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Original Article

Aberrant Expression of BLM Correlates with Malignant Progression and Immune Infiltration in Pancreatic Adenocarcinoma

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Abstract

Background and objectives: Pancreatic adenocarcinoma (PAAD) is a common malignancy in the digestive tract. Emerging studies have reported that Bloom's syndrome helicase (BLM) is closely associated with the tumor prognosis and immune microenvironment. Our study aimed to reveal BLM's potential prognosis value in PAAD.

Methods: Potential oncogenic effects and prognostic influence of BLM were explored based on the TCGA and GETx databases. Gene mutation and methylation analyses were performed on the cBioPortal website and SMART database. The ARCHS4 and JASPAR2022 databases were used to predict the upstream transcription factor targets (TFs) of BLM. Starbase was used to explore the upstream ncRNAs. The relationship of BLM with the PAAD immune infiltration and immune checkpoints was analyzed using TIMER and GEPIA databases.

Results: BLM was highly expressed and correlated with a poor prognosis in PAAD. The hypomethylation of BLM was observed in PAAD and correlated with a poor prognosis. The predicted TFs (E2F1 and ETS1) were also highly expressed and positively correlated with a poor prognosis in PAAD. LINC01133-miR-30b-5p axis was explored to be the most potential upstream ncRNAs of BLM in PAAD. Furthermore, the BLM expression was positively correlated with the PAAD immune infiltration cells. The BLM expression was also positively correlated with the expression of the immune checkpoints of PD1, PD-L1, CTLA-4, and CD47.

Conclusions: The high expression of BLM was associated with the poor prognosis of PAAD. In addition, a high BLM expression could facilitate the expression of the immune checkpoints in the immune infiltration cells, which would promote PAAD progression and affect its prognosis.

Keywords: BLM; DNA damage repair; cancer prognosis; immune microenvironment; pancreatic adenocarcinoma.

Abbreviations: BLM, Bloom's syndrome helicase; ceRNA, competitive endogenous RNA; DEGs, differentially expressed genes; HR, homologous recombination repair; ncRNA, non-coding RNA; OS, overall survival; PAAD, pancreatic adenocarcinoma; RFS, relapse free survival; TFs, transcription factors; TME, tumor microenvironment.

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Introduction

Pancreatic adenocarcinoma (PAAD) is a common malignant tumor in the digestive tract,[1] which is known as the "king of cancer" in the field of cancer. As one of the worst prognosis malignancies, PAAD's five-year survival was only 2–9% owing to its substantial invasion and migration abilities.[2] Despite the low incidence of PAAD, it is the fourth leading cause of cancer-related death and is expected to be the second most common cancer-related cause of death by 2030, surpassing colorectal and breast cancer.[3] Current new therapies for PAAD mainly include immunotherapy combinations, targeted DNA repair, and tumor metabolism. Although with

new treatment options, the survival rate has not improved significantly in the past few decades.[4]

The Bloom's syndrome helicase (BLM), a member of the human RecQ helicase family,[5] has typical RecQ helicase family structural features and plays a vital role in the cellular metabolic processes, such as DNA replication and recombination, transcription, repair, and maintenance of the telomeres.[6] Moreover, studies have shown that mutations in the BLM gene lead to the development of Bloom's syndrome, patient genetic instability, and susceptibility to various cancers, such as breast, lung, prostate, and other malignancies.[7] During the double-strand break (DSB) repair, BLM is rapidly recruited to the damaged DNA to participate in the homologous recombination repair (HRR),[8] and is considered one of the 50 best candidate targets for cancer treatment in the DNA damage repair pathway.[9] Regarding the relationship between BLM and PAAD, Kondo *et al*., by sequencing coding exons of HRR-related genes (including BLM), found that PAAD patients with HRR-related mutations had long median progression-free survival compared with those without mutations.[10] However, the BLM expression and regulatory mechanism in PAAD and its effect on the prognosis still remain unclear.

In recent years, the study of the tumor immune microenvironment has received wide attention. In general, tumor cells can use immune checkpoint regulation to escape the immune response.[11] Programmed death-ligand (PD-L1), the most representative immune checkpoint, has also been detected for its high expression in cancers, which is associated with a poor prognosis in breast cancer, colorectal cancer, gastric cancer, non-small cell lung cancer, and other cancers.[12] Additionally, PAAD has been described as a type of tumor with rich immunosuppressive mechanisms, and its complex tumor microenvironment (TME) has been regarded as a barrier to systemic treatment. Simultaneously, the DNA repair pathway involved by BLM is also related to the development and maturation of immune cells.[13] However, whether BLM involves regulating the immune microenvironment in PAAD has still not yet been determined.

Therefore, in this study, we focused on exploring the regulatory mechanism of BLM and researching the impact of BLM on the immune microenvironment and prognosis in PAAD. First, the pancancer and survival analysis of BLM in multiple cancer types was performed. Next, the genetic and epigenetic regulations of BLM were investigated, including the gene mutation, DNA methylation, transcription factors (TFs), and the noncoding RNA (ncRNA) in PAAD. Finally, the relationship of the expression of BLM with immune cell infiltration, biomarkers of immune cells, and immune checkpoints in PAAD were further explored. Taken together, our results suggested that the high expression of BLM regulated by abnormal methylation, the TFs, and the LINC01133-miR-30b-5p axis was associated with the poor prognosis and tumor immune infiltration in PAAD.

Materials and methods

Downloading and analysis of the cancer data

Raw data of 33 cancer types and normal samples were downloaded from the TCGA and GETx databases by UCSC Xena (https://xena.ucsc.edu/). The data were normalized using R software (the TPM values were extracted, log2(x+0.001) transformed, and combined).

Gene differential expression analysis

The R software, a tool with powerful data processing and mapping capabilities, has been widely used in genomic and transcriptomic studies.[14] In this study, the differential expression analysis was performed by R software's "limma" package.

Gene Expression Profiling Interactive Analysis (GEPIA) of the profiling of the gene expression (http://gepia.cancer-pku.cn/), an online database constructed by Peking University, China to analyze differentially expressed genes in cancer and normal tissues, has become an effective way to identify prognostic markers and molecular targets for cancer therapy.[15] The BLM and related long noncoding RNAs (lncRNAs) expression and prognosis influence in cancer was validated in this database, in addition to assessing the correlation of the immune checkpoints and BLM gene in PAAD.

BLM protein differential expression analysis

The Human Protein Atlas (HPA) (https://www.proteinatlas.org/) is an antibody-based method for immunostaining on tissues and cell lines. It can also be used for differential expression analysis of normal tissue and tumor tissue proteins.[16] The results of the immunohistochemistry of the BLM histochemical antibody (No: HPA005689) on the normal pancreas and PAAD tissues were quoted.

Gene mutation analysis

The cBioPortal website (https://www.cbioportal.org/) for cancer genomics provides a web resource for exploring, visualizing, and analyzing multidimensional cancer genomics data.[17] We used this website to analyze the types and loci of the BLM mutations in cancer.

BLM methylation analysis

The Shiny Methylation Analysis Resource Tool (SMART; http://www.bioinfo-zs.com/smartapp/) is a user-friendly and easy-to-use web application for comprehensively analyzing DNA methylation based on TCGA data.[18] Here we analyzed the differential expression of methylated BLM in different cancers and performed survival analyses.

Prediction of the potential upstream transcription factor targets of BLM

ARCHS4 (https://maayanlab.cloud/archs4/help.html) was used to predict the upstream transcription factor targets (TFs), which predicted upstream TFs based on the ChIP-seq data from the ChEA and ENCODE gene set libraries.[19] The JASPAR2022 database (https://jaspar.genereg.net/) was used to predict the binding sites for the transcription factors in the promoter regions of the genes.

Exploring the upstream ncRNAs of the BLM

Starbase (http://starbase.sysu.edu.cn/) is a database that can explore the upstream ncRNAs of the regulatory gene.[20] Here, we used Starbase to select the upstream ncRNAs regulating BLM.

Survival analysis

The Kaplan-Meier plotter (http://kmplot.com/Analysis/), an open database, is capable of conducting the survival analysis of more than 20 types of cancers.[21] We used this database to analyze the

effect of the candidate microRNAs (miRNAs) on the prognostic OS in PAAD.

Analysis of the immune cell infiltration level and immune checkpoint expression

TIMER (https://cistrome.shinyapps.io/timer/) is an online database for comprehensively analyzing tumor-infiltrating immune cells.[22] We used this database to analyze the relationship of the expression of BLM with the level of the immune cell infiltration or immune checkpoints in PAAD.

Other databases and software

UALCAN (http://ualcan.path.uab.edu/index.html) was used to verify the gene and protein expression in PAAD, and Cytoscape software was used to map the relationship of the lncRNA-miRNA/BLM network.

Statistical analysis

For the results obtained from all the databases and analysis software, p-value < 0.05 was considered a statistically significant difference. A t-test was used to compare the mean between the two groups. Correlation analysis was performed using Pearson's correlation based on bivariate normality. The survival curves were plotted with the Kaplan-Meier method and tested by the log-rank test.

Results

Pancancer analysis of the BLM expression

To explore the potential oncogenic role of BLM, the BLM expression level in 33 human cancers was analyzed by the TCGA and GTEx databases. The results displayed that BLM was highly expressed in ACC, BLCA, BRCA, CESC, CHOL, COAD, DLBC, ESCA, GBM, HNSC, KIRC, KIRP, LGG, LIHC, LUAD, LUSC, OV, PAAD, PCPG, PRAD, READ, SKCM, STAD, TGCT, THCA, UCEC, and UCS compared with the normal samples ($p < 0.05$), while there was no statistical difference in KIHC, LAML, MESO, SARC, and UVM (Fig. 1a). Subsequently, the expression of BLM was validated in the GEPIA database and found that it was also highly expressed in 14 cancers, including BLCA, BRAC, COAD, etc. (Fig. 1b). These results suggested that BLM could be a potential oncogene in human cancer carcinogenesis.

Prognosis analysis of BLM in cancer

The survival analysis, including two prognostic indices (OS and RFS), was performed on the above-mentioned cancer patients. The results indicated that the PAAD patients with a high BLM expression had a significantly poor prognosis for OS and RFS (Fig. 2). Thus, BLM could be a potential biomarker for PAAD prognosis.

The expression of the BLM protein in PAAD

The BLM protein level in the PAAD tissues was further researched from the HPA database. The high expression of the BLM protein only appeared in the endocrine cells of the PAAD tissue. However, the results did not indicate any significant differences compared to the normal tissue samples (Fig. 3a–b). In considering that the sample size of the normal tissue was too small to be compared with the HPA database, the CPTAC database was further used to verify the protein expression. Unsurprisingly, the results were consistent with our predictions, and the expression of the BLM protein was higher in the PAAD tissue (Fig. 3c).

Analysis of the genetic alteration of the BLM

For researching the genetic alteration of the BLM, we analyzed the different mutation types of BLM in cancer through the cBioPortal web. The results showed that BLM had mass mutations in multiple types of cancer, and the amplification mutations were the primary type in PAAD (Fig. 4a). The types and loci of the BLM mutation in PAAD were further explored. The missense and truncating were the dominating types, and the latter type occurred in the S1252F/Y on the HDRC domain (Fig. 4b). Thus, the mutations of BLM could be a cause of the high BLM expression in PAAD. However, mutations in the BLM gene did not significantly impact the prognosis of the PAAD patients (Fig. 4).

