddy K, Gardini A, Bussotti G, Lai F, Zytnicki M, Notredame C, Huang Q, Guigo R and Shiekhattar R. Long noncoding RNAs with enhancer-like

function in human cells. Cell 2010; 143: 46-58.
[7] Gumireddy K, Li AP, Yan JC, Setoyama T, Johannes GJ, Orom UA, Tchou J, Liu Q, Zhang L, Speicher DW, Calin GA and Huang QH. Identification of a long non-coding RNA-associated RNP complex regulating metastasis at the translational step. EMBO J 2013; 32: 2672-2684.
[8] Loayza-Puch F and Agami R. Lncing protein translation to metastasis. EMBO J 2013; 32: 2657-2658.
[9] Wang XY, Ren YL, Yang XY, Xiong XY, Han SC, Ge YX, Pan WT, Zhou LQ, Yuan QP and Yang M. miR-190a inhibits epithelial-mesenchymal transition of hepatoma cells via targeting the long non-coding RNA treRNA. FEBS Lett 2015; 589: 4079-4087.
[10] Gumireddy K, Li A, Yan J, Setoyama T, Johannes GJ, Orom UA, Tchou J, Liu Q, Zhang L, Speicher DW, Calin GA and Huang Q. Identification of a long non-coding RNA-associated RNP complex regulating metastasis at the translational step. EMBO J 2013; 32: 2672-2684.
[11] Christou N, Perraud A, Blondy S, Jauberteau MO, Battu S and Mathonnet M. E-cadherin: a potential biomarker of colorectal cancer prognosis. Oncol Lett 2017; 13: 4571-4576.
[12] Hanusova V, Krbal L, Soukup J, John S and Rudolf E. Role of E-cadherin in metastatic colorectal cancer treatment. Annals of Oncology 2017; 28.
[13] Miller CR, Ruppert AS, Fobare S, Chen TL, Liu CM, Lehman A, Blachly JS, Zhang XL, Lucas DM, Grever MR, Tallman MS, Flinn IW, Rassenti LZ, Kipps TJ, Sampath D, Coombes KR and Hertlein EK. The long noncoding RNA, treRNA, decreases DNA damage and is associated with poor response to chemotherapy in chronic lymphocytic leukemia. Oncotarget 2017; 8: 25942-25954.
[14] Han D, Wang M, Ma N, Xu Y, Jiang YT and Gao X. Long noncoding RNAs: novel players in colorectal cancer. Cancer Lett 2015; 361: 13-21.
[15] Wang DL, Chen ZC, Xu HD, He AB, Liu YC and Huang WR. Long noncoding RNA CCAT2 as a novel biomaker of metastasis and prognosis in human cancer: a meta-analysis. Oncotarget 2017; 8: 75664-75674.
[16] Su W, Xu M, Chen X, Chen N, Gong J, Nie L, Li L, Li X, Zhang M, Zhou Q. Long noncoding RNA ZEB1-AS1 epigenetically regulates the expressions of ZEB1 and downstream molecules in prostate cancer. Mol Cancer 2017; 16: 142.
[17] Yang J, Zhang YB, Liu P, Yan HL, Ma JC and Da MX. Decreased expression of long noncoding RNA MT1JP may be a novel diagnostic and predictive biomarker in gastric cancer. IInt J Clin Exp Pathol 2017; 10: 432-438.
[18] Tan M, Chuang VY, Tan TZ, Thiery JP and Huang RYJ. Transcriptional regulatory loops among SNAI1, TWIST1, ZEB1, and ZEB2 defines the epithelial-mesenchymal transition (EMT) spectrum in epithelial ovarian cancer (EOC). Cancer Research 2015; 75.