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Research article

The global landscape of immune-derived lncRNA signature in colorectal cancer

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ABSTRACT

Background: Colorectal cancer (CRC) is a highly heterogeneous cancer. This heterogeneity has an impact on the efficacy of immunotherapy. Long noncoding RNAs (lncRNAs) have been found to play regulatory functions in cancer immunity. However, the global landscape of immune-derived lncRNA signatures has not yet been explored in colorectal cancer.

Methods: In this study, we applied DESeq2 to identify differentially expressed lncRNAs in colon cancer. Next, we performed an integrative analysis to globally identify immune-driven lncRNA markers in CRC, including immune-associated pathways, tumor immunogenomic features, tumor-infiltrating immune cells, immune checkpoints, microsatellite instability (MSI) and tumor mutation burden (TMB).

Results: We also identified dysregulated lncRNAs, such as LINC01354 and LINC02257, and their clinical relevance in CRC. Our findings revealed that the differentially expressed lncRNAs were closely associated with immune pathways. In addition, we found that RP11-354P11.3 and RP11-545G3.1 had the highest association with the immunogenomic signature. As a result, these signatures could serve as markers to assess immunogenomic activity in CRC. Among the immune cells, resting mast cells and M0 macrophages had the highest association with lncRNAs in CRC. The AC006129.2 gene was significantly associated with several immune checkpoints, for example, programmed cell death protein 1 (PD-1) and B and T lymphocyte attenuator (BTLA). Therefore, the AC006129.2 gene could be targeted to regulate the condition of immune cells or immune checkpoints to enhance the efficacy of immunotherapy in CRC patients. Finally, we

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identified 15 immune-related lncRNA-generated open reading frames (ORFs) corresponding to 15 cancer immune epitopes.

Conclusion: In conclusion, we provided a genome-wide immune-driven lncRNA signature for CRC that might provide new insights into clinical applications and immunotherapy.

1. Introduction

Colorectal cancer (CRC) is the second leading cause of death and the fourth most common cancer occurrence in Western countries [1–4]. Despite advancements in diagnosis and treatment, CRC remains a significant cause of cancer death worldwide. Response rates to nivolumab and pembrolizumab (both PD-1/PD-L1 inhibitors) in colon cancer patients are typically less than 50%, and identifying additional immune predictive biomarkers is urgently needed [5]. Moreover, targeting lncRNAs with immunotherapy and chemotherapy has shown promising results in the treatment of CRC [6–8].

Cancer cells, including CRC cells, tend to have a wide range of transcript variants. These variants can arise from various mechanisms, including fusion transcripts, alternative splicing, and noncoding RNAs, such as lncRNAs and circRNA. The complexity and connections of transcript molecular mechanisms have been extensively studied in various diseases, particularly for those variants linked with lncRNAs [9–11]. LncRNAs have a length of more than 200 nucleotides [12]. The current study of lncRNAs has opened up new perspectives for understanding the pathological process of colon cancer, including tumor emergence and progression. Specifically, studies have found that overexpression of LINC00662 promoted the initiation and progression of colon cancer by competitively binding with miR-340-5p, which is involved in the regulation of CLDN8/IL22 expression [13]. Furthermore, dysregulated FAM83H-AS1 showed negative correlations with Smad1/5/9 in colon cancer specimens, which are functional factors in TGF-β signaling, suggesting that certain lncRNAs function by affecting TGF-β signaling in colon cancer [14]. In addition, highly expressed LINC01234 is related to shorter survival time in colon cancer tissues [15].

Increasing evidence has demonstrated that lncRNAs regulate the tumor immune microenvironment (TIME) by regulating the inflammatory response and immune gene expression [16,17]. TIME has a profound impact on the immunotherapeutic response [18–20]. A variety of immunogenomic signatures have been found to be conducive to predicting immunotherapy response, such as tumor mutation burden (TMB) and microsatellite instability (MSI) [21–25]. Furthermore, through a comprehensive characterization of lncRNAs and immune pathways in various cancer types, a recent study [26] found that lncRNAs can tightly interact with immune-related pathways and infiltrating immune cells in various cancers. Considering the continuous development of immunotherapy, lncRNA immune epitopes are also emerging as a fascinating field in cancer immunotherapy [27]. However, the association between functional lncRNAs and immune immunogenomic signatures in CRC has not been fully characterized.

In this study, we aimed to explore immune-associated lncRNAs and their clinical relevance in CRC. To globally analyze lncRNA-immunogenomic interactions in CRC, we investigated the correlation between immune signatures and lncRNA expression. The immune signatures include immune pathways, immunogenomic signatures, tumor-infiltrating immune cells, immune checkpoints, TMB, and MSI. Moreover, we also evaluated the immune epitopes of immune-related abnormal lncRNAs in CRC. Our analysis revealed an extensive association between differential lncRNAs and immune signatures in colorectal adenocarcinoma (COAD). These findings indicate that lncRNAs may have key functions in the progression of immunotherapy for colon cancer.

2. Methods

2.1. Genome-wide lncRNA and mRNA expression in CRC

The lncRNA and gene expression of CRC were obtained from The Cancer Genome Atlas (TCGA) database (http://cancergenome.nih.gov/). The gene expression profile data were explored from the TCGA database by the R package *TCGAbiolinks_2.28.4* [28], including two measurement criteria of the fragments per kilobase of exon model per million fragments mapped (FPKM) and the read count for COAD. Based on gene annotation version GRCh38.p14 in GENCODE (https://www.gencodegenes.org/), we classified the gene expression data into lncRNA and protein-coding gene expression. The lncRNAs were further divided into different types based on their type in GENCODE. In addition, the expression profiles of genes in 27 cohorts with paired adjacent normal samples were retrieved from TCGA. Genes with a sum of coding genes and lncRNA expression less than 10 in all samples were excluded from the analysis. Patient clinical information, including survival status, stage, grade and survival data, was also obtained from the TCGA database.

2.2. Dynamic fluctuations in lncRNA expression in colon cancer

DESeq2 calculated differential expression based on read counts from NGS sequencing. We used *DESeq2 version 1.40.2* to identify the differential expression status of long noncoding genes in various cancers [29], and the "condition" parameter was set as normal and tumor groups. According to the screening criteria reported in papers [30,31], lncRNAs with $|\text{Log2-fold change}| > 1.5$ and p value < 0.05 were defined as significantly differentially expressed genes in tumor samples.

2.3. Prognostic risk estimation in CRC patients

Cox proportional hazards models were used to determine whether differentially expressed lncRNAs (DE-lncRNAs) were significantly associated with overall survival (OS) in colon cancer patients. The formula of the model is as follows:

$$h(t) = h_0(t)*\exp(\beta_1*x_1 + \beta_2*x_2 + \ldots + \beta_n*x_n)$$

h(t) represents the hazards function at time t. $h_0(t)$ represents the probability that an individual will have an event at time t in the absence of risk factors. $\beta_1, \beta_2, \ldots, \beta_n$ are the hazard coefficients of lncRNAs in cancer samples. x_1 and $x_2, \ldots x_n$ are the values of lncRNA expression in colon cancer patients.