Analysis of the methylation level of the BLM promoter in PAAD

Methylation is an essential epigenetic regulatory mechanism for DNA. Therefore, a pancancer

analysis for the methylation level of the CpG site in the BLM promoter was performed (Fig. 5a). We found that the methylation level of the BLM promoter was significantly reduced in PAAD (Fig. 5b) and was positively correlated with OS (Fig. 5c–d). These findings indicated that the low methylation level of BLM could cause the high expression of BLM and affect the prognosis in PAAD patients.

Prediction of the transcription factor targets in the BLM upstream

TFs usually have competitive inhibitory relationships with DNA methylation and play an essential role in the gene transcription process. Therefore, we used the ARCHS4 resource to investigate the upstream TFs of the BLM further. Consequently, 48 upstream TFs of BLM were predicted in humans (Supplementary Table S1). Simultaneously, the JASPAR2022 database was used to verify whether these 48 TFs had binding sites in the BLM promoter, and 23 of them were revealed to have binding sites (Supplementary Table S2). Subsequently, the correlation analysis in PAAD showed that seven TFs (E2F4, E2F1, ETS1, MYCN, ELK1, IRF1, and FOXP3) were positively correlated with the BLM expression (Fig. 6a) and that five TFs (E2F1, ETS1, ELK1, IRF1, and FOXP3) were also highly expressed in PAAD (Fig. 6b). Therefore, these five TFs might be the most potential upstream TFs of BLM. Similarly, survival analysis was also performed, in which only E2F1 and ETS1, consistent with BLM, were correlated with a poor prognosis (OS or RFS) in PAAD (Fig. 6c).

Prediction and analysis of the ncRNAs upstream of the BLM

It is widely believed that ncRNAs, including miRNAs and lncR-

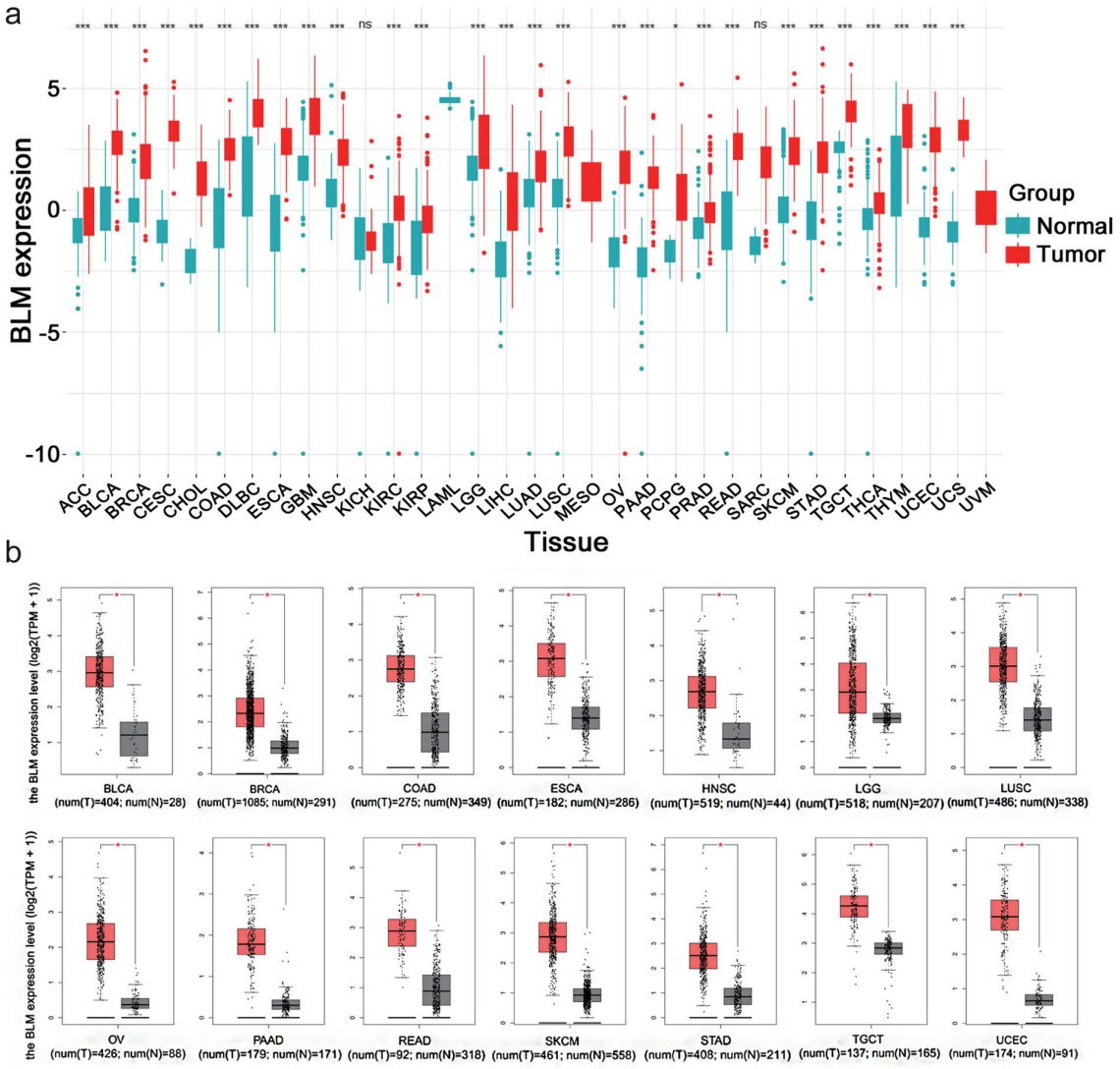


Fig. 1. Pancancer analysis of rgw BLM expression. (a) BLM expression in 33 human cancers based on the TCGA and GTEx databases. (b) BLM expression in BACL, BRCA, COAD, ESCA, HNSC, LGG, LUSC, OV, PAAD, READ, SKCM, STAD, TGCT, and UCEC based on the GEPIA database. ns, not significant; *p value is <0.05; **p value is <0.01; ***p value is <0.001. BLM, Bloom's syndrome helicase.

NAs, can regulate gene expression and are considered an epigenetic regulatory mechanism. Therefore, based on the Starbase database, we predicted the possible upstream miRNAs of BLM. Finally, we selected 11 miRNAs that were negatively correlated with the BLM expression (miRNA usually suppresses the target gene expression) (Fig. 7a). To intuitively visualize this situation, we drew the relationship network of these 11 miRNAs with BLM using Cytoscape software (Fig. 7b). Then, we determined these miRNAs' expression and prognostic value individually in PAAD. Only miR-30b-5p was downregulated in PAAD, and its downregulation was positively correlated with the poor prognosis and disease course (Fig. 7c–e). These findings suggested that miR-30b-5p could be the most potential regulatory miRNA for the BLM in PAAD.

According to the competitive endogenous RNA (ceRNA) hypothesis, lncRNA could increase the mRNA expression by competitively binding to a shared miRNA.[23] Consequently, we used the Starbase database to predict the upstream lncRNAs binding to miR-30b-5p and selected 12 lncRNAs (Fig. 8a–b). The correlation of these lncRNAs with BLM in PAAD was verified, and only LINC00657 (NORAD), LINC00707, LINC02535, and LINC01133 were positively correlated with BLM (Fig. 8c). To further verify whether they were also highly expressed in PAAD, we performed a differential expression analysis on the GEPIA database, and only LINC00657 and LINC01133 were confirmed (Fig. 8d). Finally, these two lncRNAs were defined as candidates for further prognostic analysis. LINC01133, consistent with the BLM, significantly correlated with the poor prognosis (OS) and progression in PAAD (Fig. 8e–f). For the preceding reasons, LINC01133 was selected as the most likely upstream lncRNA in the miR-30b-5p/BLM axis.

Differential expression analysis for the BLM related-genes in PAAD

The PAAD patients were divided into high and low expression

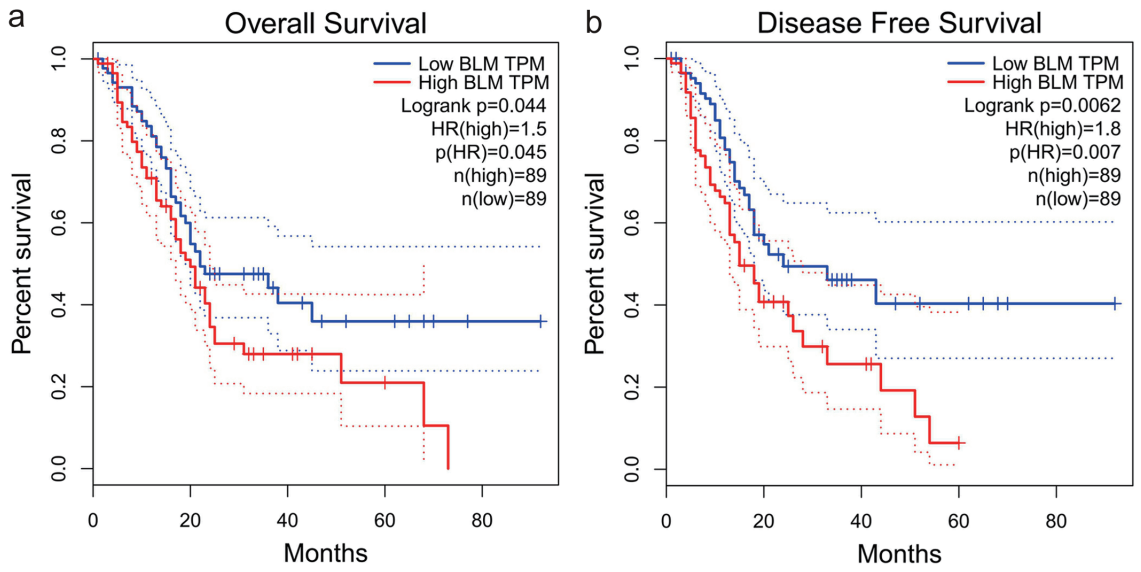


Fig. 2. Prognostic analysis of BLM in PAAD based on the GEPIA database. (a) BLM was analyzed for the overall survival (OS). (b) BLM was analyzed for disease-free survival (RFS). BLM, Bloom's syndrome helicase; PADD, pancreatic adenocarcinoma.

