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Original Article

Tumor-Educated Platelet LncRNA MALAT-1; Expression Levels and Clinical Potential as a Biomarker in Colorectal Cancer

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ABSTRACT

Background:
Long non-coding RNAs (lncRNAs) have more and more investigated as novel and valuable biomarkers in cancers. We aimed to examine the expression and potential diagnostic value of two oncogenic lncRNAs, MALAT-1 and ROR, in tumor-educated platelets (TEPs) of patients with colorectal cancer (CRC).

Materials and Methods:
The expression levels of TEP lncRNAs were analyzed in 68 patients with CRC and 68 healthy individuals by a quantitative real-time PCR (qPCR). The differential expression of lncRNAs was examined, and diagnostic values were obtained.

Results:
TEP lncRNA MALAT-1 expression levels were significantly increased (P=0.044) in patients with CRC compared to healthy individuals. The expression levels of lncRNA ROR did not show a significant change (P=0.672) between patients with CRC and healthy individuals. Moreover, a significant correlation between MALAT-1 and tumor differentiation and lymphovascular invasion (P<0.05) was detected. TEP lncRNA MALAT-1 showed a diagnostic potential by sensitivity 63% and specificity 66% with AUC 0.66.

Conclusion:
LncRNA MALAT-1 is upregulated in TEPs from patients with CRC and has potential clinical value as a cancer biomarker.

Keywords: Tumor-educated platelet, LncRNA, MALAT-1, Colorectal cancer

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INTRODUCTION

Colorectal cancer (CRC) is one of the most common cancers that affects people worldwide (1). Patients with CRC have a 5-year relative survival rate that varies from 90% for those detected at the early stages (I, II) to 14% for those diagnosed at the late stages (III, IV). This indicates that primary screening and early cancer diagnosis are critical to the survival of patients with CRC (2, 3). The fecal occult blood test (FOBT), fecal immunochemical test (FIT), and colonoscopy are the current screening techniques for CRC. However, due to their high cost, potential risks, or poor patient compliance, these approaches are often insufficient for patients with CRC (4, 5).

Proteins and nucleic acids from circulating tumor cells are increasingly studied as non-invasive sources for identification of novel diagnostic biomarkers (6, 7). Megakaryocytes give rise to platelets, the well-known effector cells in coagulation, which are a vast aggregate of blood cells (1). Recent research has revealed that platelets may play a functional role in the spread and development of cancer (8-10). There are a lot of circulating RNAs in these blood cells, both coding and non-coding (ncRNAs) (1). Cancer research has shown increasing interest in ncRNAs, such as long non-coding RNAs (lncRNAs) and microRNAs (miRNAs), and in their critical roles in the initiation and spread of tumors (11, 12).

It has been demonstrated that the ncRNA MALAT-1 (metastasis-associated lung adenocarcinoma transcript 1) functions as an oncogene in various malignancies, potentially promoting cell invasion and proliferation (13, 14). This lncRNA, which controls miRNAs, may have a significant impact on important signaling pathways that direct cellular functions (1). In several cancers, there has also been evidence of upregulation of the lncRNA MALAT1 and a link between its expression levels and clinicopathological characteristics, as well as patient survival rates (1). The clinical value of lncRNA MALAT-1 as a diagnostic and prognostic biomarker in various malignancies, including CRC, has been documented (13, 15, 16). The long non-coding regulator of reprogramming (ROR) was initially discovered to control cellular functions in stem cells (SCs) (1). LncRNA-ROR has the potential to regulate numerous biological processes, such as cell division and proliferation (1). Additionally, studies have demonstrated an association between lncRNA-ROR dysregulation and tumor invasion and metastasis in patients with CRC (17). Recent studies have indicated that altered platelet RNA patterns and protein repertoires could be served as potential prognostic and diagnostic biomarkers in numerous malignancies, including CRC (12, 18).

