Identification of prognostic immune-related lncRNA signature predicting the overall survival for colorectal cancer

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Abstract

Background

Long non-coding RNA (lncRNA) is an important regulator of gene expression and serves fundamental role in immune regulation. The present study aimed to develop a novel immune-related lncRNA signature to accurately assess the prognosis of patients with colorectal cancer (CRC).

Methods

Transcriptome data and clinical information of patients with CRC were downloaded from The Cancer Genome Atlas (TCGA), and the immune-related mRNAs were extracted from immunomodulatory gene datasets IMMUNE RESPONSE and IMMUNE SYSTEM PROCESS based on the Molecular Signatures Database (MSigDB). Then, the immune-related lncRNAs were identified by a correlation analysis between immune-related mRNAs and lncRNAs. Subsequently, univariate, lasso and multivariate Cox regression were used to identify an immune-related lncRNA signature in training cohort, and the predict ability of the signature was further confirmed in the testing cohort and the entire TCGA cohort. Finally, the lncRNA-mRNA co-expression network was established to explore the biological role of the immune-related lncRNA signature.

Results

In total, 272 Immune-related lncRNAs were identified, five of which were applied to construct an immune-related lncRNA signature based on univariate, lasso and multivariate Cox regression analyses. The signature divided patients with CRC into low- and high-risk groups, and patients with CRC in high-risk group had poorer overall survival than those in low-risk group. Univariate and multivariate Cox regression analyses confirmed that the signature could be an independent prognostic factor in human CRC. Furthermore, functional enrichment analysis revealed that the immune-related lncRNA signature was significantly enriched in immune process and tumor classical pathways.

Conclusions

The present study revealed that the novel immune-related lncRNA signature could be exploited as underlying molecular biomarkers and therapeutic targets for the patients with CRC.

1. Introduction

Colorectal cancer (CRC) is among the worst malignancies in digestive system and ranks second in terms of cancer-related death [1]. Every year, there were more than 1.8 and 0.9 million estimated new CRC cases and deaths worldwide [2]. Recently, with the development of research, the pathophysiological and

molecular mechanisms underlying the onset, invasion and metastasis of CRC has been extensively explored, and significant progress has been made in the diagnosis and treatment of CRC. However, due to the deficiency of effective early diagnostic and therapeutic biomarkers, the prognosis of patients with CRC is still poor. The 5-year survival rate for patients diagnosed with early stage is about 90%, while the 5-year survival rate sharply declines to 12% for patients diagnosed with advanced stage [3]. Considering the current situation of the diagnosis and treatment of CRC, it is imperative to seek more effective assessment models to evaluate the prognosis of CRC patients and provide optimized strategies for clinical CRC treatment.

In the past few years, accumulating studies have indicated that disorder of the immune response in the tumor microenvironment (TME) was significantly correlated with the development of cancer [4]. Immune cells are able to suppress the proliferation, invasion, and metastasis of cancer cells in the TME by perturbing the molecular signal pathway [5]. Nevertheless, some cancer cells also can inhibit the immune responses and create the environments suitable for tumor growth, while these effects could be reversed by immunotherapy [6]. Therefore, immunotherapy provides the unprecedented opportunity to effective treatment of cancers because of the important regulatory role of the immune cells in the TME [7]. Long non-coding RNAs (lncRNAs) are defined as transcripts with nucleotide more than 200 in length and have no ability to encode protein [8]. Previous studies have indicated that lncRNA can regulate gene expression at multiple levels, such as epigenetic, transcriptional or post-transcriptional regulation, and dysfunction of lncRNA is closely associated with the progression of multiple diseases, including malignancies [9–11]. Moreover, recent studies also indicate that lncRNA play a significant regulatory role in different phases of tumor immunity [12]. Hence, immune-related lncRNA deserves widely attention.

In the present work, we firstly screened the immune-related lncRNAs based on the co-expression network of the immune-related mRNAs and lncRNAs. Subsequently, we constructed and validated a reliable signature for the prediction of prognosis in patients with CRC. Finally, we investigated the possible biological function of the prediction signature. The immune-related lncRNA signature may serve as potential predictive indicator for CRC survival, but also can be utilized as promising immunotherapeutic targets.

