A prognostic five immune-related long noncoding RNA signature for patients with colon cancer

Kankan Zhao

Zhujiang Hospital

Mengchuan Wang

Zhujiang Hospital

Houlong Kang

Zhujiang Hospital

Aiguo Wu (✉ wagtyz@sina.com)

Zhujiang Hospital <https://orcid.org/0000-0002-6793-8779>

Primary research

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Abstract

Background: Our study aimed to identify immune related long non-coding RNAs (LncRNAs) to serve as potential prognostic indicators and immune therapeutic targets in patients with colon cancer.

Methods: The Cancer Genome Atlas (TCGA) and Molecular Signatures Databases (MSigDB) database were used to identify immune related lncRNAs in patients with colon cancer. The least absolute shrinkage and selection operator (LASSO) analysis, and multivariate Cox proportional hazards regression analysis were employed to screen prognostic lncRNAs and construct immune-related multi-lncRNA signature. We used time-dependent receiver operating characteristic curve to assess the performance of this signature in colon cancer by calculating the area under the curve (AUC). Univariate and multivariate Cox regression analysis were performed to verify the independence of the prognostic value of this signature in colon cancer.

Results: Five immune related lncRNAs (AC025575.2, AL161729.4, ELFN1-AS1, LINC00513, MIR210HG) were found to be significantly associated with overall survival (OS) of patients with colon cancer. Then, we developed a five immune-related lncRNA signature. According to this signature, patients were ranked into a high risk group (n = 208) and a low risk group (n = 209). Kaplan-Meier curve and log-rank method showed that patients in high risk group had worse OS than patients in low risk group ($P = 5.5644 \times 10^{-5}$). AUC for predicting 3 year survival and 5 year survival was 0.776 and 0.762 respectively, which indicated good performance of this signature. Finally, this five immune-related lncRNA signature was demonstrated to be independently associated with prognosis of patients with colon cancer.

Conclusion: We developed a five immune-related lncRNA signature as a prognostic biomarker for patients with colon cancer.

Introduction

Colon cancer ranks fourth in terms of incidence among all cancers according to statistics from International Agency for Research on Cancer (IARC) [1]. Despite advancement in diagnosis and treatment methods, the prognosis is still unsatisfactory. Tumor microenvironment (TME), one of the hallmarks of cancer, is defined as the cellular and physical environment surrounding the primary cancer [2]. The imbalance of immune status in TME plays an important role in the development and progression of cancer. Due to molecular alterations in the tumor cells and immune cells which are responsible for tumor cell recognition and killing, TME may exhibits immunosuppressive characteristics. Tumor immunosuppression may assist the immune escape of cancer cells, resulting in tumor progression [3, 4]. Immunotherapy targeted tumor immunosuppression mainly focus on blocking immune checkpoint receptors, enhancing T cell recognition and neutralizing inhibitory molecules. However, immunological nonresponse and resistance are major obstacles to tumor eradication. Thus, it is essential to find novel biomarkers as potential prognostic indicators and immune therapeutic targets.

Long noncoding RNAs (LncRNAs), a class of noncoding RNAs longer than 200 nucleotides, can bind to chromatin, DNA sequences, RNA and protein, which makes them versatile in regulating gene expression at transcriptional, post-transcriptional and epigenetic levels [5, 6]. Dysregulated lncRNAs have been reported to be implicated in a variety of cancer types including colon cancer [7]. Many studies reported that lncRNAs are key regulators in the tumor microenvironment, epithelial-mesenchymal transition (EMT), microbiota, metabolism, immune cell differentiation and immune system function in cancer [8–10]. Therefore, we aim to screen potential and valuable immune related lncRNAs to serve as potential prognostic indicators and immune therapeutic targets.

In the present study, we identified immune related lncRNAs in patients with colon cancer by applying The Cancer Genome Atlas (TCGA) and Molecular Signatures Databases (MSigDB). In addition, we developed a novel five immune-related lncRNA signature that could predict prognosis of patients with colon cancer. We also demonstrated that the five immune-related lncRNA signature was independently associated with overall survival (OS) of patients with colon cancer. Our analysis suggests potential roles of lncRNAs in colon cancer pathogenesis and progression.