For individual lncRNA, cancer samples were classified into two groups (high-expression groups and low-expression groups) based on the median expression level as the cutoff value. The overall survival time of the two groups was further compared using Kaplan-Meier analysis. Differences in the distribution of the survival times were explored according to the log-rank test, and p values below 0.05 were defined as survival-related lncRNAs.

2.4. Computation of immune pathways and cancer hallmark pathway scores

A total of 1811 human immune-related genes and 17 immune-related pathways were retrieved from the ImmPort project (https://www.immport.org/home). We downloaded the hallmark gene sets from the Molecular Signature Database (MSigDB, https://www.gsea-msigdb.org/gsea/msigdb) [32]. Subsequently, gene set variation analysis (*GSVA, version 1.48.3*) was applied to calculate the pathway activity scores of each COAD patient [33], and the parameters were set as follows: mx.diff = FALSE, verbose = FALSE, parallal.sz = 1, method = "ssgsea".

2.5. Construction of colon cancer risk scoring model based on immune-associated lncRNAs

LASSO regression models were applied using the R package *glmnet version 4.1-8* to reduce dimensionality and select significant genes. First, survival models for LASSO were constructed with expression data and patient follow-up data. Next, the model was cross-validated, and the genes screened by LASSO were extracted for multivariate Cox regression analysis. Finally, the risk score for each sample was predicted based on the *prediction* function. We generated the LaRisk score via regression coefficients according to the following formula:

$$\text{LaRisk score} = \sum_i^n Gene_i * Coef_i$$

where $Gene_i$ represents the expression level and $Coef_i$ represents the LASSO coefficient of the target gene.

2.6. Enrichment of lncRNAs in immune function signatures

Spearman's correlation coefficient is non-parametric, robust, applicable to ordered data, and less affected by outliers. It has wider applicability than the Pearson correlation coefficient. The enrichment of lncRNAs in 50 cancer hallmark pathways and 17 immune-related pathways was evaluated by calculating Spearman's correlation between the individual lncRNAs and the pathway gene features. The Spearman's correlation coefficient (R) of each candidate immune-related regulatory pair identified was calculated as:

$$R = 1 - \frac{6\sum_{i=1}^{n}(R_i - Q_i)^2}{n(n^2 - 1)}$$

Rank the values of two paired variables in order separately. Here, x_i represents $gene_i$, and y_i represents the score matrix of the pathway. R_i represents the rank of x_i, Q_i represents the rank of y_i, and R_i -Q_i is the difference between the ranks of x_i and y_i.

LncRNA-gene pairs with correlation coefficients greater than 0.3 and p values less than 0.05 were defined as significant pairs. Then, lncRNAs were classified into significant regulatory pairs as highly correlated lncRNAs. In addition, the scores of 25 tumor immunogenomic features were obtained from the study [19] to explore the association between individual lncRNAs and immune features in CRC. Specifically, correlations between individual lncRNAs and tumor immunogenomic feature scores were calculated by Spearman correlation. LncRNA-immunogenomic features with correlation coefficients greater than 0.10 and p value < 0.05 were retained for analysis.

2.7. Estimation of immune cell subgroups in colon cancer

The CIBERSORT algorithm (R script version 1.03) [34] is a machine learning method based on linear support vector regression for assessing the proportion of 22 immune cells in tissues or cells. Under the parameter setting perm = 100 and a cutoff of $p < 0.05$, this experiment simulated the transcriptional signature matrix of 22 immune cells, such as T cells, B cells, eosinophils, monocytes, macrophages, mast cells, dendritic cells and neutrophils. Next, Spearman's correlation between the expression of individual lncRNAs and immune cell proportions was analyzed to identify the immune cells associated with the lncRNAs in CRC. The screening criteria were defined as a correlation coefficient between lncRNAs and immune cells greater than 0.3 and p value less than 0.05.

2.8. Functional immune peptides translated from immune-related lncRNAs

A comprehensive resource of immunogenic epitopes presented by human leukocyte antigen (HLA) derived from noncoding regions was obtained from a study [35]. Next, we used immunogenic epitopes to evaluate whether immune-related lncRNAs can translate into short functional peptides. Moreover, the tumor immune dysfunction and exclusion (TIDE) computational framework (http://tide.dfci.harvard.edu) was applied to calculate the possibility of immune escape and the benefit of immunotherapy [36].

3. Results

3.1. De-lncRNAs and their clinical relevance in CRC

We obtained matched samples for 27 cancer types from the TCGA database, with a total of 10,662 samples (Supplementary Fig. 1). First, a total of 480 CRC samples and 41 normal tissue samples were explored to screen out dysregulated lncRNAs in CRC patients. In total, 15,984 lncRNAs were detected in all samples. The differential expression analysis showed that there were 1801 upregulated lncRNAs and 563 downregulated lncRNAs in the CRC samples (Fig. 1A, Supplementary Table 1). In addition, we analyzed whether the De-lncRNAs were also dysregulated in 26 other cancer types. The results showed that most lncRNAs were dysregulated in at least two cancers. Among them, 366 lncRNAs were dysregulated in more than 11 cancer types and defined as pan-cancer De-lncRNAs. A total of 1872 (79.2%) lncRNAs were dysregulated in 2–10 cancer types and defined as intermediate De-lncRNAs. Only 126 lncRNAs were identified as CRC-specific De-lncRNAs (Fig. 1B–Supplementary Table 2). Subsequently, we downloaded the disease-associated lncRNAs from the Lnc2cancer [37] and LncRNAdisease [38] databases and found that when compared with other De-lncRNAs, the pan-cancer De-lncRNAs were often associated with disease (Fig. 1C).