2. Materials And Methods

2.1 Data Acquisition and Processing

The entire RNA-sequencing profile data of COAD and READ in the format of Fragments Per Kilobase Million (FPKM) were downloaded from The Cancer Genome

Atlas (TCGA, <https://gdc.cancer.gov/>). Then, the protein-coding genes and lncRNAs were classified by using gene annotation function of the GENCODE project (<https://www.gencodegenes.org/>) [13]. In addition, the patient's complete clinical information, including survival time, survival status, age, gender, TNM stage, AJCC stage were also downloaded from TCGA. The patients with incomplete clinical data

were excluded. There is no need for approval by the ethics committee because of all the data involved in the present study were obtained from the public database.

2.2 Identification of Immune-related lncRNAs

The Immune-related mRNAs were extracted from immunomodulatory gene datasets IMMUNE RESPONSE (M19817) and IMMUNE SYSTEM PROCESS (M13664) based on the Molecular Signatures Database (MSigDB, <https://www.gsea-msigdb.org/gsea/msigdb/>) [14]. Then, the correlation between lncRNAs and Immune-related mRNAs was calculated by using the Pearson correlation. lncRNAs with Pearson correlation coefficient > 0.5 and $P < 0.001$, and interacted with more than two Immune-related mRNA were identified as Immune-related lncRNAs and selected for further analysis. In addition, to enhance the reliability of our study, the entire expression cohort of Immune-related lncRNAs was randomly divided into the training cohort and the testing cohort by the “caret” package in R.

2.3 Construction of an Immune-related lncRNA Signature Associated with Prognosis

To identify the survival-related lncRNAs, the prognostic significance of Immune-related lncRNAs were analyzed by univariate Cox regression. Those Immune-related lncRNAs with $P < 0.01$ in univariate analysis were included in the LASSO regression analysis to minimize the over-fitting. Then, we conducted a multivariate Cox analysis to construct the risk signature calculated as: Risk score = $\sum (\beta_i \times \text{Expi})$ [15]. Where the β_i and Expi represented the coefficient index and the gene expression level, respectively. Patients were divided into high- and low-risk groups according to the median value of the risk score.

2.4 Prediction Analysis of Risk Score Signature

To evaluate the prognostic value of the Immune-related lncRNA signature in human CRC, we depicted the survival curve between high- and low-risk groups by using the Kaplan-Meier method with log-rank test. Additionally, we applied time-dependent receiver operating characteristic (ROC) curve and area under the ROC curve (AUC) to estimate the diagnostic efficacies. Similarly, we evaluate the prognostic value of the Immune-related lncRNA signature in the testing cohort and entire TCGA cohort to verify the reliability of the signature. To further confirm the clinical value of the Immune-related lncRNA signature, we assessed the associations between the signature and clinical parameters. Finally, we compared the prognostic value of clinical parameters and risk score to determine whether the signature can be used as an independent prognostic factor.

2.5 Construction of Predictive Nomogram

By using “rms”, “foreign”, and “survival” packages in R, we constructed a nomogram consisting of relevant clinical parameters and risk score based on the multivariate Cox regression analysis to predict survival for the patients with CRC. The index of concordance (C-index), calibration curves, and ROC curves were applied to investigate the calibration and the discrimination of the nomogram [16].

2.6 Co-expression Analysis and Enrichment Analyses

To further investigate the biological function of the Immune-related lncRNA signature, we constructed a co-expressed lncRNA-mRNA network by using Pearson correlation based on the correlation coefficient threshold > 0.5 , and the corresponding $P < 0.001$. After that, we used the “clusterProfiler” package in R to carry out Gene Ontology (GO) enrichment analysis, including biological process (BP), cell components (CC) and molecular function (MF) for the co-expressed network. The Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis is also carried out by the same tool.