Materials And Methods

The gene expression quantification data (HTSeq-FPKM) and corresponding clinic data of patients with colon cancer were retrieved from the TCGA colon adenocarcinoma (COAD) project (<http://cancer.genome.nih.gov>). We excluded patients without lncRNA sequence data and patients having a survival time of 0 day. The immune related mRNAs were downloaded from the MSigDB (<http://www.broadinstitute.org/gsea/msigdb/index.jsp>, version 7.0) [11]. To identify immune related lncRNAs in colon cancer, we performed Pearson correlation analysis for each of the immune related mRNAs with lncRNAs based on the sequence data of the TCGA COAD project. The lncRNAs with $|\text{Pearson coefficient}| > 0.7$ were considered to be immune related lncRNAs.

Identification of immune related lncRNA signature in colon cancer

These immune related lncRNAs entered into the least absolute shrinkage and selection operator (LASSO) method analysis to select the best candidates by employing glmnet package in R software (version 3.6.1) [12]. Then, we conducted multivariate Cox proportional hazards regression analysis to develop the immune-related multi-lncRNA signature. Based on this immune-related lncRNA signature, we calculated the risk score for each patient by integrating the lncRNAs expression profiles and relative multivariate Cox regression coefficient. Using the median risk score, patients were divided into a high risk group and low risk group. The Kaplan-Meier curve and log-rank method were performed to evaluate the OS difference between the high-risk group and low-risk group. Distribution of risk scores, survival status of patients with colon cancer, and expression profiles of prognostic lncRNAs were exhibited using R software. We used time-dependent receiver operating characteristic (ROC) curve to assess the sensitivity and specificity of the immune-related lncRNA signature by calculating the area under the curve (AUC) [13,14].

Independence of multi-lncRNA signature for predicting prognosis of patients with colon cancer

To verify the independence of the prognostic value of the immune-related lncRNA signature based risk score and clinicopathological factors (including age, sex, TNM stage, pathological stage), univariate and multivariate Cox regression analysis were performed to explore their associations with OS of patients with colon cancer. P-value < 0.05 was considered to be statistically significant.

Results

Identification of immune related lncRNAs in colon cancer

A total of 417 patients with baseline data and gene expression quantification data were included. The detailed clinicopathological information was presented in Table 1. Based on the data of MSigDB (Immune system process M13664, Immune response M19817) and expression quantification data of TCGA COAD project, we identified 311 immune related lncRNAs in patients with colon cancer by applying Person correlation analysis (Table S1).

Table 1
Clinicopathological characteristics of
patients with colon cancer

Characteristics	Number of cases (%)
Age	118(28.3)
<60	299(71.7)
≥ 60	
Gender	
Female	193(46.3)
Male	224(53.7)
T stage	
Tis	1(0.2)
T1	9(2.2)
T2	74(17.7)
T3	284(68.1)
T4	49(11.8)
N stage	
N0	247(59.2)
N1	98(23.5)
N2	72(17.3)
M stage	
M0	311(74.6)
M1	57(13.7)
Mx	49(11.8)
Risk score	
Low risk	209(50.1)
High risk	208(49.9)

Identification of immune related lncRNA signature in colon cancer

These immune related lncRNAs were further assessed for their association with prognosis of patients with colon cancer. We identified 19 lncRNAs using LASSO Cox analysis (Figure 1). Then, multivariable Cox proportional hazards regression analysis were performed to identify the optimal immune related

lncRNAs. The results showed that five lncRNAs (AC025575.2, AL161729.4, ELFN1-AS1, LINC00513, MIR210HG) significantly affected OS of patients with colon cancer (Figure 2). We constructed an immune-related signature by integrating the five lncRNAs expression profiles and corresponding Cox regression coefficient. According to this five immune-related lncRNA signature, we calculated the risk score for each patient and ranked them into a high risk group (n = 208) and a low risk group (n = 209) based on the median of risk score (Table S2). The Kaplan-Meier curve showed patients in the high risk group have significantly worse OS than patients in the low risk group ($P = 5.5644 \times 10^{-5}$, Figure 3). Distribution of risk score, survival status of patients with colon cancer, and the expression profiles of the five prognostic lncRNAs were shown in Figure 4. As Figure 4 showed, the mortality was much higher for patients with high risk score than those with low risk score and patients in the high risk group have a tendency to higher expression of AC025575.2, AL161729.4, ELFN1-AS1 and MIR210HG, and lower expression of LINC00513. The prognostic power of the five-lncRNA signature was evaluated by calculating the area under the ROC curve (AUC). The results showed that the AUC of five-lncRNA signature for predicting 3 year survival and 5 year survival was 0.776 and 0.762 respectively, which indicated good performance (Figure 5).