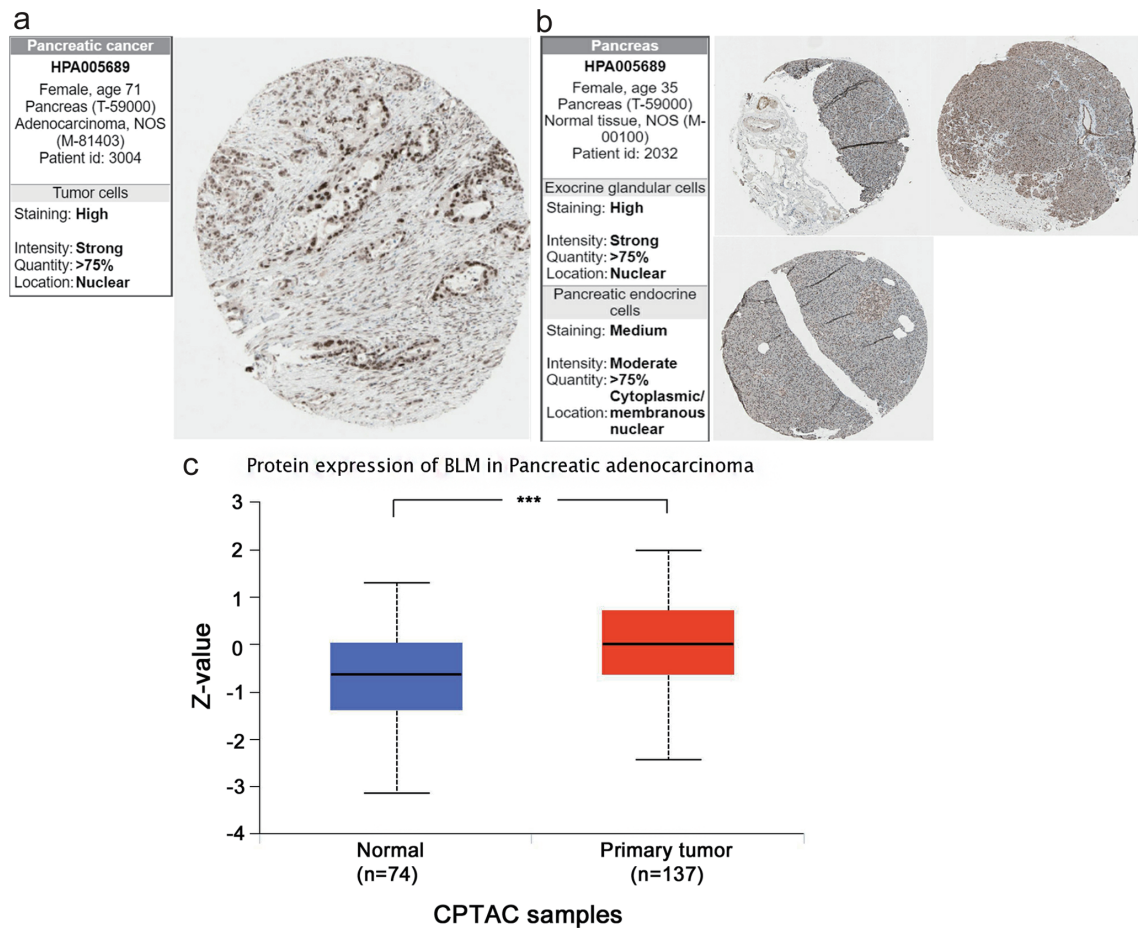


Fig. 3. The expression of the BLM protein in PAAD. (a–b) The BLM protein expression was observed in PAAD and the normal pancreas tissue based on the HPA database. (c) The BLM protein differential expression was observed from the CPTAC database. **p* value is <0.05; ***p* value is <0.01; ****p* value is <0.001. BLM, Bloom's syndrome helicase; PADD, pancreatic adenocarcinoma.

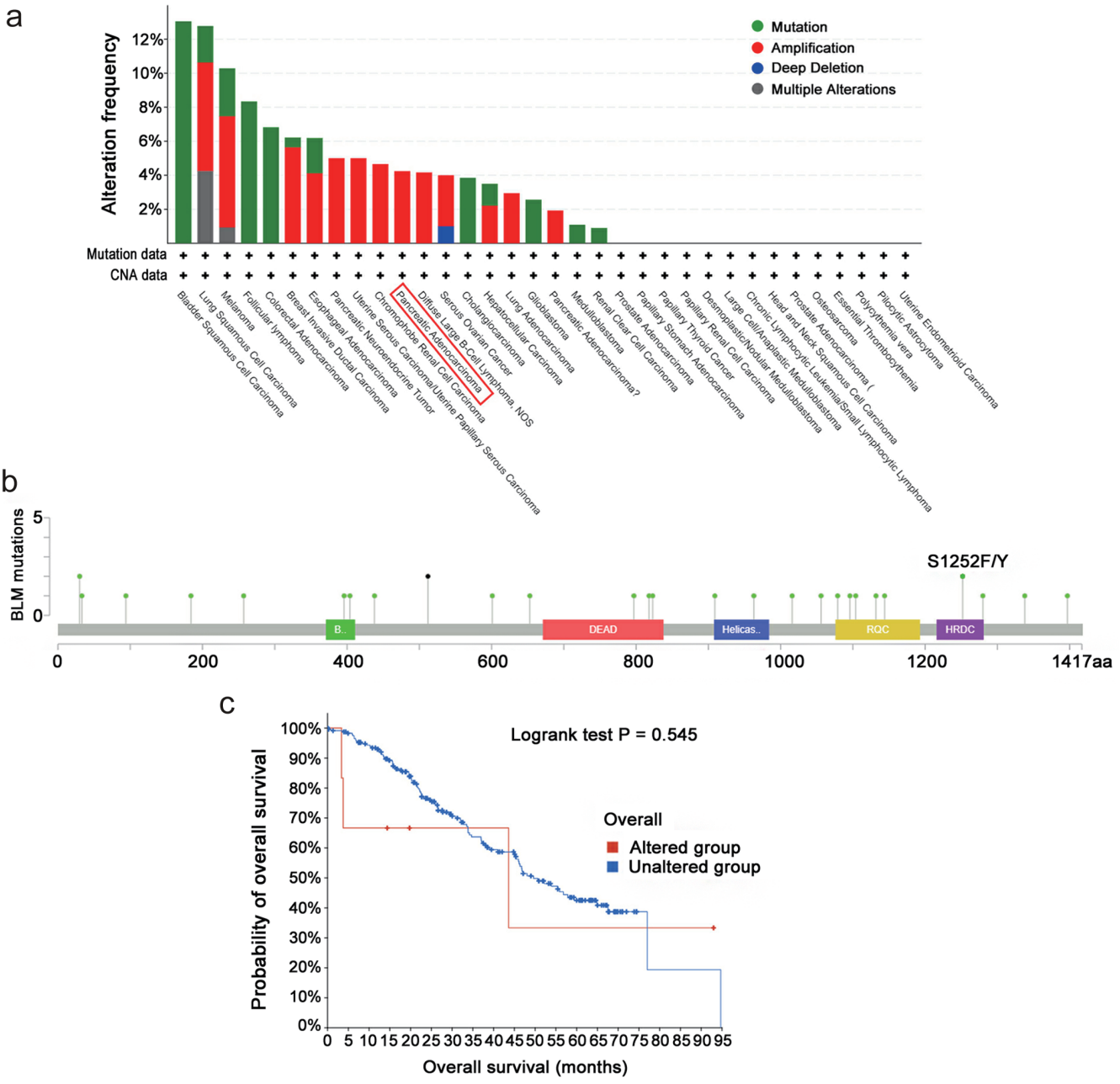


Fig. 4. The BLM gene mutations and the prognosis analysis based on the cBioPortal web. (a) The BLM mutation features were observed in different cancers. (b) The mutation locus of BLM in PAAD. (C) The survival analysis of the BLM mutations in PAAD. BLM, Bloom's syndrome helicase; PADD, pancreatic adenocarcinoma.

groups according to the BLM expression. Differential expression analysis was performed to explore the BLM's potential carcinogenic mechanism. A total of 106 differentially expressed genes (DEGs) with $|\log FC| \geq 1$ and $p < 0.001$ between the two groups were determined, as shown in the heatmap and the volcano plot (Fig. 9a–b). Subsequently, a GO and KEGG functional enrichment analysis for DEGs revealed these genes were involved in some biological processes and pathways closely related to cancer development, including maintaining chromatin stability, cell cycle, and p53 signaling pathway (Fig. 9c–d).

Relationship of immune cell infiltration with BLM in the PAAD cells

As mentioned in the introduction, there were some associations between BLM and the cancer's immune microenvironment. Thus, by first comparing the expression of BLM in different immune cells from PAAD and the normal samples, we found that BLM was highly expressed in various immune cells from PAAD (Fig. 10a). To validate the regulatory role of BLM on the immune cells in PAAD, the level of immune cell infiltration at different BLM

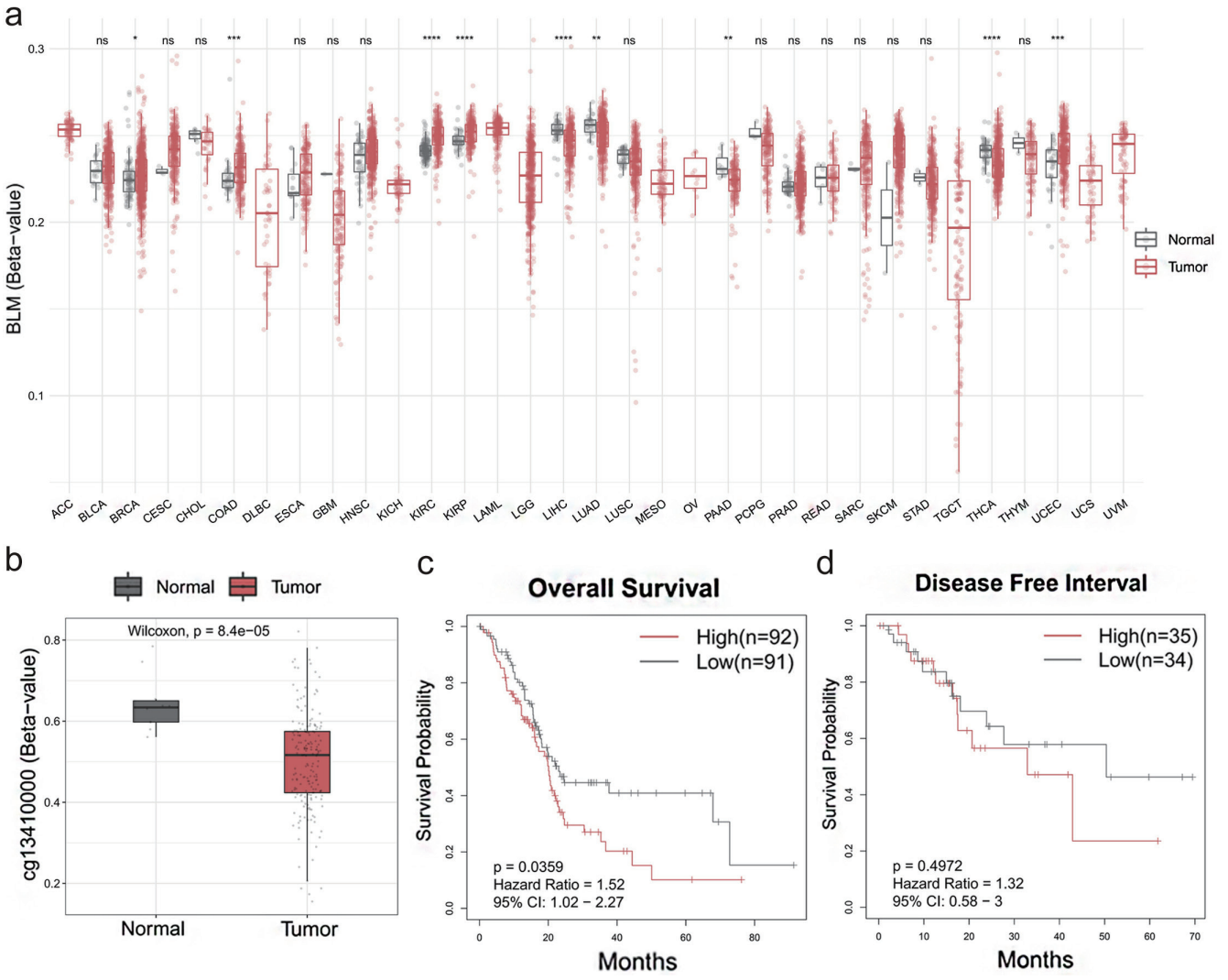


Fig. 5. The methylation level of BLM and the survival analysis based on the SMART database. (a) The pancancer analysis for BLM methylation. (b) The methylation level of BLM in PAAD compared with the normal samples. (c–d) The survival analysis of BLM methylation in PAAD. ns, not significant; **p* value is <0.05; ***p* value is <0.01; ****p* value is <0.001. BLM, Bloom's syndrome helicase; PADD, pancreatic adenocarcinoma.