Furthermore, the Cox proportional hazards model showed that the majority (154/180) of De-lncRNAs were risk factors for poor survival in CRC patients. The rest (26/180) were found to be protective factors (Supplementary Table 3). Among the top highly

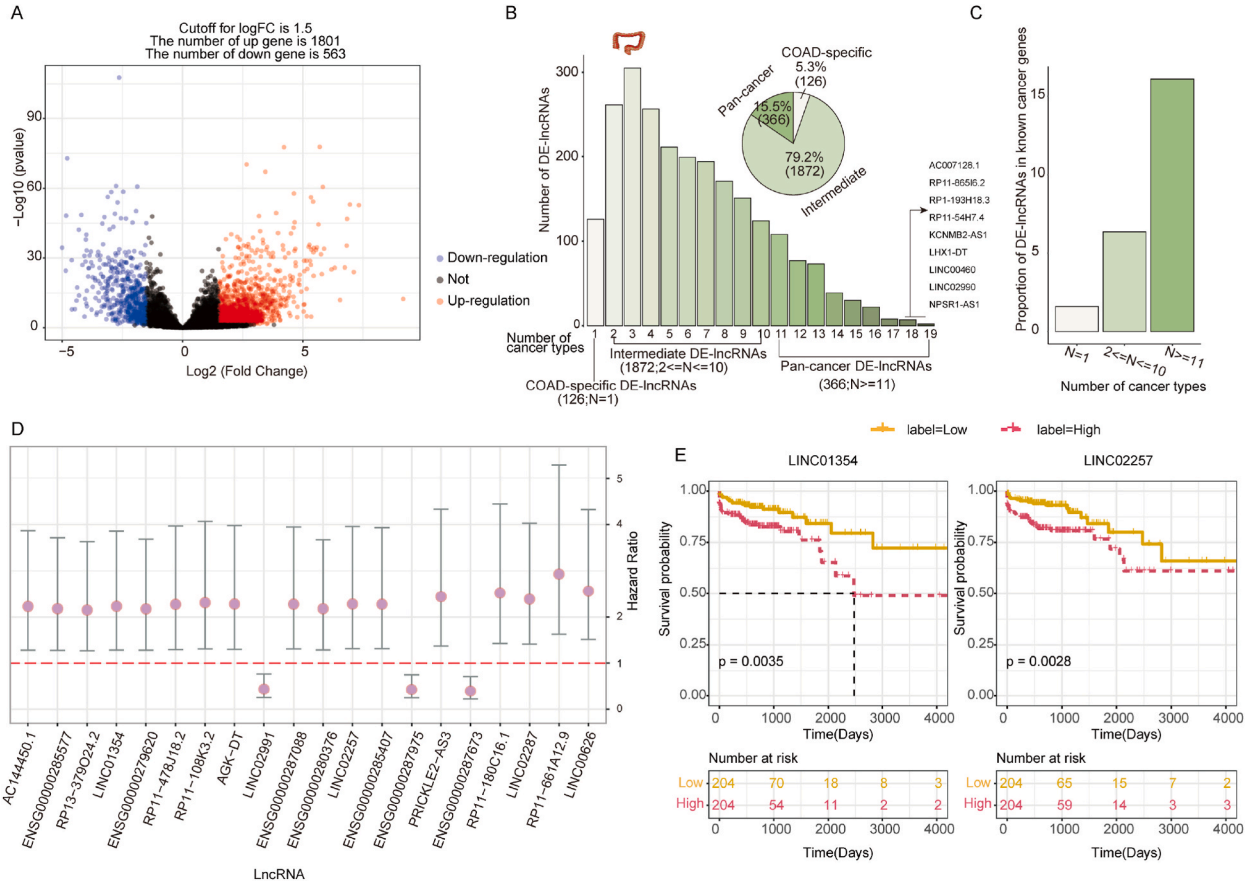

Fig. 1. Differential expression and clinical relevance of lncRNAs in CRC. (A) Volcano plot showing the differential lncRNA distribution in CRC samples compared to normal samples. (B) The expression changes of the differential lncRNAs of CRC across 18 TCGA cancer types. (C) The proportion of DE-lncRNAs in known cancer genes. (D) Forest plot showing the survival hazard ratios for the most significant differential lncRNAs according to the univariate regression analysis. (E) Kaplan–Meier curve of LINC01354 and LINC02257 in a COAD cohort.

expressed De-lncRNAs, LINC01354 and LINC02257 were significantly associated with patient prognosis (Fig. 1D and E). Consistent with previous studies, LINC01354 was upregulated in CRC, while knockdown of LINC01354 inhibited epithelial-mesenchymal transition (EMT) and cell proliferation phenotype formation in CRC cells [39]. Similarly, LINC02257 has also been found to be a prognostic biomarker in CRC [40]. These results suggest that De-lncRNAs play key roles in CRC development.

3.2. *De-lncRNA is closely associated with immune pathways in colorectal cancer*

After evaluating the impact of De-lncRNAs on 17 immune pathways, 524 De-lncRNAs were shown to be closely associated with immune pathways under a set correlation coefficient |R| of 0.3 or higher (Fig. 2A, Supplementary Table 4). As a result, 1129 lncRNA-immune pairs were defined as positively correlated, while 765 were negatively correlated. Out of the 524 De-lncRNAs, 272 were upregulated lncRNAs, and 252 were downregulated lncRNAs. The Fisher test showed that 9 immune pathways, including cytokine receptors, transforming growth family beta (TGFb) family member receptors, and TGFb family members, were significantly enriched with De-lncRNAs (Fig. 2B). Studies have shown that the TGFb signaling pathway is frequently altered in CRC [41] and therefore may play a regulatory role in the development of De-lncRNAs in TGFb signaling. Subsequently, we explored the associations between 50 hallmark gene sets and De-lncRNAs to explore the impact on biological functions. The findings indicate that RP11-863P13.3 and epithelial mesenchymal transition (EMT) exhibited the highest correlation and enrichment levels. These findings indicate that RP11-863P13.3 and EMT may have a potential association in CRC (Supplementary Table 5). Furthermore, we also found that the 485 identified lncRNAs significantly regulate cancer hallmark-related pathways and immune-related pathways (Fig. 2C). For important lncRNA-pathway pairs, we found a subset of lncRNAs suggested exclusive enrichment in immune-related hallmarks (Supplementary Table 5), with the "COMPLEMENT" pathway having the highest number of significantly associated lncRNAs, followed by the "ALLOGRAFT REJECTION" pathway (Fig. 2D).

Least absolute shrinkage and selection operator (LASSO) is a regression technique for variable selection and regularization to improve the prediction accuracy and interpretability of statistical models. LASSO regression adds a regularization penalty, and data values are shrunk toward the central point. The algorithm is well suited for models with high multicollinearity. These models force many coefficients to zero, resulting in variable elimination. To identify the set of key immune lncRNA genes in colon cancer that can be