2.7 Statistical analysis

The statistical analyses were performed via the R software (v 4.0.2). The correlations of gene expression were evaluated by Pearson’s correlation coefficient. Cox regression and Lasso regression were used to evaluate the prognostic significance of the Immune-related lncRNA signature and clinicopathological data. Chi-square test was used to evaluate the differences of clinicopathological features between two groups of patients. Kaplan-Meier curves and log-rank tests were utilized to evaluate the survival data. The predictive accuracy of the risk model was determined by ROC curves. Wilcoxon test was mainly utilized for comparing two groups, and Kruskal-Wallis test was used for two or more groups. Fisher’s exact test was applied to evaluate the significant GO terms and KEGG enrichment pathways. P -value < 0.05 was considered as statistically significant.

3. Results

3.1 Identification of Immune-related lncRNAs

In total, 14,142 lncRNAs and their expression profiles were obtained from the TCGA database, and the list of 331 Immune-related mRNAs was identified from the MSigDB. Then, 272 Immune-related lncRNAs were screened according to the pre-defined threshold. The entire expression profile of the Immune-related lncRNAs was randomly divided into the training cohort and the testing cohort by using the “caret” package in R. As shown in Table 1, there was no significant difference in clinical parameters between the training cohort and the testing cohort. Therefore, the expression profiles of the 272 Immune-related lncRNAs in the training cohort were selected for subsequent study.

Table 1
Clinical features of the patients with CRC in each cohort.

Variables	Training cohort (n = 253)	Testing cohort (n = 253)	<i>P</i>	Entire TCGA cohort (n = 506)
Age (years)				
≤ 65	120	105	0.2104	225
> 65	133	148		281
Gender				
Female	105	125	0.0898	230
Male	148	128		276
Survival status				
Alive	209	206	0.8169	415
Dead	44	47		91
Tumor invasion (T)				
T1	7	7	0.8897	14
T2	45	46		91
T3	174	170		344
T4	27	29		56
Unknow	0	1		1
Lymph node (N)				
N0	150	145	0.6741	295
N1	63	61		124
N2	40	46		86
Unknow	0	1		1
Metastasis (M)				
M0	214	213	0.2475	427
M1	38	35		73
Unknow	1	5		6
Tumor stage				
Stage I	44	46	0.5482	90

Variables	Training cohort (n = 253)	Testing cohort (n = 253)	<i>P</i>	Entire TCGA cohort (n = 506)
Stage II	100	88		188
Stage III	66	74		140
Stage IV	38	35		73
Unknow	5	10		15

3.2 Construction of the Immune-related lncRNA Signature

We carried out univariate Cox regression model to assess the prognostic significance of the 272 Immune-related lncRNAs in the training cohort and found that 13 lncRNAs were significantly associated with the overall survival (OS) of CRC patients ($P < 0.01$, Fig. 1A). Subsequently, we performed Lasso regression with a 10-fold cross-validation on these lncRNAs in order to avoid over-fitting the prognostic signature. As a result, 10 genes were remained from 13 significant prognosis related lncRNAs in univariate Cox regression model by using Lasso regression model (Fig. 1B&C). Then, a total of five lncRNAs were identified as independent prognostic factors by Multivariate Cox regression model, and its regression coefficients (β) were also determined for further analysis (Fig. 1D and Table 2). Finally, based on the expression levels of these five lncRNAs, the risk score of each patient was calculated as follow: riskScore = $(0.22928 \times \text{Exp}_{HCG11}) + (0.52785 \times \text{Exp}_{MIR181A2HG}) + (0.46034 \times \text{Exp}_{NIFK-AS1}) + (0.05573 \times \text{Exp}_{SNHG7}) + (0.65167 \times \text{Exp}_{ZEB1-AS1})$.

Table 2
Multivariate Cox results of lncRNAs based on TCGA data.

lncRNA	Coefficient (β)	HR	95% CI of HR
HCG11	0.22928	1.258	1.058–1.494
MIR181A2HG	0.52785	1.695	1.059–2.712
NIFK-AS1	0.46033	1.585	0.932–2.693
SNHG7	0.05573	1.057	1.015–1.102
ZEB1-AS1	0.65167	1.919	1.111–3.314
HR, Hazard Ratio; CI, Confidence Interval.			