copy numbers in PAAD was examined using the TIMER database. There were significant differences in the immune infiltration level of the B cells and CD4+ cells under the different BLM copy number variations (Fig. 10b). Furthermore, a correlation analysis of BLM with immune cell infiltration level was performed. The immune infiltration of the B cells, neutrophil cells, and dendritic cells were positively correlated with BLM in PAAD (Fig. 10c–d). Therefore, BLM could facilitate the level of the PAAD immune infiltration.

Relationship of the immune cell biomarkers with BLM in PAAD

To further validate the role of BLM in PAAD immunity, we examined the correlation of BLM with the expression of the immune cell biomarkers in PAAD by using the GEPIA database. Unsurprisingly, the BLM was positively correlated with most immune cell biomarkers (Table 1). These results supported the relationship of BLM with immune cell infiltration in PAAD.

Relationship of BLM with the immune checkpoints in PAAD cells

To explore whether BLM may exert its oncogenic and prognostic role through immune mechanisms, we analyzed the critical immune checkpoints, PD1, PD-L1, CTLA-4, and CD27 responsible for tumor immune escape. From the TIMER database, we found that the BLM expression was positively correlated with the PD1 (PDCD1), PD-L1 (CD274), CTLA-4, and CD27 gene expressions in PAAD (Fig. 11a–c). Simultaneously, these results were confirmed in the GEPIA database (Fig. 11d–f). Consequently, the tumor immune escape could be involved in BLM-mediated PAAD carcinogenesis.

Discussion

There was the highest incidence of PAAD in all pancreatic tu-

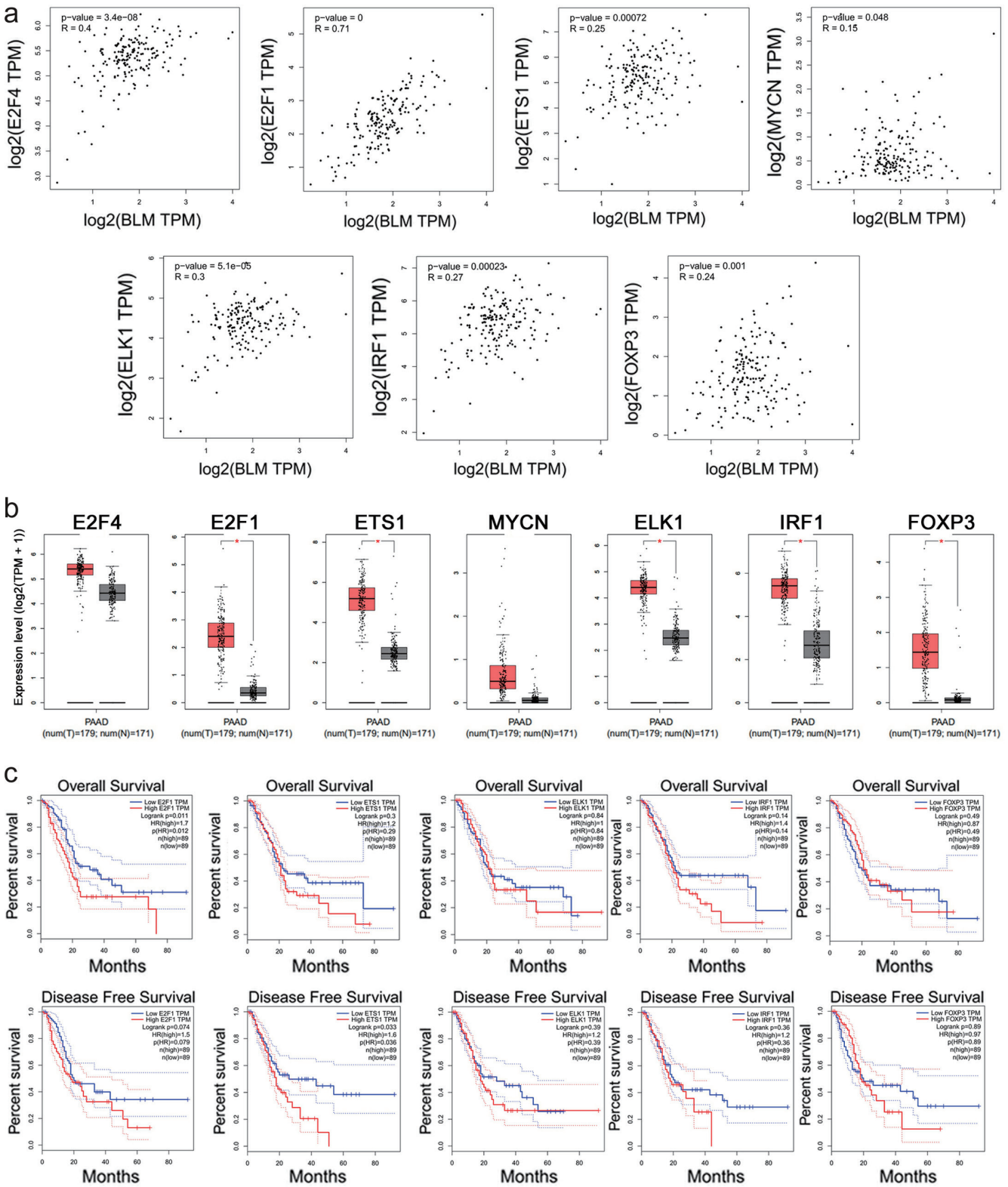
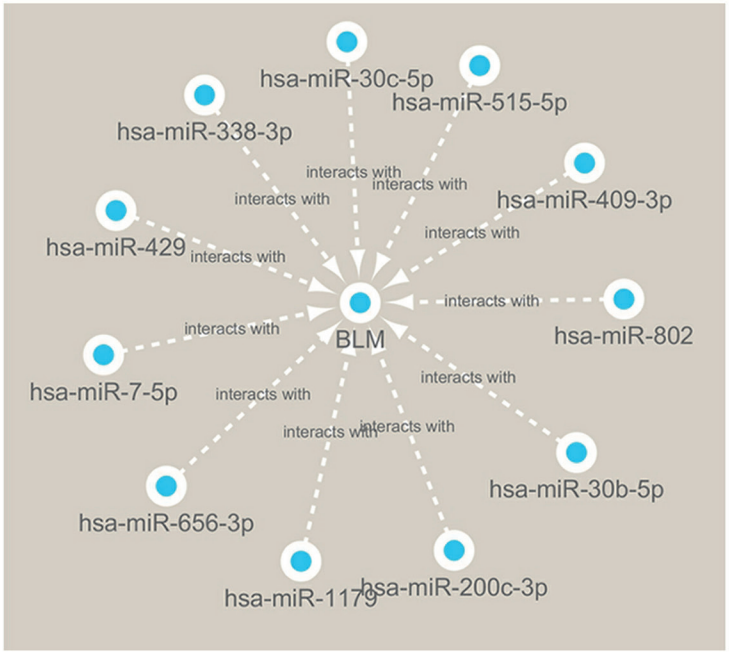


Fig. 6. The prediction and survival analysis of the upstream TFs of BLM based on the ARCHS4 resource and JASPAR2022 database. (a) The correlation analysis of BLM and the selected TFs in PAAD. (b) The differential expression analysis of the TFs that were positively correlated with BLM in PAAD. (c) The survival analysis (OS and RFS) of the most potential upstream TFs of BLM in PAAD. **p* value is <0.05; ***p* value is <0.01; ****p* value is <0.001. BLM, Bloom's syndrome helicase; PAAD, pancreatic adenocarcinoma; TF, transcription factor.

a

Gene	miRNA	R-value	*P-value*
BLM	hsa-miR-7-5p	-0.278	1.76E-04
BLM	hsa-miR-338-3p	-0.272	2.44E-04
BLM	hsa-miR-1179	-0.264	3.67E-04
BLM	hsa-miR-656-3p	-0.194	9.39E-03
BLM	hsa-miR-515-5p	-0.184	1.41E-02
BLM	hsa-miR-30b-5p	-0.171	2.29E-02
BLM	hsa-miR-30c-5p	-0.166	2.64E-02
BLM	hsa-miR-200c-3p	-0.166	2.72E-02
BLM	hsa-miR-802	-0.163	2.93E-02
BLM	hsa-miR-409-3p	-0.162	3.13E-02
BLM	hsa-miR-429	-0.149	4.77E-02

b



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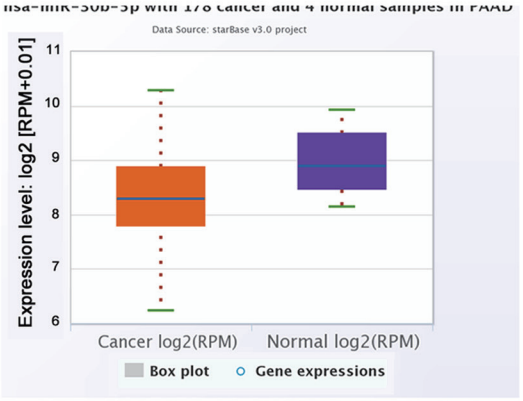

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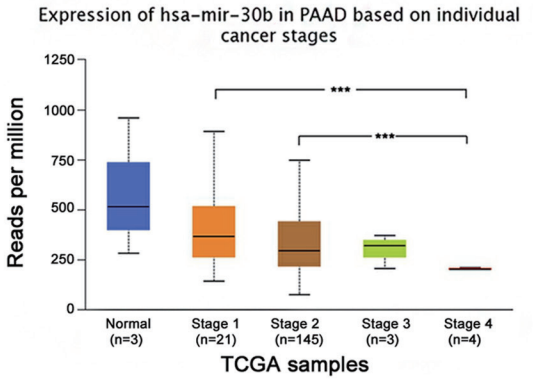