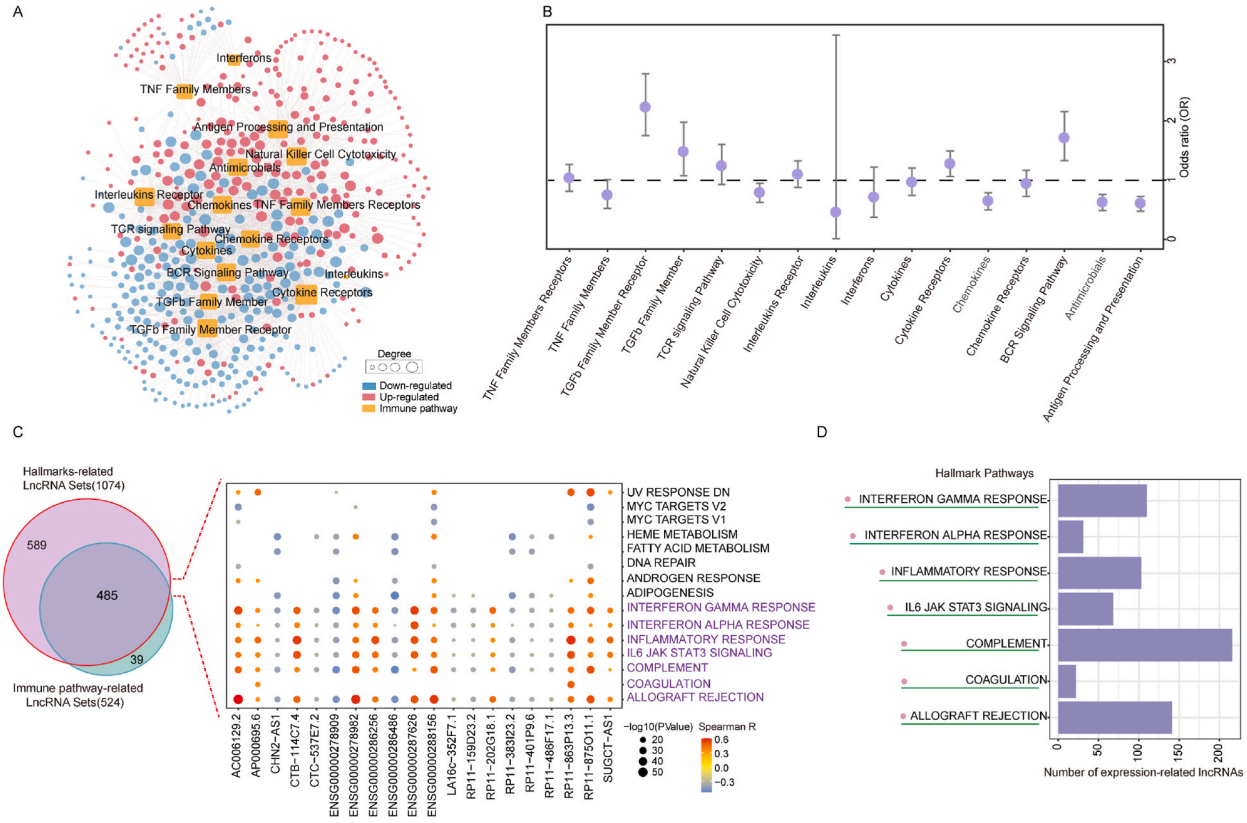

Fig. 2. Correlation between the CRC differential lncRNAs and immune pathways and biological hallmarks. (A) The upregulated (red dots) and downregulated (blue dots) lncRNAs significantly associated with the 16 immune pathways (orange squares). The size of the dots represents the number of gene interactions. (B) Forest plot showing the enrichment status of the differential lncRNAs for the different immune pathways. (C) Venn diagram showing the intersection of the De-lncRNAs in cancer hallmarks and immune pathways. Bubble plots representing the subset of lncRNAs significantly enriched in immune-related hallmarks. (D) The number of De-lncRNAs enriched in immune-related hallmarks.

used for patient prognostic assessment, thirty-eight immune-related De-lncRNAs were screened to define the LaRisk score by LASSO regression (Fig. 3A and B). Finally, 6 lncRNAs were selected for the construction of the LaRisk score formula. The formula of LaRisk was (0.424)*LINC01977 + (0.739)*FGF13-AS1 + (0.346)*RP4-594A5.1 + (0.781)*RP11-108K3.2 + (1.202)*RP11-342A23.2 + (0.286)*RP11-426C22.4 (Fig. 3C and D). Four of the six lncRNAs have been shown to have important functions in cancer progression, e.g., infiltration of M2-like tumor-associated macrophages (TAM2) induces SMAD3 upregulation of LINC09177 transcription, which in turn activates the TGF-β/SMAD3 pathway and promotes lung adenocarcinoma tumorigenesis [42]. In contrast, FGF13-AS1 expression is downregulated in breast cancer, and functional studies have shown that FGF13-AS1 inhibits cancer cell proliferation, migration, and invasion by impairing glycolysis and stemness properties [43]. In this study, the 6 De-lncRNAs were all risk factors for COAD. The high LaRisk and low LaRisk groups were defined based on the median value of LaRisk (training and testing set cutoffs: 0.439 and 0.919, respectively). We found a clear difference in survival status between the high LaRisk and low LaRisk groups in the COAD sets (Fig. 3E).

To identify predictors of OS of COAD patients by clinical pathologic characteristics, univariate and multivariate Cox proportional hazards regression were performed. The basic clinical characteristics of colon cancer patients are shown in Table 1. As shown in Supplementary Figs. 2A–B, LaRisk, age, pathologic_stage, pathologic_T and pathologic_N were independent risk factors affecting patient prognosis. Multivariate Cox analysis was applied to explore in depth the effects of clinical parameters. The results revealed increased hazard ratios (HRs) for the following features: older age, higher pathologic_N stage and LaRisk score ($p < 0.05$). The results show the efficiency of LaRisk in predicting survival in COAD patients.

3.3. *De-lncRNAs serve as potential active markers for the immunogenomic signatures of CRC*

Since a subset of lncRNA gene sets were significantly associated with immune pathways, we examined the association between the lncRNA activity scores and 25 immunogenomic signatures retrieved from the study of Vesteinn Thorsson et al. [19]. Most (17/25) of the lncRNA activity scores were significantly positively correlated with immunogenomic signatures, while the rest (8/25) were generally negatively correlated with signatures (Supplementary Table 6). Interestingly, we found that the "BCR Shannon", "BCR Richness", and "Intratumor Heterogeneity" had higher amounts of related lncRNAs (Fig. 4A). Except for some De-lncRNAs shared among immune genomic signatures, most De-lncRNAs were only correlated with individual immune genomic features (Fig. 4B). In