In cancer patients' circulation, there may be a biological cross-talk between circulating tumor cells and platelets (1). Furthermore, the tumor microenvironment (TME) may reciprocally impact circulating blood cells like platelets (1). These interactions may create tumor-educated platelets (TEPs) by generating a signal to modify RNA splicing or enhance platelet absorption of tumor-spliced mRNAs (19, 20). Therefore, TEPs may differ from healthy persons in terms of their altered RNA pattern, which may comprise nucleic acid components (1).

We can consider TEP RNAs, including lncRNAs, as newly developed minimally invasive biomarkers for the diagnosis and prognosis of CRC. However, the significance of TEP lncRNAs as CRC biomarkers remains unclear. Our goal was to evaluate the expression levels and potential diagnostic value of ROR and MALAT-1, two oncogenic TEP lncRNAs, in platelets from people with CRC.

MATERIALS AND METHODS

Blood sample collection

A total of 68 whole-blood samples were collected from patients with CRC who received no chemotherapy or radiotherapy, and 68 blood samples from healthy individuals between November 2021 and July 2023. Criteria for selection of the healthy control group included no malignancy (or benign polyps) in the intestine, no drugs or chemotherapy within 2 months of sampling, and no other disease or malignancy. An attempt was also made to match the control samples to the patient group by age and sex. Informed consent was obtained from all participants. The malignancy was confirmed in all patients by pathological examination, and clinical and pathological features, including tumor size, differentiation, clinical stage, and lymphovascular invasion. The study was approved by the Ethics Committee of Iran University of Medical Sciences (Ethical code: IR.IUMS.REC.1399.1389) and conducted in accordance with the ethical guidelines.

Blood processing and platelet separation

Whole blood (5-8 mL) was collected from each participant in EDTA-2K vacutainer tubes. All blood samples (5 mL) were immediately centrifuged at 140 g for 12 min at room temperature, and platelet-rich plasma (PRP) was separated from peripheral mononuclear cells (PBMC). Then, the PRP was centrifuged at 360 g for 12 min to precipitate the platelets. Platelet pellets were washed with PBS, then resuspended in DEPC-treated water and stored at -80 °C for RNA extraction.

RNA extraction from platelets and cDNA synthesis

Total RNA was extracted from isolated platelets using RNX Plus Solution (RNX Plus, Sinnaclon, Iran). The quality and quantity of total RNAs were checked using electrophoresis and a NanoDrop 2000 spectrophotometer (Thermo Fisher

Scientific, Waltham, MA, USA). In total, 2 µg total RNA was used for cDNA synthesis using the PrimeScript™ RT reagent Kit (Takara, Japan). cDNA was synthesized according to the manufacturer's instructions and was stored at -20 °C for future use.

LncRNA expression analysis by real-time PCR

A quantitative real-time PCR was accomplished for lncRNA expression analysis using SYBR®Premix Ex Taq™ II (Takara, Japan) on the 7500 Real-Time PCR System (Applied Biosystems, CA, USA). The PCR cycles were repeated 40 times. The expression of lncRNAs MALAT-1 and ROR was normalized against β-Actin as a housekeeping internal control. The amplification reactions were completed using specific primers shown in Table 1. The relative expression of lncRNAs was calculated using the $2^{-\Delta\Delta CT}$ method.

Statistical analysis

SPSS software version 19.0 (IBM Corp., Armonk, NY, USA) was used for completing the independent samples t-test and Mann-Whitney U test. Receiver operating characteristic (ROC) analysis was used to assess the diagnostic value of lncRNAs. P values <0.05 were reported as statistically significant.