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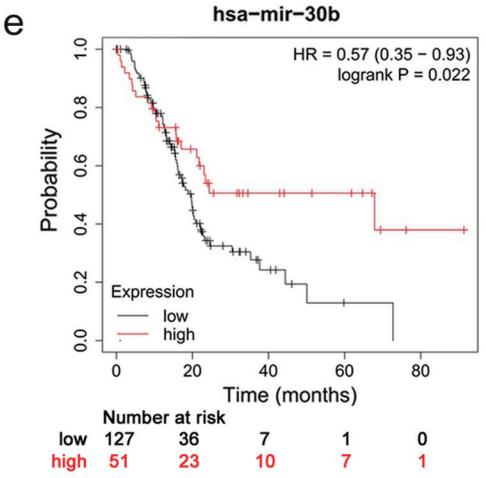


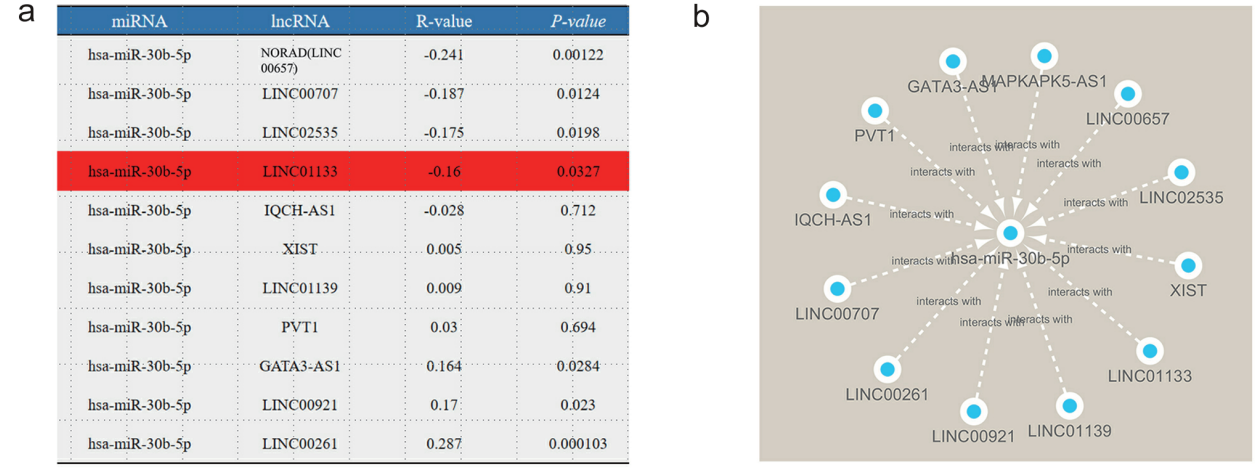
Fig. 7. The prediction and analysis of the upstream miRNAs of BLM. (a) The miRNAs with a negative correlation of BLM based on the Starbase. (b) The miRNAs-BLM relationship network drawn by using Cytoscape software. (c) The differential expression of miR-30b-5p in PAAD. (d) The expression of miR-30b-5p in the different courses of PAAD based on the UALCAN database. (e) The miR-30b-5p prognostic survival curve in PAAD depicted by the Kaplan-Meier plotter. **p* value was <0.05; ***p* value was <0.01; ****p* value was <0.001. BLM, Bloom's syndrome helicase; PAAD, pancreatic adenocarcinoma.

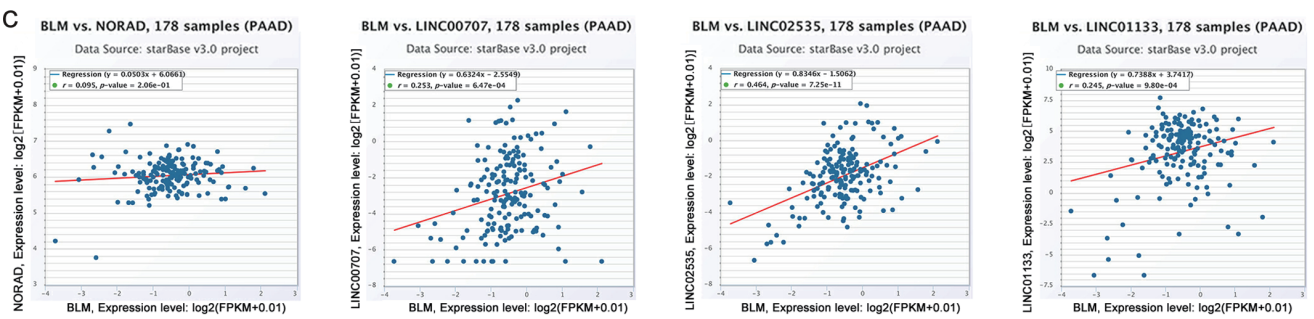
mors accounting for approximately 85% of all cases.[24] Due to the lack of unique clinical symptoms in the early stage, the early detection and treatments for PAAD patients are more difficult, and the five-year survival rate of PAAD is the lowest among all pancreatic tumor types.[25] Therefore, early prevention and effective judgment of prognosis are the keys. Although significant progress had been made in early diagnosis, the overall survival rate of PAAD has not improved in recent years.[26,27] This requires us to exhaustively study its carcinogenic mechanisms, identify potential molecular markers, and find new therapeutic targets. In recent years, the role of BLM in DNA damage repair and the carcinogenesis mechanism has gradually received attention. However, the regulatory mechanism and oncogenic role in PAAD still remain unknown.

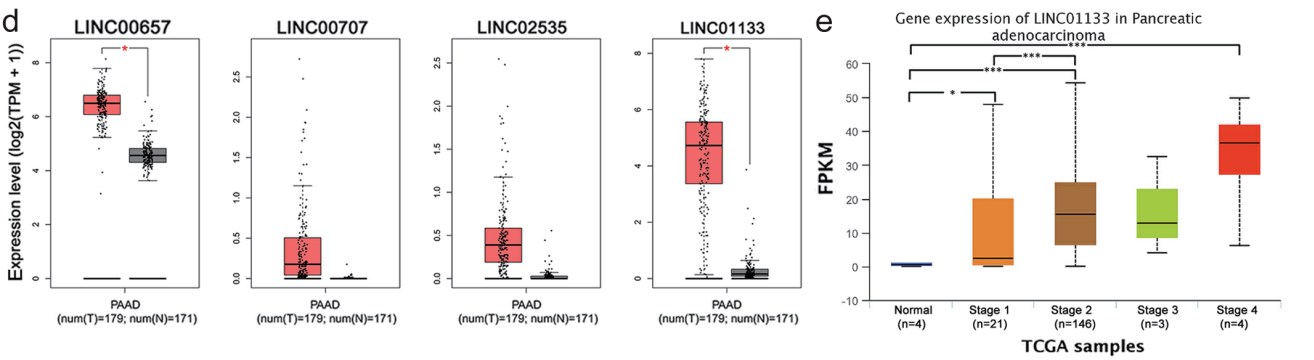
In this study, a pancancer analysis using the TCGA and GETx databases showed that BLM was highly expressed in most cancers, which meant that BLM had extensive oncogenic effects. We further performed a prognostic analysis in cancer patients through

a

miRNA	lncRNA	R-value	*P-value*
hsa-miR-30b-5p	NORAD(LINC 00657)	-0.241	0.00122
hsa-miR-30b-5p	LINC00707	-0.187	0.0124
hsa-miR-30b-5p	LINC02535	-0.175	0.0198
hsa-miR-30b-5p	LINC01133	-0.16	0.0327
hsa-miR-30b-5p	IQCH-AS1	-0.028	0.712
hsa-miR-30b-5p	XIST	0.005	0.95
hsa-miR-30b-5p	LINC01139	0.009	0.91
hsa-miR-30b-5p	PVT1	0.03	0.694
hsa-miR-30b-5p	GATA3-AS1	0.164	0.0284
hsa-miR-30b-5p	LINC00921	0.17	0.023
hsa-miR-30b-5p	LINC00261	0.287	0.000103







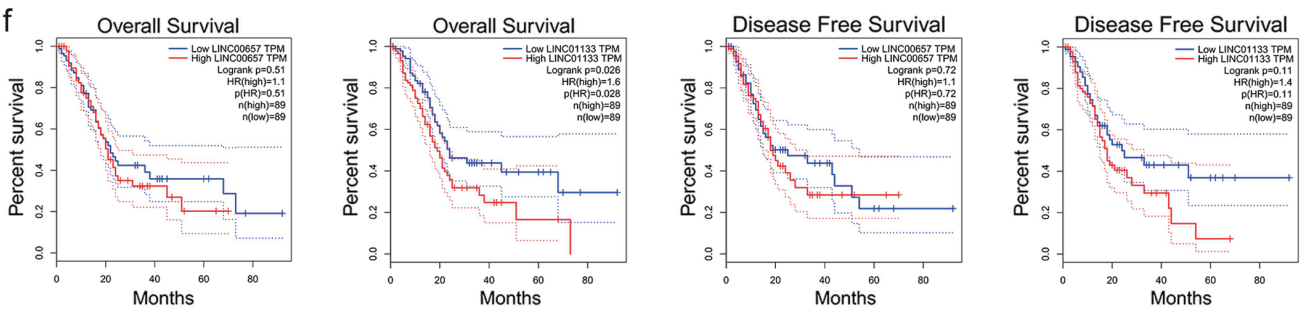


Fig. 8. The prediction and analysis of the upstream lncRNAs of miR-30b-5p. (a) lncRNAs with a negative correlation of miR-30b-5p based on Starbase. (b) The lncRNAs-miRNA relationship network drawn by using Cytoscape software. (c) The correlation analysis of the related lncRNAs and BLM in PAAD based on Starbase. (d) The expression of the related lncRNAs in PAAD based on the GEPIA database. (e) The LINC01133 expression in different courses of PAAD based on the UALCAN database. (f) The prognostic survival curves (OS and RFS) of LINC00657 (NORAD) and LINC01133 in PAAD based on the GEPIA database. **p* value is <0.05; ***p* value is <0.01; ****p* value is <0.001. PAAD, pancreatic adenocarcinoma.