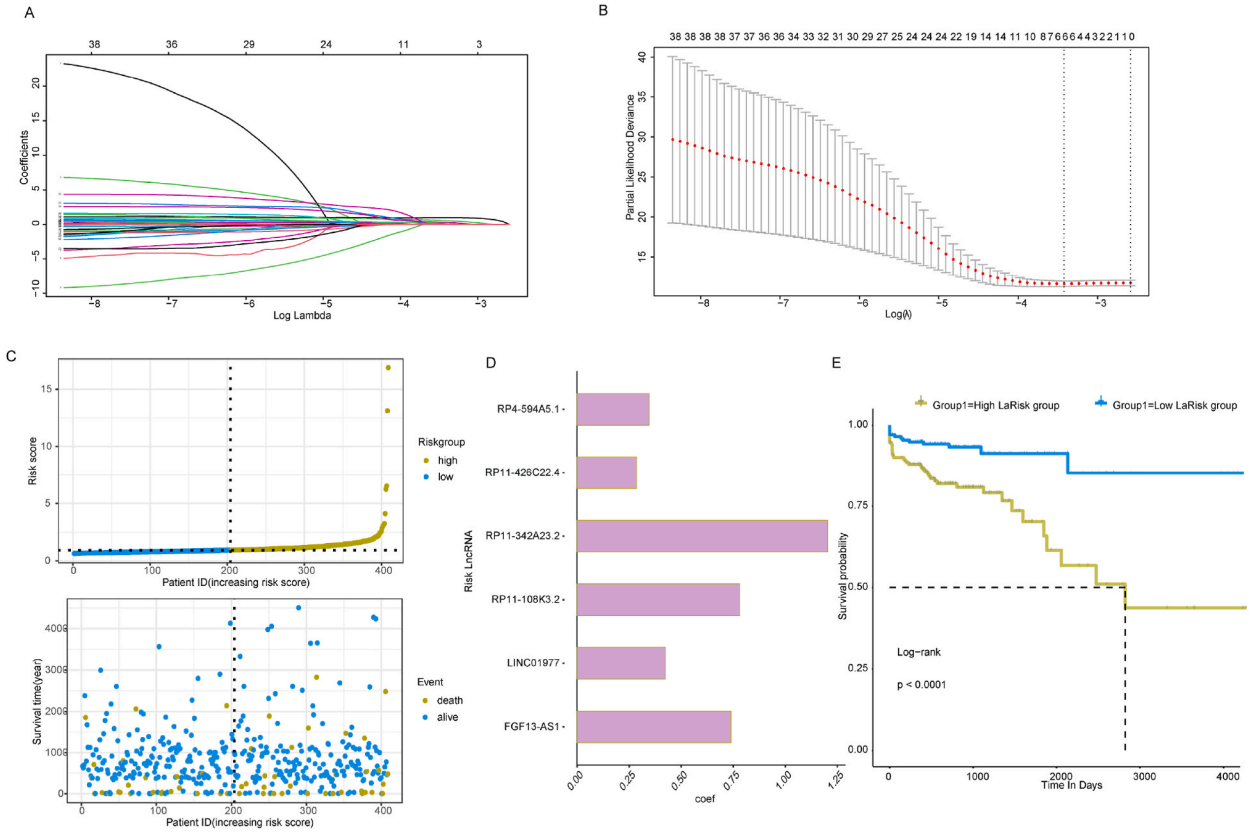

Fig. 3. Construction of the LaRisk score to predict the prognosis of COAD patients. (A) Coefficient of the lactate signature in the LASSO model. (B) LASSO analysis of the lactate signature with the minimum lambda value. (C) The distributions of risk scores and OS status of key immune signatures. (D) The distribution of LASSO regression correlation coefficients for six key immune lncRNAs. (E) Kaplan–Meier curves of the high- and low-risk groups in the COAD cohort.

Table 1
Clinicopathological characteristics of colon cancer patients.

Characteristics	Levels	Overall
Stage	Stage I	74 (16.7%)
	Stage II	176 (39.7%)
	Stage III	128 (28.9%)
	Stage IV	65 (14.7%)
T	T1	9 (2.0%)
	T2	75 (16.9%)
	T3	305 (68.8%)
	T4	54 (12.2%)
N	N0	259 (58.5%)
	N1	104 (23.5%)
	N2	80 (18.1%)
M	M0	334 (75.4%)
	M1	65 (14.7%)
	MX	44 (9.9%)
Gender	Female	210 (47.4%)
	Male	233 (52.6%)
Age	Age >65	263 (59.4%)
	Age <65	180 (40.6%)
OS	Mean ± SD	841.4 ± 789.7
living	Dead	96(21.7%)
	Alive	347(78.3%)

particular, most of the lncRNAs associated with "BCR Shannon" and "BCR Richness" were specifically positively correlated with the two immunogenomic signatures. Furthermore, we found that RP11-354P11.3 and RP11-545G3.1 were significantly correlated with 10 signatures, of which 8 were shared signatures (Fig. 4C). In addition, they also had the largest number of correlations with immunogenomic signatures among the evaluated lncRNAs. Overall, these results suggest that a subset of De-lncRNAs may serve as immunogenomic signature activity markers for CRC.

3.4. The interaction of immune lncRNAs with tumor-infiltrating immune cells (TIICs) and immune checkpoints indicates new targets for CRC immunotherapy

To estimate the interaction between the infiltration of immune cells and lncRNA features, we profiled the abundance of 22 TIICs using the CIBERSORT algorithm. Subsequently, we analyzed the correlation between lncRNAs and the TIIC scores. The lncRNAs were significantly correlated in at least 2 immune-infiltrating cell types. Based on these findings, we created a list of 50 De-lncRNAs (Supplementary Table 7). Among the important lncRNA immune cell interactions, resting mast cells had the highest positive correlation with the distinct lncRNAs, followed by M0 and M2 macrophages (Fig. 5A). Specifically, the analysis showed that cancer patients with high expression levels of RP11-733O18.1 displayed a significantly greater abundance of M2 macrophage infiltration than those with low expression levels ($P = 6.1E\text{-}16$, as shown in Fig. 5B). However, cancer patients with high expression of CTD-3184A7.4 exhibited significantly reduced levels of infiltrating M0 macrophages ($P = 1.1E\text{-}10$, as illustrated in Fig. 5B).

Since immune checkpoint blockade (ICB) therapy has shown valuable efficacy in cancer treatment, we also examined the relationship between lncRNAs and immune checkpoints in colorectal cancer. Notably, our results demonstrated that the immune checkpoint receptor-ligand pairs were differentially expressed in CRC cancer (Fig. 5C). Additionally, these De-lncRNAs were also significantly associated with the gene expression of immune checkpoints (Fig. 5D and Supplementary Table 8). Our analysis identified two clusters of these lncRNAs that exhibited a significant positive correlation with various immune checkpoint genes, including RP11-1070N10.3, AC006129.2, and ENSG00000278982. Conversely, a small number of lncRNAs were found to be negatively associated with immune checkpoint genes, including RVSTM2A-OT1 and PVR. Among the identified immune checkpoint genes, AC006129.2 exhibited the strongest positive correlation with CD96 molecule (CD96), CD28 molecule (CD28), and B and T lymphocyte associated (BTLA). Meanwhile, AC006129.2 showed a significant positive correlation with programmed cell death protein 1 (PD-1) (Fig. 5E). Overall, these results suggest that AC006129.2 may mediate the expression of PD-1, CD96, CD28, and BTLA. Therefore, lncRNAs could be potential targets to regulate the levels of immune cells or immune checkpoints, thereby enhancing the efficacy of immunotherapy in CRC patients.