RESULTS

Upregulation of TEP lncRNA MALAT-1 in patients with CRC

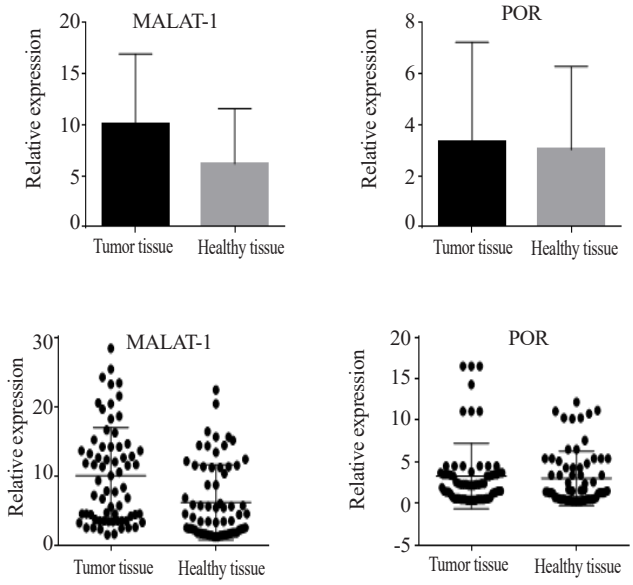
The expression levels of TEP lncRNAs in CRC were evaluated by a quantitative real-time PCR in 68 patients with CRC and 68 healthy individuals. The results showed that the expression levels of TEP lncRNA MALAT-1 were significantly upregulated in patients with CRC in comparison with the healthy individuals (P=0.044, Figure 1). However, the expression levels of the TEP lncRNA ROR did not differ significantly between the patients with CRC and healthy individuals (P=0.672).

TEP lncRNA MALAT-1 correlated with the clinicopathological features of patients with CRC

The correlation between TEP lncRNA MALAT-1 expression levels and clinicopathological features of CRC was examined in 68 patients. It was found that the expression levels of TEP lncRNA MALAT-1 correlated with the clinicopathological features, including tumor differentiation (P=0.023) and lymphovascular invasion (P=0.004).

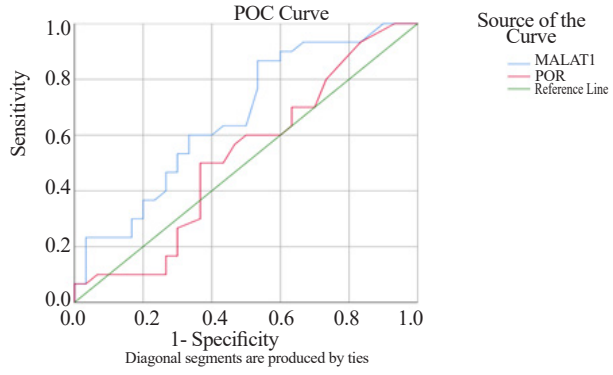
TEP lncRNA MALAT-1 as a potential diagnostic marker for patients with CRC

The ROC analysis was performed in 68 patients with CRC and 68 healthy individuals to evaluate the diagnostic value of the TEP lncRNA MALAT-1. According to the results, the area under the ROC curve (AUC) for TEP lncRNA MALAT-1 was 0.66 (Table 2), with a sensitivity of 63% and specificity of 66%, and a cut-off value of 5.6 (Figure 2, Table 3). The analytic power indicated the clinical potential of TEP lncRNA MALAT-1 as a diagnostic marker for patients with CRC.



The expression levels of TEP lncRNA MALAT-1, but not ROR, were significantly dysregulated in patients with CRC compared with healthy individuals (P=0.044 and P=0.672, respectively).

Figure 1. The relative expression levels of TEP lncRNAs in 68 patients with CRC and 68 healthy individuals



The AUC of TEP lncRNA MALAT-1 in CRC was 0.66 with optimal sensitivity of 63% and optimal specificity of 66%, and a cut-off value of 5.6.

Figure 2. The ROC curves of TEP lncRNA MALAT-1 in CRC.