3.3 The Prognostic Influence of the Established Signature in TCGA

The CRC patients were divided into low-risk group and high-risk group according to the median risk score. We assessed the predictive performance of the five-lncRNA immune signature by time-dependent ROC curves and found that the AUC for 1-, 3-, and 5-year OS were 0.790, 0.799, and 0.760, respectively (Fig. 2A). Survival status analysis showed that the patient mortality rate increased with the increase of

risk score (Fig. 2B). The Kaplan-Meier log-rank test revealed that patients in the high-risk group suffered shorter survival time than those in the low-risk group ($P < 0.05$, Fig. 2C). Then, we applied the testing cohort to validate the prognostic ability of the five-lncRNAs immune signature. Time-dependent ROC curves showed that the AUC for 1-, 3-, and 5-year OS were 0.651, 0.633, and 0.690, respectively (Fig. 2D). Survival status analysis showed that the patient mortality rate increased with the increase of risk score (Fig. 2E). Kaplan-Meier curves showed that patients in the high-risk group suffered shorter survival time than those in the low-risk group ($P < 0.05$, Fig. 2F). Similarly, we verified the prognostic ability of this Immune-related lncRNA signature in the entire TCGA cohort. Time-dependent ROC curves showed that the AUC for 1-, 3-, and 5-year OS were 0.710, 0.716, and 0.799, respectively (Fig. 2G). Survival status analysis showed that the patient mortality rate increased with the increase of risk score (Fig. 2H). Kaplan-Meier curves showed that patients in the high-risk group suffered shorter survival time than those in the low-risk group ($P < 0.05$, Fig. 2I). These findings above suggested that the Immune-related lncRNA signature was competent for predicting the prognosis of CRC patients.

3.4 Clinical Values of the Immune-related lncRNA Signature

We assessed the association between the signature and clinical parameters to further confirm the clinical values of the Immune-related signature. As a result, the risk scores roughly increased with increasing T stage (Fig. 3A), N stage (Fig. 3B), M stage (Fig. 3C), and American Joint Committee on Cancer (AJCC) tumor stage (Fig. 3D). These findings revealed that this Immune-related lncRNA signature may be associated with the metastasis and progression of human CRC. Then, we applied univariate and multivariate Cox regression model to determine whether the signature can be used as an independent prognostic factor in CRC. The hazard ratio (HR) of risk score and 95% CI were 1.205 and 1.135–1.280 in univariate Cox regression analysis ($P < 0.001$), and 1.189 and 1.109–1.274 in multivariate Cox regression analysis ($P < 0.001$), respectively. This Immune-related lncRNA signature could serve as prognostic factor for CRC independent of clinicopathological factors.

3.5 Construction of the Prognostic Nomogram

To better predict the prognosis of patients with CRC, we constructed a nomogram by integrating risk score and other clinicopathologic risk factors and found that risk score and TNM stage were the largest risk factors for OS of CRC patients (Fig. 4A). The AUC of OS rate showed that risk score (0.778) and stage (0.769) had a certain prediction ability (Fig. 4B). In addition, the calibration plot showed a satisfactory agreement between predictive and actual values at the probabilities of 3- and 5-year survival (Fig. 4C). These findings indicated that the nomogram held good accuracy in predicting the survival of CRC patients.

3.6 lncRNA-mRNA Co-expression Network Analysis

Based on the correlation coefficient threshold > 0.5 , and the corresponding $P < 0.001$, we found that these five Immune-related lncRNAs had obvious correlation with 24 mRNAs, and the lncRNA-mRNA co-expression relationship network was constructed and visualized by using Cytoscape software (Fig. 5A).

Subsequently, GO enrichment analysis showed that the mRNAs co-expressed with the Immune-related lncRNA signature mainly enriched in immunomodulatory pathways, such as myeloid cell differentiation, regulation of hemopoiesis, regulation of leukocyte differentiation (Fig. 5B). KEGG pathway enrichment analysis showed these mRNAs mainly enriched in NF- κ B signaling pathway, RIG-I-like receptor signaling pathway, IL-17 signaling pathway, and TNF signaling pathway (Fig. 5C).