3.5. Genome instability and tumor mutational burden reveal lncRNAs as immunotherapy biomarkers

Cancer-associated lncRNA-derived epitopes can enable the immune system to fight tumor cells and be increasingly targeted by immunotherapy. Based on a recent study by Xu et al. [27], we identified 15 lncRNA-generated short open reading frames (sORFs) in 50 immune lncRNAs corresponding to 15 cancer immune epitopes that are likely to serve as novel targets for cancer immunotherapy (Fig. 6A, Supplementary Table 9). TMB and MSI are usually used as biomarkers to identify patients who will benefit from ICB therapy [44–49]. Therefore, *TCGAbiolinks was used* [50] to obtain somatic mutations. The *maftools package* [51] was applied to explore the mutation profiles and calculate the TMB score in CRC patients. The analysis revealed the overall distribution of TMB in colorectal

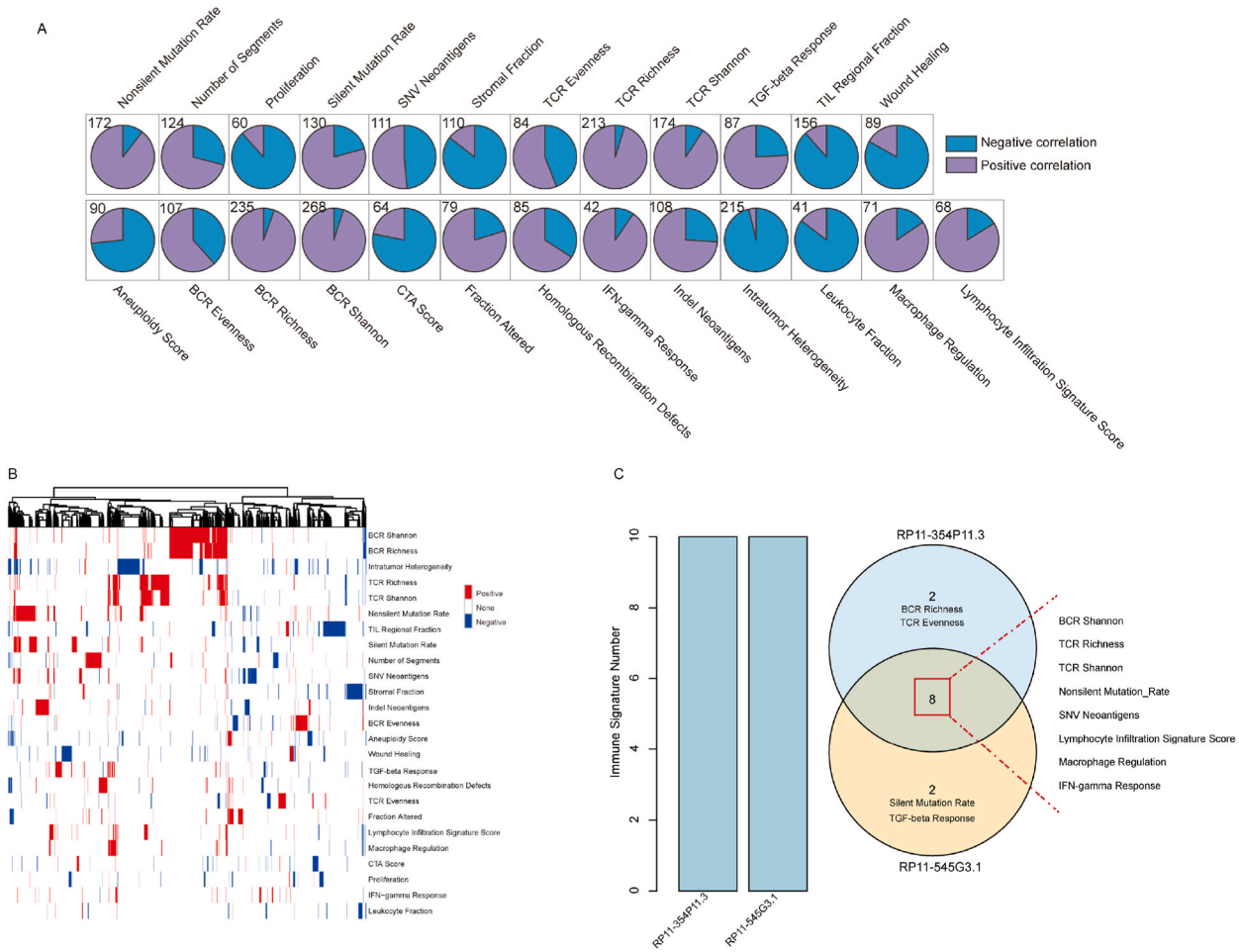

Fig. 4. Association analysis between the De-lncRNA and immunogenomic signatures. (A) The number of differentially expressed lncRNAs positively or negatively correlated with immunogenomic signatures. (B) Heatmap showing the landscape of significantly correlated lncRNAs across different immunogenomic signatures. (C) Analysis of the two lncRNAs most correlated with immunogenomic signatures, RP11-354P11.3 and RP11-545G3.1.

cancer patients (Supplementary Figs. 3A–B). At the same time, comparing the TMB of CRC with other cancers, we found that the proportion of somatic mutations in CRC was higher than that of partial cancers (Supplementary Fig. 3C).

Moreover, we also calculated the correlation of immune lncRNAs with TMB and MSI. The results revealed that immune lncRNAs were significantly correlated with TMB and MSI (Fig. 6B). ENSG00000287626, AP000695.6, and LINC01705 were positively correlated with two immunotherapy indicators, while RP11−519G16.5 and ENSG00000280032 were significantly negatively correlated with TMB and MSI. High expression of RP11-426C22.4 and LINC01354 and low expression of ENSG00000287743 and ENSG00000280032 were correlated with unfavorable survival in CRC patients (Fig. 6C). The tumor immune dysfunction and exclusion (TIDE) score was applied to assess the possibility of immune escape. A higher score represents a higher likelihood of immune escape, indicating a lower benefit to the patient from immunotherapy. According to the response predicted by the TIDE pipeline, the CRC patients were classified into response and non-response groups. As shown in Supplementary Fig. 4A, most lncRNAs (40/50) showed significantly lower expression levels in the response group. The response group also tended to have lower TIDE scores (Supplementary Fig. 4B). These results indicate that lncRNAs can be used as immune epitopes, effective targets, or prognostic markers for ICB therapy or immunotherapy in CRC patients.