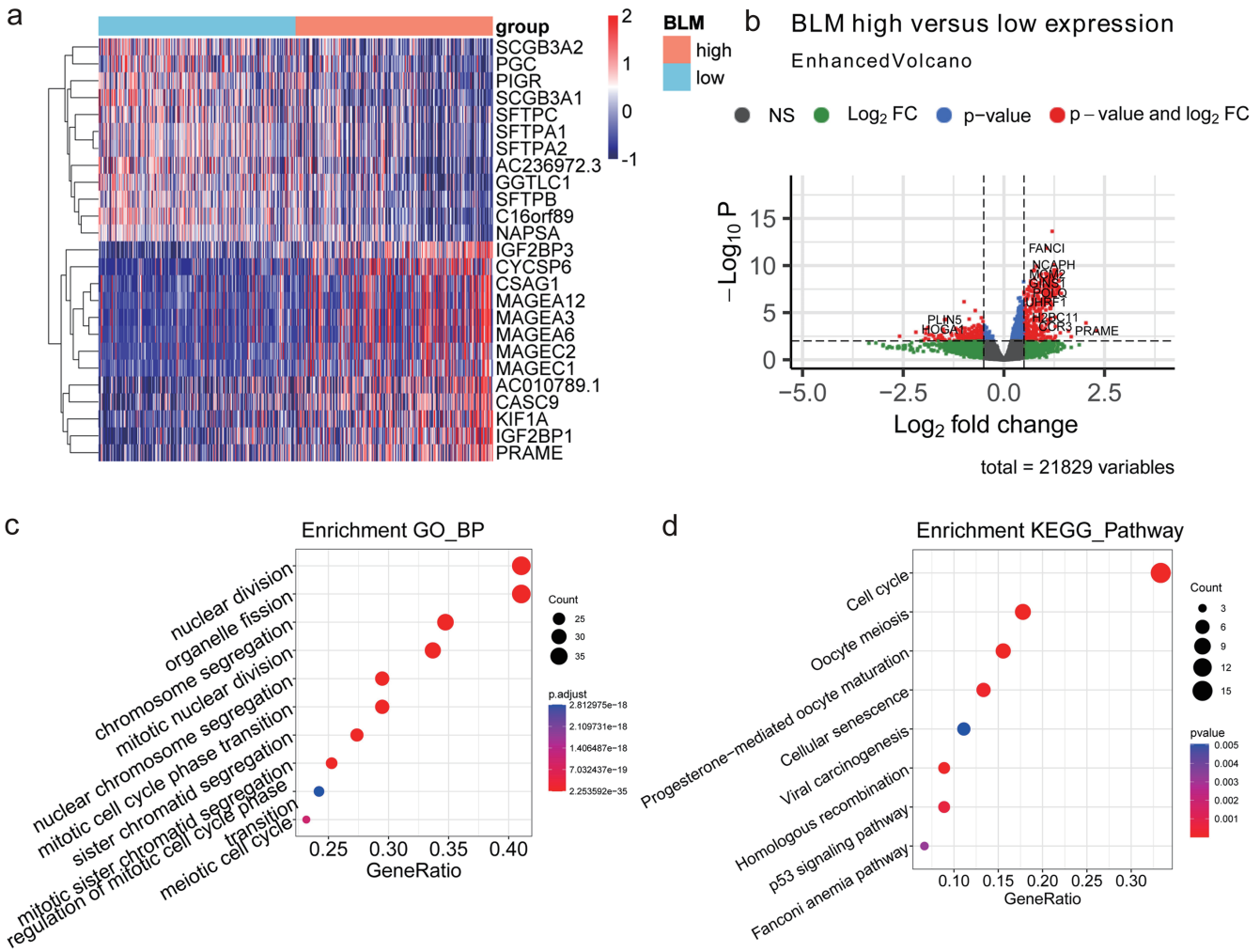


Fig. 9. The differential expression analysis for the high and low BLM expression in PAAD. (a) The heatmap of the DEGs in PAAD (blue: low expression level; orange: high expression level). (b) The differential expression of the volcano plot based on the DEGs. Function enrichment analysis of the DEGs of GO (c) and KEGG (d). BLM, Bloom's syndrome helicase; DEGs, differentially expressed genes; PAAD, pancreatic adenocarcinoma.

two survival indicators (OS and FRS), and found that the high expression of BLM had a significantly poor prognosis for the PAAD patients. Consequently, BLM could also be a prognostic marker of PAAD. To investigate the regulatory mechanisms of the high BLM expression in PAAD, we explored both the genetic and epigenetic regulations. As with most cancers and their mechanisms of origin, PAAD is also initiated by mutations in the proto-oncogene, tumor suppressor genes, and DNA repair genes. Both activating and inactivating mutations in these genes lead to cancer progression.[28] Moreover, Bononi *et al*. found that mutations in DNA damage repair genes were present in various cancers.[29] Thus, we analyzed the frequency and type of the BLM mutations and found many amplification mutations in BLM, and that structural mutation appeared on S1252F/Y of the HDRC domain, which could partly be responsible for the high BLM expression. At the same time, the mutations in BLM had no significant effect on the prognosis of the PAAD patients.

As for the epigenetic aspects, DNA methylation is a stable epigenetic modification that contributes to the spatiotemporal regulation of the gene expression. Traditionally, dense promoter DNA methylation has been associated with the transcriptional repression.[30] Therefore, we verified whether the methylation level was also changed in the BLM promoter region. As expected, the methylation level of the BLM promoter was significantly reduced in PAAD compared with the normal tissues, thus suggesting that the high BLM expression could be regulated by hypomethylation. Next, we also explored the potential transcription factor targets of BLM, which could be bound to specific gene sequences to play a role in regulating their expression and form gene regulatory networks (GRNs).[31] Combined with the target prediction and correlation analyses, we identified the five most likely potential upstream TFs of BLM. We found that TFs (E2F1 and ETS1), consistently with BLM, marked a poor prognosis in the PAAD patients.

In addition to DNA methylation and TFs, the effect of ncRNA on mRNA is also an essential epigenetic regulatory mechanism. The ceRNA hypothesis suggests that multiple RNAs are involved in the regulatory network of ceRNA, including lncRNA, miRNA, circular RNA, and mRNA. Among them, miRNA can bind to the untranslated region of mRNA to inhibit the translation of mRNA.[32] In contrast, lncRNA competitively binds to miRNA through microRNA response elements (MREs) and inhibits the

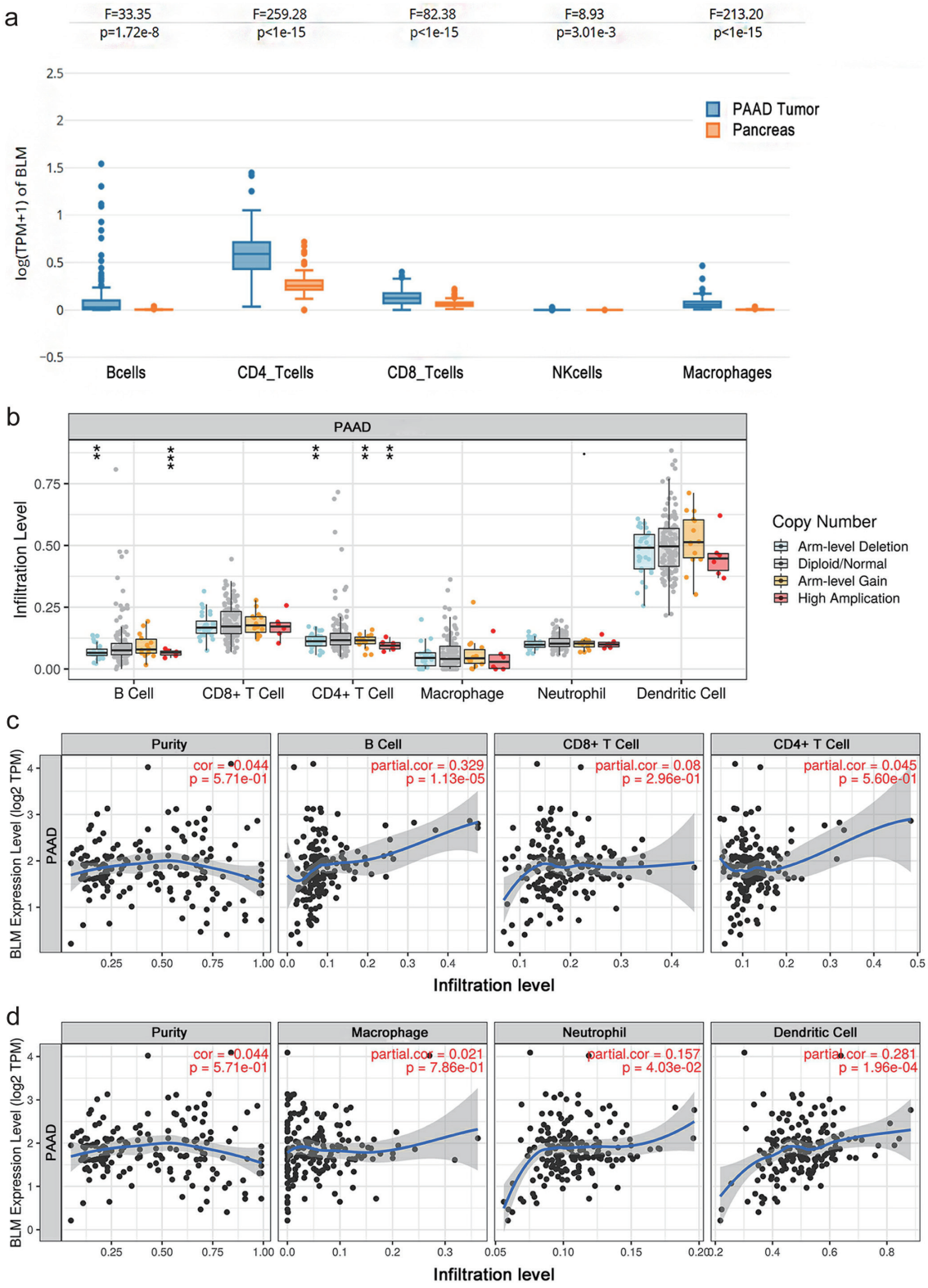


Fig. 10. The relationship of the immune cell infiltration with BLM in the PAAD cells. (a) The differential expression of BLM in different immune cells by comparing PAAD versus the normal tissue samples based on the GEPIA database. (b) The relationship of different BLM copy numbers with the immune cell infiltration level in PAAD based on the TIMER database. (c–d) The correlation analysis of BLM with the different levels of immune cell infiltration in PAAD based on the TIMER database. **p* value <0.05; ***p* value <0.01; ****p* value <0.001. BLM, Bloom's syndrome helicase; PADD, pancreatic adenocarcinoma.