Table 1. Oligonucleotide sequences used as specific primers for lncRNAs and internal control

LncRNA/Gene	Primer sequence (5' ⟶ 3')
MALAT-1	Forward: AAAGCAAGGTCTCCCCACAA Reverse: GGTCTGTGCTAGATCAAAAGGCA
ROR	Forward: CATTTTCCATCCTGCTGTTCAGAGT Reverse: GGCTCTTTCTCTCCTGTGGTTTCAT
β-Actin	Forward: AGAGCTACGAGCTGCCTGAC Reverse: AGCACTGTGTTGGCGTACAG

lncRNAs: long non-coding RNAs; MALAT-1: metastasis-associated lung adenocarcinoma transcript 1; ROR: regulator of reprogramming

Table 2. The ROC curve values of TEP lncRNAs

Variables	AUC	95% CI	P Value
Lnc-MALAT-1	0.66	0.52-0.80	0.044
Lnc-ROR	0.52	0.37-0.67	0.672

Lnc-ROR: long non-coding regulator of reprogramming; TEP: tumor-educated platelet; Lnc-MALAT-1: long non-coding metastasis-associated lung adenocarcinoma transcript 1

Table 3. Diagnostic value of TEP lncRNAs for patients with CRC

lncRNA	Cut off (fold change)	Sensitivity (%)	95%CI	Specificity (%)
Lnc-MALAT-1	5.6	63	0.52-0.80	66
Lnc-ROR	1.45	56	0.37-0.67	54

Lnc-ROR: long non-coding regulator of reprogramming; TEP: tumor-educated platelet; Lnc-MALAT-1: long non-coding metastasis-associated lung adenocarcinoma transcript 1; CRC: colorectal cancer

DISCUSSION

LncRNAs frequently play a role in the initiation and spread of cancer, and their dysregulation may serve as valuable clinical indicators (21). Researchers are increasingly studying nucleic acids and circulating tumor-derived proteins as suitable molecular sources for biomarker identification (22,23). Numerous studies have validated that circulating lncRNAs can function as prognostic and diagnostic biomarkers for various malignancies, including CRC (11,21,24,25). It has been revealed that platelet cells are an appropriate repository of circulating nucleic acids, including RNAs, offering a popular blood-based biopsy for the identification of cancer biomarkers (25). When cells interact with tumor cells, transcriptional patterns and RNA splicing may change. This could cause TEPs to form (18,25). Consequently, TEPs incorporate nucleic acid components derived from an altered RNA pattern, which could potentially differentiate patients with cancer from healthy individuals. In this context, dysregulated TEP nucleic acids have been shown to have diagnostic and prognostic significance in cancers (11). A recent study revealed an elevated expression of TIMP metallopeptidase inhibitor 1 (TIMP1) in TEPs of patients with CRC compared to healthy individuals. This finding suggests that the platelets of patients with cancer have an altered RNA signature (25).

In the present study, we examined the expression levels of two oncogenic TEP lncRNAs as clinical biomarkers in patients with CRC. We found that platelets from patients with CRC had significantly higher MALAT-1 lncRNA expression (P=0.044) than those from healthy individuals. There was also an interesting association between TEP lncRNA MALAT-1 and clinicopathological features, such as lymphovascular invasion and tumor differentiation. We assessed the diagnostic value of TEP lncRNA MALAT-1 using ROC analysis, yielding an AUC of 0.66, 63% sensitivity, and 66% specificity. The analysis suggested that TEP lncRNA MALAT-1 has clinical promise as a diagnostic marker for patients with CRC.

lncRNA MALAT-1 is a major biological effector in the development of CRC. Several investigations have revealed that MALAT1 is often overexpressed in a variety of human

malignancies. According to functional investigations, this lncRNA may promote the growth and invasion of tumor cells (14, 26). Furthermore, several investigations have established its noticeable overexpression in patients with CRC, exploring it as a potential prognostic and diagnostic biomarker (27, 28). Additionally, the overexpression of lnc-MALAT-1 has been investigated in clinical blood samples from patients with CRC (29). In this regard, a substantial correlation between MALAT-1 expression levels and tumor clinical stage and metastasis has been reported. Furthermore, findings suggested a correlation between MALAT-1 overexpression and low survival rates for patients with CRC (28). Our results showed that TEP lncRNA MALAT-1 expression was significantly higher in patients with CRC than in healthy individuals. Additionally, there was a significant correlation among lymphovascular invasion, tumor differentiation, and the expression levels of the TEP lncRNA MALAT-1.