Discussion

Colorectal cancer, which causes severe health burden worldwide, is the most frequently diagnosed malignant tumor of the digestive system. Although surgical resection has been proven to be the only curable therapy for CRC patients diagnosed at localized stage, the prognosis of patients diagnosed at advanced stage remains largely unsatisfactory [17]. Accumulating studies have indicated that dysregulation of immune system involved in the initial and progression of malignant tumor and may be one of the factors affecting the poor outcome of cancer [18][19]. In recent years, with the development of molecular biology and a greater understanding of mechanisms underlying the immune system, effective anti-tumor immunotherapy is now a reality [20]. Emerging immunotherapy methods such as immune checkpoint inhibitors (ICIs) have developed rapidly in the treatment of tumor and achieved a certain therapeutic effect [21]. It is now well-established that patients with mismatch-repair-deficient (dMMR): microsatellite instability-high (MSI-H) tumors tend to be more sensitive to anti-tumor immunotherapy methods such as ICIs [22]. However, for the vast majority (~ 85%) of CRC patients whose tumors are MMR-proficient (pMMR): microsatellite stable (MSS), immunotherapy currently provides little benefit [22] [23]. Therefore, identifying novel biomarkers for immunotherapy is needed.

Previous studies have revealed that lncRNA serves as an important regulator of the immune response in multiple types cancers. For example, lncRNA *SATB2-AS1* has been reported to regulate TH1-type chemokines expression and immune cell density in CRC, thereby inhibiting tumor metastasis [24]. A recent study reported that lncRNA *MALAT1* promoted tumorigenesis and immune escape of diffuse large B cell lymphoma by sponging miR-195 to induce apoptosis of CD8 + T cells [25]. *GATA3-AS1* prevented the PD-L1 protein against the ubiquitination through *miR-676-3p/COPS5* axis, thereby facilitating immune escape in triple-negative breast cancer [26]. These findings suggested that immune-related lncRNAs may be better prognostic biomarkers and serve as potential targets for anti-cancer therapy.

In this study, a total of 272 immune-related lncRNAs were identified through constructing a co-expression network of immune-related genes and lncRNAs. Then, a five immune-related lncRNAs signature comprised of *HCG11*, *MIR181A2HG*, *NIFK-AS1*, *SNHG7*, and *ZEB1-AS1* was constructed based on univariate Cox regression, lasso regression, and multivariate Cox regression analyses. Subsequently, survival analysis revealed that the immune-related lncRNAs signature could distinguish patients with favorable prognosis from those with poor prognosis, and the signature could accurately predict the prognosis of the patients at 1 year (AUC = 0.790), 3 years (AUC = 0.799), and 5 years (AUC = 0.760). In addition, univariate Cox regression and multivariate Cox regression analysis revealed that the signature is an independent prognostic factor for CRC. Among these five candidate lncRNAs, *SNHG7* has been

reported to be expressed at high levels in CRC samples compared to normal control [27]. *SNHG7* promoted proliferation, metastasis, and inhibited apoptosis of CRC cells via sponging *miR-34a* to regulate PI3K/Akt/mTOR pathway [28]. It was reported that *ZEB1-AS1* expression was significantly higher in CRC tissues than in adjacent normal tissues, and patients with high *ZEB1-AS1* expression showed poorer prognosis than those with low expression [29]. In addition, *ZEB1-AS1* activated the Wnt/ β -catenin signaling pathway by sponging miR-181-5p, thereby promoting proliferation of CRC cells *in vitro* [30]. The roles of *HCG11*, *NIFK-AS1*, and *MIR181A2HG* in human CRC had not been previously reported. LncRNA *HCG11* has been experimentally demonstrated to facilitate proliferation and migration of gastric cancer cells through *miR-1276/CTNNB1/Wnt* pathway [31]. *NIFK-AS1* was reported to function as sponge for miR-146a to suppress the M2-like polarization of macrophages, thereby inhibiting the estrogen-induced proliferation, migration and invasion of endometrial cancer cells [32]. A recent study has identified *MIR181A2HG* as reliable component of an immune-related signature to predict overall survival of patients with bladder cancer [33]. Although these three lncRNAs have not been studied in human CRC, our results revealed that they are also correlated with the prognosis of patients with CRC. Thus, more experimental studies on their roles in human should be done in the future.