Table 1. The correlation analysis of BLM with the biomarkers of the immune cells in PAAD determined by the GEPIA database

Immune cell	Biomarker	R-value	*p* value
B cell	CD19	0.28	0.00013***
	CD79A	0.29	0.00011***
CD8+ T cell	CD8A	0.14	0.063
	CD8B	0.17	0.02*
CD4+ T cell	CD4	0.18	0.015*
M1 macrophage	NOS2	0.1	0.17
	IRF5	0.16	0.029*
	PTGS2	0.11	0.16
M2 macrophage	CD163	0.27	0.00022***
	VSIG4	0.23	0.0019***
	MS4A4A	0.17	0.02*
Neutrophil	CEACAM8	-0.051	0.5
	ITGAM	0.067	0.37
	CCR7	0.25	0.00061***
Dendritic cell	HLA-DPB1	0.15	0.052
	HLA-DQB1	0.073	0.33
	HLA-DRA	0.15	0.043*
	HLA-DPA1	0.13	0.072
	CD1C	0.089	0.23
	NRP1	−0.091	0.22
	ITGAX	0.067	0.38

**p* value <0.05; ***p* value <0.01; ****p* value <0.001. 1PTGS2, Prostaglandin-Endoperoxide Synthase 2; BLM, Bloom's syndrome helicase; CCR7, C-C Motif Chemokine Receptor 7; CD163, Hemoglobin Scavenger Receptor; CD19, B-Lymphocyte Surface Antigen B4; CD1C, T-Cell Surface Glycoprotein CD1c; CD4, T-Cell Surface Glycoprotein CD4; CD79A, B-Cell Antigen Receptor Complex-Associated Protein; CD8A, T-Cell Surface Glycoprotein CD8 Alpha Chain; CD8B, T-Cell Surface Glycoprotein CD8 Beta Chain; CEACAM8, CEA Cell Adhesion Molecule 8; HLA-DPA1, Major Histocompatibility Complex, Class II, DP Alpha 1; HLA-DPB1, Major Histocompatibility Complex, Class II, DP Beta 1; HLA-DQB1, Major Histocompatibility Complex, Class II, DQ Beta 1; HLA-DRA, Major Histocompatibility Complex, Class II, DR Alpha; IRF5, Interferon Regulatory Factor 5; ITGAM, Integrin Subunit Alpha M; ITGAX, Integrin Subunit Alpha X; MS4A4A, Membrane Spanning 4-Domains A4A; NOS2, Nitric Oxide Synthase 2; NRP1, Neuropilin; PAAD, pancreatic adenocarcinoma; VSIG4, V-Set and Immunoglobulin Domain Containing 4.

downregulation of mRNA mediated by miRNA.[33] Many lncRNA-miRNA/mRNA axes have been confirmed to be involved in regulating tumor development.[34] Therefore, we next explored the regulatory axis of the miRNA-lncRNA upstream of BLM by combining the consistency of the correlation and prognostic analysis, and finally determined that the LINC01133-miR-30b-5p axis could be involved in the regulation of BLM in PAAD. Moreover, the LINC01133-miR-30b-5p axis regulates Rab3D in renal cell carcinoma (RCC).[35]

It is well-known that DNA repair defects can lead to immunodeficiency, as DNA repair is essential for developing antigen receptors expressed in both the B and T lymphocytes. These antigen receptors are formed by the recombination of the variables (V), diversity (D), and the (J) genes at the antigen receptor locus.[36] During the V, D, and J recombination, non-homologous end joining (NHEJ) and class-switch recombination (CSR) DNA repair pathway connections would be required.[36] BLM is also a helicase involved in DNA repair, and immune cells are an essential factor affecting pancreatic cancer's pathogenesis and disease severity.[37] Therefore, we next further analyzed the relationship of BLM with the immune cell infiltration in PAAD. The results showed that BLM expression was positively correlated with the infiltration level of the multiple immune cells, including the B cells, neutrophil, and dendritic cells. Simultaneously, combined with the analysis of the immune markers, we could reasonably speculate that BLM promotes the infiltration of the immune cells in PAAD.

To further explore whether BLM exerts its oncogenic and influencing prognostic effects through the immune system, we considered the infiltration level of the immune cells and the 'immune checkpoints' mechanism. A higher level of immune cell infiltration usually suggests a good prognosis.[38] In contrast, the immune checkpoint mechanism indicates that tumor cells can utilize checkpoint-regulated inhibitory effects to evade the immune response.[39] PD-1, the most common immune checkpoint, is expressed in T lymphocytes and other immune cells, while PD-L1 is one of its ligands, and the binding of PD-1 and PD-L1 causes inhibitory signals in the T cells.[40] Immune checkpoint CTLA-4 is a receptor with inhibitory effects on T lymphocyte activation.[41] CD27, a co-stimulatory immune-checkpoint receptor, was expressed on a broad range of T-cells, NK-cells, and B-cells.[42] Our results demonstrated that the high expression of BLM was correlated with the most immune cells infiltration level in PAAD, while the prognosis in PAAD with the high level of BLM was poor. Therefore, we

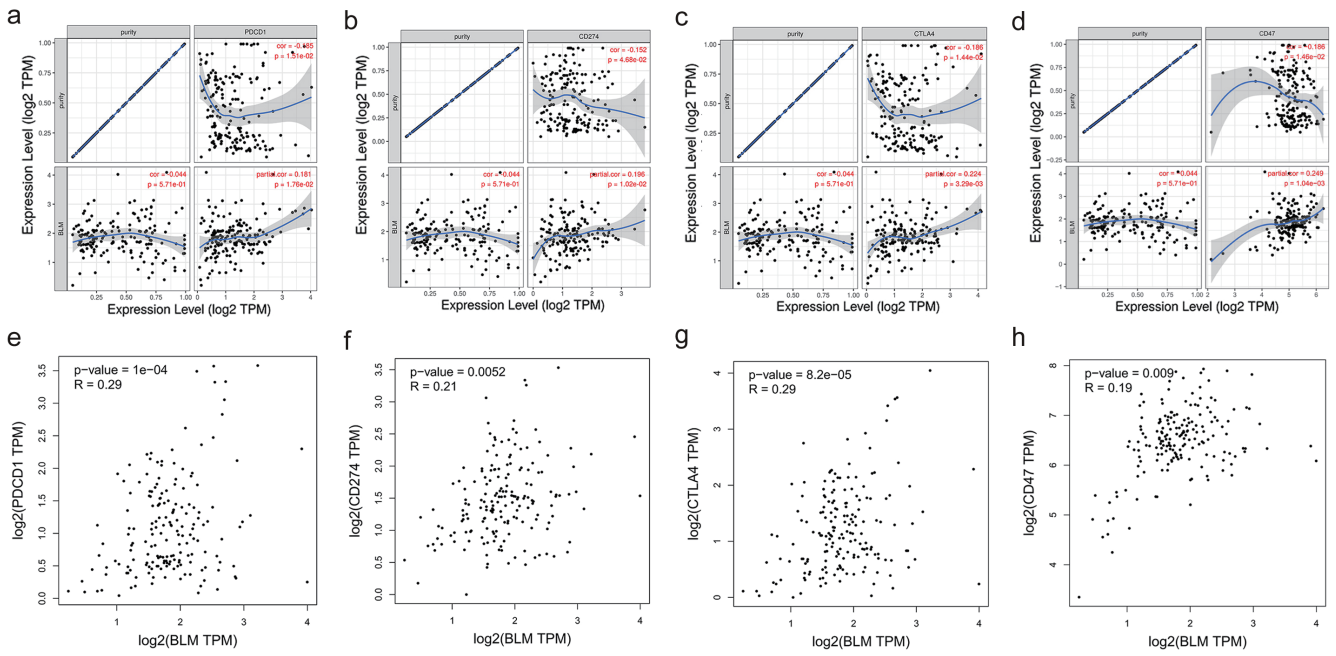


Fig. 11. The relationship of BLM with the immune checkpoints in PAAD. (a–c) Based on the TIMER database, the correlation analysis of BLM with the immune checkpoints, PD1 (PDCD1), PD-L1 (CD274), CTLA-4, and CD27 in the PAAD. (d–f) The correlation analysis of BLM with the immune checkpoints of PD1 (PDCD1), PD-L1 (CD274), CTLA-4, and CD27 in PAAD based on the GEPIA database. BLM, Bloom's syndrome helicase; PAAD, pancreatic adenocarcinoma.

validated the relationship of BLM with PD-1, PD-L1, CTLA-4, and CD27 in PAAD. Not unexpected, BLM showed a significant correlation with all three immune checkpoints. These results could explain why PAAD patients with a high level of BLM had a poor prognosis.

Taken together, the high expression of BLM regulated by gene mutation, hypomethylation, TFs, and the LINC01133-miR-30b-5p/axis was associated with a poor prognosis of PAAD. This may promote cancer development by regulating the immune checkpoints, consequently affecting its prognosis (Fig. 12). However, this deduction would need to be confirmed by biological experiments in the future.

Conclusions

Our study revealed the critical role of BLM as a potential biomarker in the PAAD prognosis and immune microenvironment by providing new targets for the treatment and prognosis research of PAAD.

Supporting information

Supplementary material for this article is available at https://doi.org/10.14218/CSP.2022.00015.

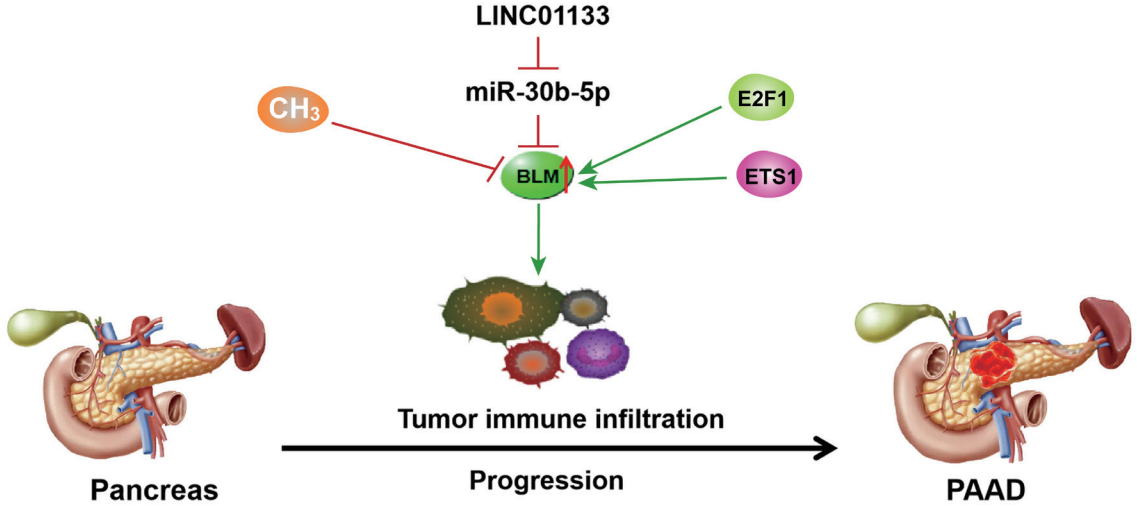


Fig. 12. The model of BLM in the carcinogenesis of PAAD. BLM, Bloom's syndrome helicase; PAAD, pancreatic adenocarcinoma.

Supplementary Table 1. Upstream TFs of BLM in humans based on ARCHS4 resource.

Supplementary Table 2. The binding sides of 48 related TFs in BLM based on JASPAR2022 database.

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Conflict of interest

The authors declare no competing interests.

Author contributions

QL designed the study protocol and wrote the manuscript. A-HS and PZ were responsible for the literature review and the manuscript discussion. H-XH and Y-NZ contributed to the statistical analyses. J-FY gave valuable suggestions. B-RH designed, advised, and revised the manuscript. All authors contributed to the article and approved the submitted version.

Data sharing statement

Public data sets used for this study can be found in the Cancer Genome Atlas (https://portal.gdc.cancer.gov/).

References

[1] Gonzalez RS, Raza A, Propst R, Adeyi O, Bateman J, Sopha SC, *et al*. Recent Advances in Digestive Tract Tumors: Updates From the 5th Edition of the World Health Organization "Blue Book". Arch Pathol Lab Med 2021;145(5):607–626. doi:10.5858/arpa.2020-0047-RA, PMID:32886739.