4. Discussion

The heterogeneity of CRC poses a significant obstacle to the efficacy of immunotherapy. Although lncRNAs have been found to regulate key functions in cancer-related immunity, the impact of immune-derived lncRNA signatures on prognosis has not yet been explored in CRC. In this study, we comprehensively characterized immune-derived lncRNAs in CRC. The relationship between lncRNA expression and immune-related pathways, tumor immunogenomic features, tumor-infiltrating immune cells, immune checkpoints, TMB and MSI was discussed. Our analysis revealed the association between COAD De-lncRNAs and various immune signatures. Most of

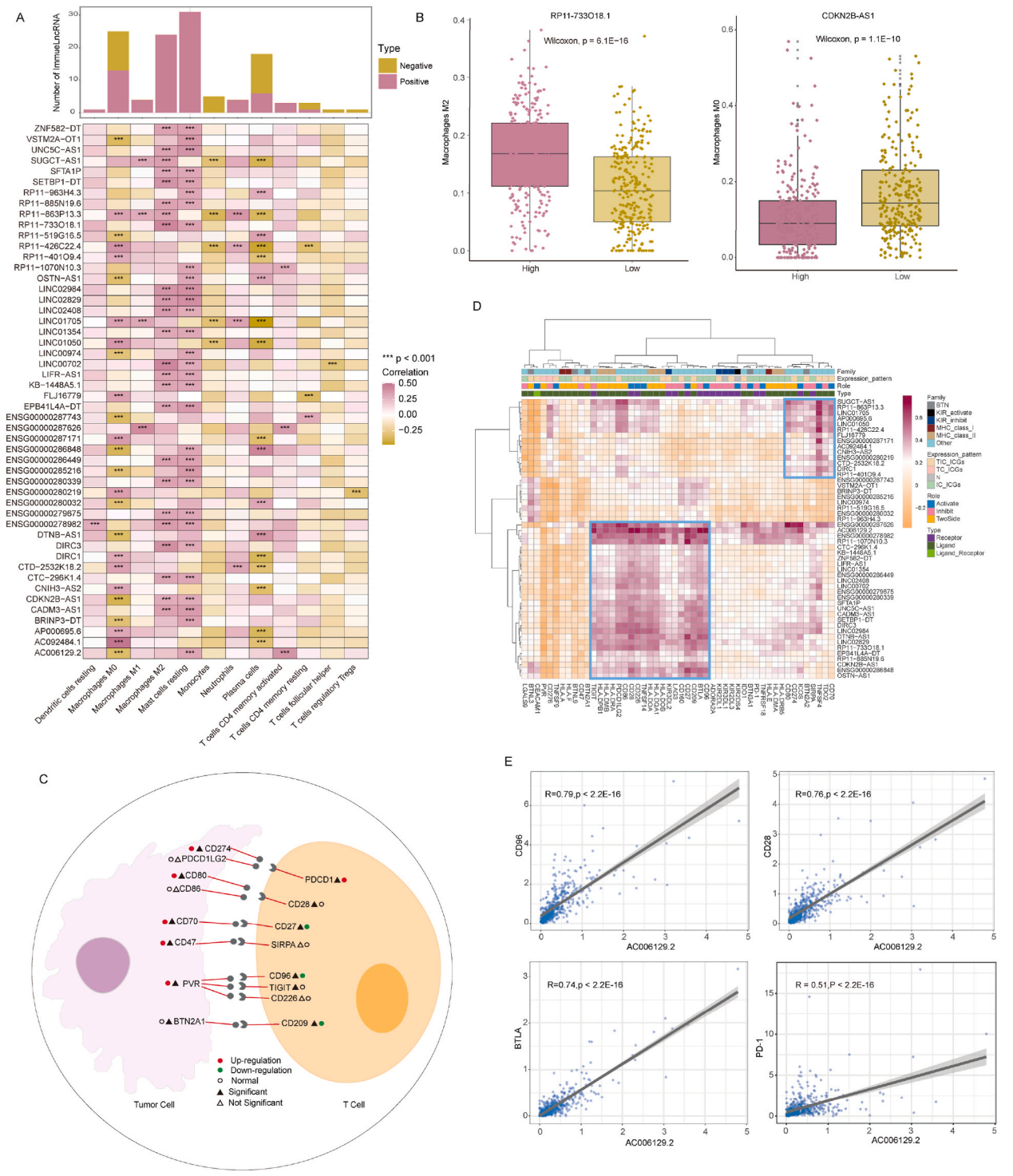

Fig. 5. Interactions between lncRNAs and TIICs and immune checkpoints. (A) The correlations between the significant De-lncRNAs and the major immune cell types. The colors indicate the strength of the correlation coefficients. (B) Comparison of the relative abundance of M2 or M0 macrophages between CRC samples with high and low expression levels of RP11-733O18 and CTD-3184A7.4. (C) Differential expression of immune checkpoint receptor ligands in CRC. (D) Correlation heatmap of a set of differential lncRNAs with immune checkpoint genes. (E) Correlation analysis between AC006129.2 and immune checkpoint gene expression levels, such as CD96, CD28, BTLA, and PD-1, in COAD samples.