Independence of the five-lncRNA signature with prognosis of patients with colon cancer

The results of univariate Cox regression analysis indicated that the five-lncRNA signature based risk score, age, stage, T stage, N stage, M stage were significantly related to OS of patients with colon cancer (Figure 6A). Multivariate Cox regression analysis showed that age, T stage, and five-lncRNA signature independently related to OS of patients with colon cancer (Figure 6B).

Discussion

In the present study, we identified 311 immune-related lncRNAs in patients with colon cancer. Then, using Lasso analysis and Cox regression, we constructed a five immune-related lncRNA signature with good performance. According to the five immune-related lncRNA signature, patients with colon cancer can be classified into a high risk group and a low risk group with significantly different OS. Finally, this five immune-related lncRNA signature were demonstrated to independently predict the prognosis of patients with colon cancer through univariate and multivariate Cox regression analysis.

Among the five lncRNAs, AC025575.2, and AL161729.4 are novel transcripts. The functions of them are unknown. ELFN1-AS1 is antisense lncRNA. It has been reported that ELFN1-AS1 was highly expressed in many cancer types including lung cancer, uterus cancer, and ovarian cancer [15]. In addition, Dong et al. found that ELFN1-AS1 promoted COAD cell proliferation and migration, and might be a potential target for COAD treatment [16]. Our results are consistent with their findings that ELFN1-AS1 played a tumor promoting role in COAD. LINC00513 is intergenic lncRNA and played important roles in lupus pathogenesis by promoting IFN signaling pathway [17]. However, little is known about the role of LINC00513 in cancer. MIR210HG belongs to microRNA non-coding host gene. Many studies have reported the functions of MIR210HG in various cancer types. Wang et al. found that MIR210HG promoted cervical cancer cell proliferation, invasion and epithelial mesenchymal transition (EMT) through regulating MIR210HG/miR-503-5p/TRAF4 axis [18]. MIR210HG could sponge miR-126-3p to promote

breast cancer cell invasion and metastasis through regulating mucin-1c and EMT pathway [19]. Similarly, MIR210HG could sponge miR-503 to function as oncogenic lncRNA in osteosarcoma [20]. MIR210HG was also demonstrated to facilitate progress of lung cancer [21], glioma [22], and hepatocellular carcinoma [23]. Moreover, two articles using bioinformatics methods identified MIR210HG was associated with poor OS of patients with colon cancer [24, 25], which was in accordance with the results of our study. Our results imply potential functions of the five immune-related lncRNA signature in the development of colon cancer, suggesting them as important biomarkers and therapeutic targets. However, the predicted functions of the five-lncRNA signature could not only be interpreted through this method. Thus, studies remain need to be conducted to validate the biological function of these five lncRNAs and explore additional targets and strategies for treatment. Admittedly, our study has some limitations because it was based on the high-throughput RNA-sequencing profiles and data analysis only. The roles of these five lncRNA deserve further in vitro and in vivo studies. In conclusion, we developed the five immune-related lncRNA signature as a potential prognostic indicator for patients with colon cancer.

Declarations

Ethical approval and informed consent

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

All data and material used to support the findings of this article are included within this article and supplementary information files.

Competing interest

The authors declare that they have no competing interests.

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Author's contributions

KZ and MW performed the research, analyzed data, and drafted the manuscript. HK participated in data analysis. AW participated in research design. All authors read and approved the final manuscript.

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Figures

Figure 1

Selection of candidate lncRNAs through LASSO analysis. (A) LASSO coefficient profiles of differentially expressed lncRNAs. (B) “Leave-one-out-cross-validation” for parameter selection in the LASSO model. LASSO, least absolute shrinkage and selection operator.

Figure 2

Forest plot of the five immune related lncRNAs for affecting the prognosis of patients with colon cancer according to the results of multivariable Cox proportional hazards regression analysis.

Survival curve of risk score(P=5.564e-05)

Figure 3

Kaplan-Meier plots of OS among patients in the high risk group and low risk group according to the five immune-related lncRNA signature. OS, overall survival.

Figure 4

Risk score distribution, survival status and expression profiles of the five lncRNAs among patients in the high risk group and low risk group according to the five immune-related lncRNA signature.

Figure 5

ROC curve of the five immune related lncRNA signature for predicting 3 year survival and 5 year survival of patients with colon cancer. ROC, receiver operating characteristic.

Figure 6

Forest plots of the five immune related lncRNA signature and clinicopathological factors for predicting prognosis of patients with colon cancer. (A) Univariate Cox proportional hazards regression analysis. (B) Multivariate Cox proportional hazards regression analysis.

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