[2] Tang R, Zhang Y, Liang C, Xu J, Meng Q, Hua J, *et al*. The role of m6A-related genes in the prognosis and immune microenvironment of pancreatic adenocarcinoma. PeerJ 2020;8:e9602. doi:10.7717/peerj.9602, PMID:33062408.

[3] Ilic M, Ilic I. Epidemiology of pancreatic cancer. World J Gastroenterol 2016;22(44):9694–9705. doi:10.3748/wjg.v22.i44.9694, PMID:27956793.

[4] Henriksen A, Dyhl-Polk A, Chen I, Nielsen D. Checkpoint inhibitors in pancreatic cancer. Cancer Treat Rev 2019;78:17–30. doi:10.1016/j.ctrv.2019.06.005, PMID:31325788.

[5] Newman JA, Savitsky P, Allerston CK, Bizard AH, Özer Ö, Sarlós K, *et al*. Crystal structure of the Bloom's syndrome helicase indicates a role for the HRDC domain in conformational changes. Nucleic Acids Res 2015;43(10):5221–5235. doi:10.1093/nar/gkv373, PMID:25901030.

[6] Croteau DL, Popuri V, Opresko PL, Bohr VA. Human RecQ helicases in DNA repair, recombination, and replication. Annu Rev Biochem 2014;83:519–552. doi:10.1146/annurev-biochem-060713-035428, PMID:24606147.

[7] Nimonkar AV, Genschel J, Kinoshita E, Polaczek P, Campbell JL, Wyman C, *et al*. BLM-DNA2-RPA-MRN and EXO1-BLM-RPA-MRN constitute two DNA end resection machineries for human DNA break repair. Genes Dev 2011;25(4):350–362. doi:10.1101/gad.2003811, PMID:21325134.

[8] Shi J, Chen WF, Zhang B, Fan SH, Ai X, Liu NN, *et al*. A helical bundle in the N-terminal domain of the BLM helicase mediates dimer and potentially hexamer formation. J Biol Chem 2017;292(14):5909–5920. doi:10.1074/jbc.M116.761510, PMID:28228481.

[9] Wang CX, Zhang ZL, Yin QK, Tu JL, Wang JE, Xu YH, *et al*. Design, Synthesis, and Evaluation of New Quinazolinone Derivatives that Inhibit Bloom Syndrome Protein (BLM) Helicase, Trigger DNA Damage at the Telomere Region, and Synergize with PARP Inhibitors. J Med Chem 2020;63(17):9752–9772. doi:10.1021/acs.jmedchem.0c00917, PMID:32697083.

[10] Kondo T, Kanai M, Kou T, Sakuma T, Mochizuki H, Kamada M, *et al*. Association between homologous recombination repair gene mutations and response to oxaliplatin in pancreatic cancer. Oncotarget 2018;9(28):19817–19825. doi:10.18632/oncotarget.24865, PMID:29731985.

[11] Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012;12(4):252–264. doi:10.1038/nrc3239, PMID:22437870.

[12] Ohaegbulam KC, Assal A, Lazar-Molnar E, Yao Y, Zang X. Human cancer immunotherapy with antibodies to the PD-1 and PD-L1 pathway. Trends Mol Med 2015;21(1):24–33. doi:10.1016/j.molmed.2014.10.009, PMID:25440090.

[13] Schoenaker MHD, Henriet SS, Zonderland J, van Deuren M, Pan-Hammarström Q, Posthumus-van Sluijs SJ, *et al*. Immunodeficiency in Bloom's Syndrome. J Clin Immunol 2018;38(1):35–44. doi:10.1007/s10875-017-0454-y, PMID:29098565.

[14] Sepulveda JL. Using R and Bioconductor in Clinical Genomics and Transcriptomics. J Mol Diagn 2020;22(1):3–20. doi:10.1016/j.jmoldx.2019.08.006, PMID:31605800.

[15] Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res 2017;45(W1):W98–W102. doi:10.1093/nar/gkx247, PMID:28407145.

[16] Lei Y, Yu T, Li C, Li J, Liang Y, Wang X, *et al*. Expression of CAMK1 and its association with clinicopathologic characteristics in pancreatic cancer. J Cell Mol Med 2021;25(2):1198–1206. doi:10.1111/jcmm.16188, PMID:33342045.

[17] Gao JJ, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, *et al*. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 2013;6(269):pl1. doi:10.1126/scisignal.2004088, PMID:23550210.

[18] Li Y, Ge D, Lu C. The SMART App: an interactive web application for comprehensive DNA methylation analysis and visualization. Epigenetics Chromatin 2019;12(1):71. doi:10.1186/s13072-019-0316-3, PMID:31805986.

[19] Lachmann A, Torre D, Keenan AB, Jagodnik KM, Lee HJ, Wang L, *et al*. Massive mining of publicly available RNA-seq data from human and mouse. Nat Commun 2018;9(1):1366. doi:10.1038/s41467-018-03751-6, PMID:29636450.

[20] Li JH, Liu S, Zhou H, Qu LH, Yang JH. starBase v2.0: decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. Nucleic Acids Res 2014;42(Database issue):D92–D97. doi:10.1093/nar/gkt1248, PMID:24297251.

[21] Győrffy B, Surowiak P, Budczies J, Lánczky A. Online survival analysis software to assess the prognostic value of biomarkers using transcriptomic data in non-small-cell lung cancer. PLoS One 2013;8(12):e82241. doi:10.1371/journal.pone.0082241, PMID:24367507.

[22] Li T, Fan J, Wang B, Traugh N, Chen Q, Liu JS, *et al*. TIMER: A Web Server for Comprehensive Analysis of Tumor-Infiltrating Immune Cells. Cancer Res 2017;77(21):e108–e110. doi:10.1158/0008-5472.CAN-17-0307, PMID:29092952.

[23] Thomson DW, Dinger ME. Endogenous microRNA sponges: evidence and controversy. Nat Rev Genet 2016;17(5):272–283. doi:10.1038/nrg.2016.20, PMID:27040487.

[24] Taffel MT, Luk L, Ream JM, Rosenkrantz AB. Exploratory Study of Apparent Diffusion Coefficient Histogram Metrics in Assessing Pancreatic

Malignancy. Can Assoc Radiol J 2019;70(4):416–423. doi:10.1016/j.carj.2019.07.001, PMID:31604596.
[25] Liu Y, Xu G, Li L. LncRNA GATA3-AS1-miR-30b-5p-Tex10 axis modulates tumorigenesis in pancreatic cancer. Oncol Rep 2021;45(5):59. doi:10.3892/or.2021.8010, PMID:33760161.
[26] Deng T, Yuan Y, Zhang C, Zhang C, Yao W, Wang C, *et al*. Identification of Circulating MiR-25 as a Potential Biomarker for Pancreatic Cancer Diagnosis. Cell Physiol Biochem 2016;39(5):1716–1722. doi:10.1159/000447872, PMID:27639768.
[27] Staal B, Liu Y, Barnett D, Hsueh P, He Z, Gao C, *et al*. The sTRA Plasma Biomarker: Blinded Validation of Improved Accuracy Over CA19-9 in Pancreatic Cancer Diagnosis. Clin Cancer Res 2019;25(9):2745–2754. doi:10.1158/1078-0432.CCR-18-3310, PMID:30617132.
[28] Naqvi AAT, Hasan GM, Hassan MI. Investigating the role of transcription factors of pancreas development in pancreatic cancer. Pancreatology 2018;18(2):184–190. doi:10.1016/j.pan.2017.12.013, PMID:29289465.
[29] Bononi A, Goto K, Ak G, Yoshikawa Y, Emi M, Pastorino S, *et al*. Heterozygous germline *BLM* mutations increase susceptibility to asbestos and mesothelioma. Proc Natl Acad Sci U S A 2020;117(52):33466–33473. doi:10.1073/pnas.2019652117, PMID:33318203.
[30] Koroknai V, Szász I, Hernandez-Vargas H, Fernandez-Jimenez N, Cuenin C, Herceg Z, *et al*. DNA hypermethylation is associated with invasive phenotype of malignant melanoma. Exp Dermatol 2020;29(1):39–50. doi:10.1111/exd.14047, PMID:31602702.
[31] Lambert SA, Jolma A, Campitelli LF, Das PK, Yin Y, Albu M, *et al*. The Human Transcription Factors. Cell 2018;172(4):650–665. doi:10.1016/j.cell.2018.01.029, PMID:29425488.
[32] McGuire A, Brown JA, Kerin MJ. Metastatic breast cancer: the potential of miRNA for diagnosis and treatment monitoring. Cancer Metastasis Rev 2015;34(1):145–155. doi:10.1007/s10555-015-9551-7, PMID:25721950.
[33] Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP. A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? Cell 2011;146(3):353–358. doi:10.1016/j.cell.2011.07.014, PMID:21802130.
[34] Tang JM, Yu B, Li YX, Zhang WW, Alvarez AA, Hu B, *et al*. TGF-β-activated lncRNA LINC00115 is a critical regulator of glioma stem-like cell tumorigenicity. EMBO Rep 2019;20(12):e48170. doi:10.15252/embr.201948170, PMID:31599491.
[35] Zhai X, Wu Y, Wang Z, Zhao D, Li H, Chong T, *et al*. Long Noncoding RNA LINC01133 Promotes the Malignant Behaviors of Renal Cell Carcinoma by Regulating the miR-30b-5p/Rab3D Axis. Cell Transplant 2020;29:963689720964413. doi:10.1177/0963689720964413, PMID:33054325.
[36] Peters JM. How DNA loop extrusion mediated by cohesin enables V(D)J recombination. Curr Opin Cell Biol 2021;70:75–83. doi:10.1016/j.ceb.2020.11.007, PMID:33422934.
[37] Zheng L, Xue J, Jaffee EM, Habtezion A. Role of immune cells and immune-based therapies in pancreatitis and pancreatic ductal adenocarcinoma. Gastroenterology 2013;144(6):1230–1240. doi:10.1053/j.gastro.2012.12.042, PMID:23622132.
[38] Badalamenti G, Fanale D, Incorvaia L, Barraco N, Listì A, Maragliano R, *et al*. Role of tumor-infiltrating lymphocytes in patients with solid tumors: Can a drop dig a stone? Cell Immunol 2019;343:103753. doi:10.1016/j.cellimm.2018.01.013, PMID:29395859.
[39] Sioud M. Releasing the Immune System Brakes Using siRNAs Enhances Cancer Immunotherapy. Cancers (Basel) 2019;11(2):E176. doi:10.3390/cancers11020176, PMID:30717461.
[40] Borch TH, Donia M, Andersen MH, Svane IM. Reorienting the immune system in the treatment of cancer by using anti-PD-1 and anti-PD-L1 antibodies. Drug Discov Today 2015;20(9):1127–1134. doi:10.1016/j.drudis.2015.07.003, PMID:26189934.
[41] Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA, Sharpe AH. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. Immunity 1995;3(5):541–547. doi:10.1016/1074-7613(95)90125-6, PMID:7584144.
[42] Grant EJ, Nüssing S, Sant S, Clemens EB, Kedzierska K. The role of CD27 in anti-viral T-cell immunity. Curr Opin Virol 2017;22:77–88. doi:10.1016/j.coviro.2016.12.001, PMID:28086150.