Functional enrichment analysis revealed that the novel immune-related lncRNAs signature was mainly enriched in immunomodulatory pathways. Furthermore, KEGG enrichment analysis also revealed that the novel signature involved in various classical tumor-related pathways, which participate in the initial and progression of CRC, including NF- κ B signaling [34], TNF signaling [35], RIG-I-like receptor signaling [36], IL-17 signaling [37] and JAK/STAT signaling [38]. Therefore, these results above suggested that the novel signature may plays important role in human CRC.

Inevitably, several limitations existed in the present study should be point out. First, the novel immune-related lncRNAs signature was only validated in the TCGA dataset because of data limitations, and external validation will be needed to enhance the reliability of the novel signature. Second, the construction and evaluation of the novel signature depended on the public database, functional experiments should be conducted to further explore the functional role of the novel signature in human CRC.

Conclusions

In summary, the present study constructed a novel immune-related signature which was composed of five immune-related lncRNAs to predict the prognosis of patients with CRC, and found it was an independent prognostic factor for human CRC. The results of this study may provide new insights into the prognostic evaluation of CRC and may provide novel therapeutic targets for clinical treatment of patients with CRC.

Abbreviations

AJCC, American Joint Committee on Cancer; AUC, area under the ROC curve; BP, biological process; CC, cell components; CRC, Colorectal cancer; dMMR, mismatch-repair-deficient; lncRNA, Long non-coding

RNA; TCGA, The Cancer Genome Atlas; MSigDB, Molecular Signatures Database; FPKM, Fragments Per Kilobase Million; ROC, receiver operating characteristic; HR, hazard ratio; C-index, index of concordance; GO, Gene Ontology; MF, molecular function; KEGG, Kyoto Encyclopedia of Genes and Genomes; MSI-H, microsatellite instability-high; pMMR, MMR-proficient; MSS, microsatellite stable.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analyzed during current study are available in The Cancer Genome Atlas (TCGA) repository (<https://www.cancer.gov/tcga>).

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors' contributions

JL, TH and QY conceived and designed the study. JL, TH, YW and XW performed the bioinformatics analysis and interpretation of the data. JL and TH drafted the manuscript. QY agreed to be responsible for all aspects of the work to ensure that issues of accuracy or completeness of the study were properly investigated and addressed. All authors read and approved the final manuscript.

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Figures

Figure 1

Construction of immune-related lncRNAs signature associated with prognosis of patients with CRC. (A) Univariate Cox regression models identified 13 immune-related lncRNAs associated with overall survival. (B & C) Lasso regression analysis identified 10 immune-related lncRNAs. (D) Multivariate Cox regression models identified five immune-related lncRNAs to construct the prognostic signature.

Figure 2

The prognostic value of the immune-related lncRNAs signature. (A) Time-dependent ROC curve analysis, (B) risk score and survival status, and (C) Kaplan–Meier survival curve of the immune-related lncRNA signature in the training cohort. (D) Time-dependent ROC curve analysis, (E) risk score and survival status, and (F) Kaplan–Meier survival curve of the immune-related lncRNA signature in the testing cohort. (G) Time-dependent ROC curve analysis, (H) risk score and survival status, and (I) Kaplan–Meier survival curve of the immune-related lncRNA signature in the entire TCGA cohort.

Figure 3

Clinical evaluation by the riskScore assessment signature. (A) T classification (B) N classification, (C) M classification, and (D) AJCC tumor stage were significantly associated with the riskScore. (E) Univariate Cox regression and (F) multivariate Cox regression analyses revealed that the riskScore serves as an independent prognostic predictor in human CRC.

Figure 4

Construction of the prognostic nomogram. (A) Construction of the nomogram based on multivariate Cox regression models. (B) The ROC curves analysis based on riskScore and the clinicopathologic features. (C & D) The 3- and 5-years calibration plots for evaluating the agreement between the predicted and the actual prognosis for the prognostic signature.

Figure 5

The co-expression network and functional enrichment analysis of the novel immune-related lncRNAs signature. (A) The co-expression network between the immune-related genes and prognostic lncRNAs in CRC. (B) GO functional enrichment and (C) KEGG pathway enrichment analyses of the co-expression network.