In conclusion, our results indicated that platelets from patients with CRC may have elevated levels of the TEP lncRNA MALAT-1. The dysregulation of TEP lncRNA MALAT-1 is associated with clinicopathological characteristics and has the potential to serve as an effective tool in clinical settings, including cancer diagnosis. Our findings could support bigger clinical sample sizes for functional analyses and more thorough clinical research on CRC. In this regard, conducting wide-ranging clinical studies in patients with CRC can help elucidate the diagnostic relevance of TEP lncRNAs.

DECLARATIONS

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The studies involving human participants were reviewed and approved by the Ethics Committee of Iran University of Medical Sciences (Ethical code: IR.IUMS.REC.1399.1389). Written informed consent to participate in this study was provided by the participants. All methods were carried out in accordance with the Declaration of Helsinki.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this study are available within the article.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest related to this work.

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AUTHOR'S CONTRIBUTION

M.T., A.A., and SP.T. contributed to study conceptualization, data curation, and project administration. A.A. and SP.T. completed funding acquisition and resources. SNB and SBA. contributed to methodology, data curation, and formal data analysis. M.M., S.A., and A.N. contributed to clinical investigation, supervision, validation, and visualization. A.A. and Z.SE. and P.A. prepared the original draft or critical revision for important intellectual content. All authors approved the final draft.

REFERENCES:

1. Shi J, Zhang W, Tian H, Zhang Q, Men T. lncRNA ROR promotes the proliferation of renal cancer and is negatively associated with favorable prognosis. *Mol Med Rep*. 2017;16(6):9561-6.
2. Siegel RL, Miller KD, Goding Sauer A, Fedewa SA, Butterly LF, Anderson JC, et al. Colorectal cancer statistics, 2020. *CA Cancer J. Clin*. 2020;70(3):145-64.
3. Eshkiki ZS, Agah S, Tabaeian SP, Sedaghat M, Dana F, Talebi A, Akbari A. Neoantigens and their clinical applications in human gastrointestinal cancers. *World J Surg Oncol.* 2022 Sep 29;20(1):321.
4. Ladabaum U, Dominitz JA, Kahi C, Schoen RE. Strategies for colorectal cancer screening. *Gastroenterology*. 2020;158(2):418-32.
5. Vega P, Valentin F, Cubiella J. Colorectal cancer diagnosis: Pitfalls and opportunities. *World J Gastrointest Oncol*. 2015;7(12):422.
6. Marcuello M, Vymetalkova V, Neves RP, Duran-Sanchon S, Vedeld HM, Tham E, et al. Circulating biomarkers for early detection and clinical management of colorectal cancer. *Mol Aspects Med*. 2019;69:107-22.
7. Fleischhacker M, Schmidt B. Circulating nucleic acids (CNAs) and cancer—a survey. *Biochimica et Biophysica Acta*. 2007;1775(1):181-232.
8. Schlesinger M. Role of platelets and platelet receptors in cancer metastasis. *J Hematol Oncol*. 2018;11(1):1-15.
9. Bambace N, Holmes C. The platelet contribution to cancer progression. *J Thromb Haemost*. 2011;9(2):237-49.
10. Stegner D, Dütting S, Nieswandt B. Mechanistic explanation for platelet contribution to cancer metastasis. *Thromb Res*. 2014;133:S149-S57.
11. Ye B, Li F, Chen M, Weng Y, Qi C, Xie Y, et al. A panel of platelet-associated circulating long non-coding RNAs as potential biomarkers for colorectal cancer. *Genomics*.