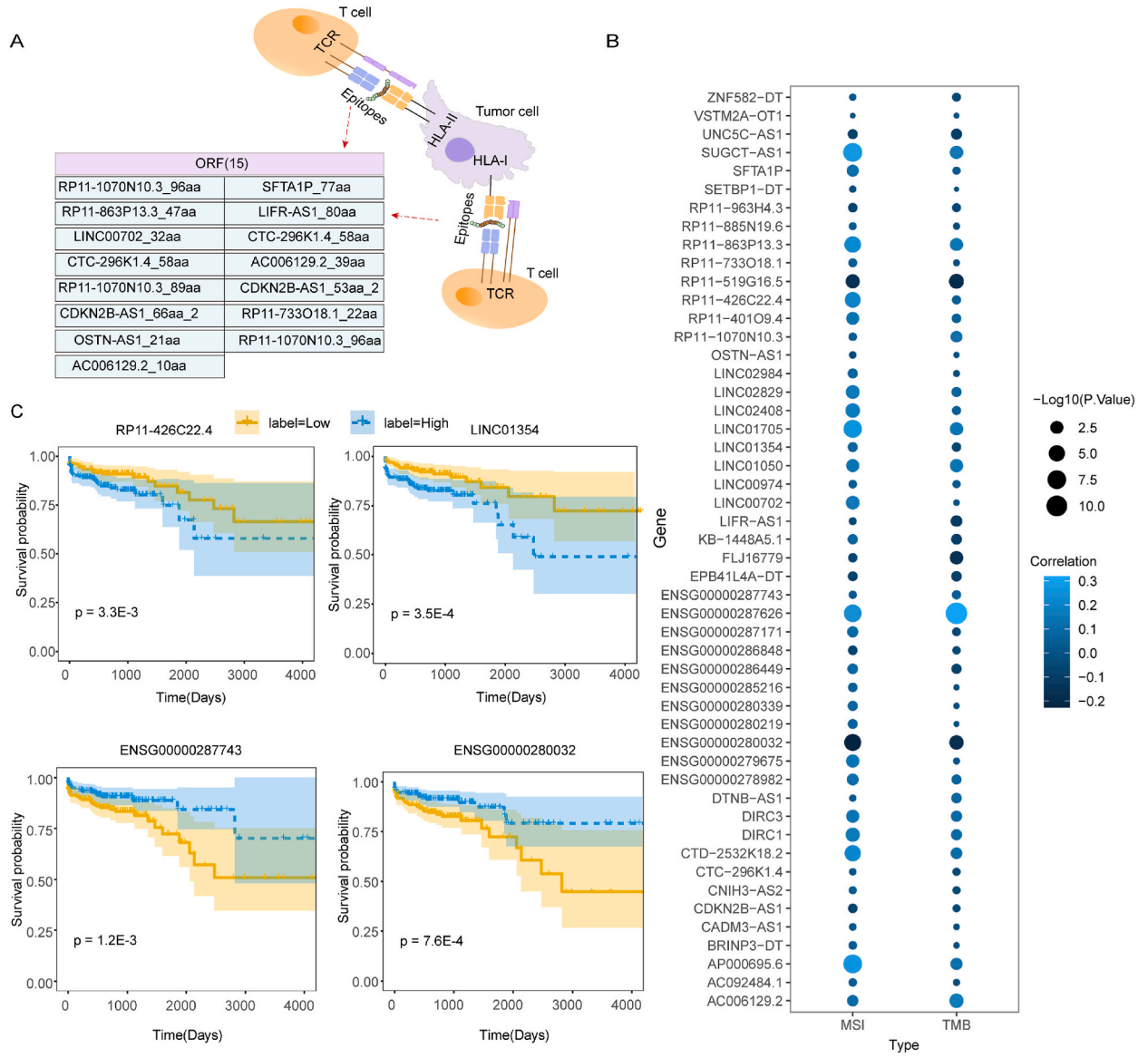

Fig. 6. Association analysis of the differential lncRNAs as biomarkers for immunotherapy. (A) Immune peptide epitopes for differential lncRNA translation. (B) Correlation between the differential lncRNAs with MSI and TMB index in CRC patients. (C) Kaplan–Meier curves for RP11-426C22.4, LINC01354, ENSG00000287743, and ENSG00000280032 in the COAD cohort.

these dysregulated lncRNAs were also widely dysregulated in various cancers. For example, KCNMB2-AS1, LINC00460, and NPSR1-AS1 were dysregulated in 18 cancer types. In particular, studies have found that the overexpression of LINC00460 in CRC can promote EMT, cell proliferation, migration and invasion and promote tumor growth and metastasis in vivo [52]. The prognosis-related lncRNAs showed a close association between the differentially expressed lncRNAs and the biomarkers, as most differentially expressed lncRNAs were identified as cancer risk factors. Although immune pathways were not enriched from the largest subset of lncRNAs, they were significantly enriched in lncRNA-immune associations. Cytokine receptors are the premier immune pathways associated with lncRNAs, suggesting that dysregulated lncRNAs are crucial in regulating cellular molecular mechanisms. For example, in liver and CRC, Lnc-DILC reduced cancer stem cell proliferation and cancer cell differentiation [53] and strongly inhibited the IL-6/JAK2/STAT3 autocrine pathway [54]. In recent years, key roles of lncRNA-regulated immune mechanisms (innate and adaptive immunity) have been revealed, especially in cancer-associated immunity [55,56]. Several lncRNAs enriched in various immune pathways have indicated their important functions in regulating cancer immunity to promote cancer development. A study found that LINC00240 overexpression promoted cervical cancer progression by inhibiting the cytotoxic level of NKT cells and by affecting the STAT3/MICA axis [57]. Another study showed that the cancer immunogenic lncRNA LIMIT, which binds the LIMIT-GBP-HSF1 axis, could regulate the function of MHC-I, thereby serving as a target for cancer immunotherapy [58].

In addition to immune-related pathways, we further explored the impact of tumor immunogenic signatures on tumor infiltrating immune cells, immune checkpoints, TMB, and MSI. These signatures reflect the immune activity of tumor samples from different aspects, including the tumor immune genome signatures that represent the genomic variation induced or likely to be induced by immune reprogramming [19,59]. Furthermore, many lncRNAs were associated with B-cell receptor (BCR) diversity indexes (BCR Richness, BCR Shannon), which further highlighted their important role in immune regulation.

Importantly, bias and confounding factors produce large deviations in the analysis and results, and reasonable handling of these deviations is an important step. We strictly screened and processed the expression values of colon cancer datasets, filtered out low-quality reads, and filtered out genes with a sum of gene expression less than 10 in all samples. In addition, multivariable Cox regression analysis followed by univariable Cox regression analysis showed that Pathologic_stage, Gender, Pathologic_M, and Pathologic_T existed as confounders in the prognosis of colon cancer patients.

In conclusion, we provided a unique collection of immune-associated lncRNAs in colon cancer, and this subset of immune lncRNAs expanded the catalog of immune-associated lncRNAs. Moreover, one important mechanism by which lncRNAs regulate gene expression is through the translation of sORFs, and studies have shown that sORF translation regulates inflammatory and immune responses [60]. We identified a set of immune-associated lncRNA-translated peptides that function as immune antigenic epitopes. In this study, bioinformatic strategies were mainly used for analysis, and the current study contributes to providing candidate biomarkers for the clinical diagnosis of colon cancer, while the functionality of the identified immune-associated lncRNAs must be verified. Next, a large amount of data and experiments could be designed to demonstrate and validate that these lncRNAs or small molecule peptides regulate certain functional pathways in colon cancer progression. We will also design experiments to verify whether targeting lncRNAs or small molecule peptides have certain inhibitory effects on colon cancer progression, and then we will construct small molecule peptides with low cytotoxicity, good cell permeability, and high specificity, which provide new perspectives in the search for new colon cancer inhibitors.

Ethics approval and consent to participate

Not applicable.

Data and code availability statement

Published datasets could be explored from the TCGA database (https://cancergenome.nih.gov/). The code involved in the data processing can be requested from the author of the paper upon request.

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CRediT authorship contribution statement

Mengying Zhang: Writing – review & editing, Writing – original draft, Project administration, Funding acquisition. **Yifei Wu:** Visualization, Data curation. **Jingyi Mou:** Software, Data curation. **Yang Yao:** Visualization, Data curation. **Pengbo Wen:** Software. **Xin Liu:** Visualization. **Shipeng Shang:** Visualization, Software. **Xingxing Kang:** Validation. **Jiaqi Tian:** Software. **Yan Liu:** Validation. **Enhui Lv:** Methodology, Formal analysis. **Liang Wang:** Writing – review & editing, Project administration, Funding acquisition.

Declaration of competing interest

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e25568.

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