2022;114(1):31-7.
12. Sol N, Wurdinger T. Platelet RNA signatures for the detection of cancer. *Cancer Metastasis Rev*. 2017;36:263-72.
13. Pei C, Gong X, Zhang Y. LncRNA MALAT-1 promotes growth and metastasis of epithelial ovarian cancer via sponging microrna-22. *Am J Trans Res*. 2020;12(11):6977.
14. Fu S, Wang Y, Li H, Chen L, Liu Q. Regulatory networks of LncRNA MALAT-1 in cancer. *Cancer Manag Res*. 2020:10181-98.
15. Xu C, Yang M, Tian J, Wang X, Li Z. MALAT-1: a long non-coding RNA and its important 3'end functional motif in colorectal cancer metastasis. *Int J Oncol*. 2011;39(1):169-75.
16. Wang J, Pan Y, Wu J, Zhang C, Huang Y, Zhao R, et al. The association between abnormal long Noncoding RNA MALAT-1 expression and cancer lymph node metastasis: A meta-analysis. *Biomed Res Int*. 2016;2016.
17. Yang P, Yang Y, An W, Xu J, Zhang G, Jie J, et al. The long noncoding RNA-ROR promotes the resistance of radiotherapy for human colorectal cancer cells by targeting the p53/miR-145 pathway. *J Gastroenterol Hepatol*. 2017;32(4):837-45.
18. Wurdinger T, In 't Veld SG, Best MG. Platelet RNA as pan-tumor biomarker for cancer detection. *Cancer Res*. 2020;80(7):1371-3.
19. Best MG, Wesseling P, Wurdinger T. Tumor-educated platelets as a noninvasive biomarker source for cancer detection and progression monitoring. *Cancer Res.* 2018;78(13):3407-12.
20. Li X, Liu L, Song X, Wang K, Niu L, Xie L, et al. TEP linc-GTF2H2-1, RP3-466P17. 2, and lnc-ST8SIA4-12 as novel biomarkers for lung cancer diagnosis and progression prediction. *J Cancer Res Clin Oncol*. 2021;147:1609-22.
21. Dastmalchi N, Safaralizadeh R, Nargesi MM. LncRNAs: potential novel prognostic and diagnostic biomarkers in colorectal cancer. *Curr Med Chem*. 2020;27(30):5067-77.
22. Danese E, Montagnana M, Lippi G. Circulating molecular biomarkers for screening or early diagnosis of colorectal cancer: which is ready for prime time? *Ann Transl Med*. 2019;7(21).
23. Shokati Eshkiki Z, Mansouri F, Karamzadeh AR, Namazi A, Heydari H, Akhtari J, et al. Chitosan and Its Derivative-Based Nanoparticles in Gastrointestinal Cancers: Molecular Mechanisms of Action and Promising Anticancer Strategies. *J Clin Pharm Ther.* 2024;2024(1):1239661.
24. Sol N, Wurdinger T. Platelet RNA signatures for the detection of cancer. *Cancer Metastasis Rev*. 2017;36(2):263-72.
25. Yang L, Jiang Q, Li D-Z, Zhou X, Yu D-S, Zhong J. TIMP1 mRNA in tumor-educated platelets is diagnostic biomarker for colorectal cancer. *Aging (Albany NY)*. 2019;11(20):8998.
26. Xu Y, Zhang X, Hu X, Zhou W, Zhang P, Zhang J, et al. The effects of lncRNA MALAT1 on proliferation, invasion and migration in colorectal cancer through regulating SOX9. *Mol Med*. 2018;24:1-15.
27. Wang Y, Xue D, Li Y, Pan X, Zhang X, Kuang B, et al. The long noncoding RNA MALAT-1 is a novel biomarker in various cancers: a meta-analysis based on the GEO database and literature. *J Cancer.* 2016;7(8):991.
28. Zheng H-T, Shi D-B, Wang Y-W, Li X-X, Xu Y, Tripathi P, et al. High expression of lncRNA MALAT1 suggests a biomarker of poor prognosis in colorectal cancer. *Int J Clin Exp Pathol*. 2014;7(6):3174.
29. Galamb O, Barták BK, Kalmár A, Nagy ZB, Szigeti KA, Tulassay Z, et al. Diagnostic and prognostic potential of tissue and circulating long non-coding RNAs in colorectal tumors. *World J Gastroenterol*. 2019;25(34):5026.