

High LOXL1-AS1 Lncrna Expression is a Prognostic Biomarker For Poor Prognosis in Cholangiocarcinoma

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1. Abstract

Cholangiocarcinoma (CHOL) is the most common malignant tumor of hepatobiliary system. The present study investigated the clinical significance and biological functions of LOXL1-AS1 in CHOL. The poor survival in CHOL has a probable prognostic molecular marker known as the LOXL1-AS1, and LOXL1-AS1 may be an important regulator of immune cell infiltration.

1.1. Background: Cholangiocarcinoma (CHOL) is the most common malignant tumor of hepatobiliary system, characterized by a poor prognosis and resistance to chemotherapeutics. LOXL1 antisense RNA 1 (LOXL1-AS1) dysregulation is critical in tumors, which serves a pivotal role in the biological characteristics but its expression and functions in CHOL are unclear.

1.2. Methods: In this study, we compared the LOXL1-AS1 mRNA expression between CHOL tissues and normal tissues. Our data was collected from The Cancer Genome Atlas (TCGA), the R and Gene Expression Profiling Interactive Analysis (GEPIA) identified

the correlation of LOXL1-AS1 with CHOL.

1.3. Results: The present study investigated the clinical significance and biological functions of LOXL1-AS1 in CHOL. LOXL1-AS1 was highly expressed in CHOL, which was associated with tumor regional lymph node metastasis and poor prognosis. Multivariate analysis revealed that the up-regulated LOXL1-AS1 expression is an independent prognostic factor for poor prognosis. Moreover, LOXL1-AS1 expression level has significant positive correlations with infiltrating levels of Tem cells and negative correlations with infiltrating levels of DC cells. In addition, interleukins, leishmania infection, cell surface interactions at the vascular wall, FC epsilon receptor signaling and immunoregulatory interactions between a lymphoid and a non-lymphoid were differentially enriched in the high LOXL1-AS1 expression phenotype pathway.

1.4. Conclusion: We conclude that the poor survival in CHOL has a probable prognostic molecular marker known as the LOXL1-AS1, and LOXL1-AS1 may be an important regulator of immune cell infiltration.

2. Introduction

Cholangiocarcinoma (CHOL) is the most common malignant tumor of hepatobiliary system, characterized by a poor prognosis and resistance to chemotherapeutics [1]. In most cases, CHOL is resistant to radiation, cytotoxic and hormone therapies, and surgery is the main treatment for CHOL [2, 3]. Because the early clinical symptoms of CHOL are not obvious, it often has peripheral infiltration and distant metastasis at the time of diagnosis [2, 4]. The average survival time of untreated CHOL after clinical symptoms is 3 to 4 months [2, 5]. Therefore, searching for new sensitive biomarkers are important for early diagnosis of CHOL.

LOXL1-AS1(LOXL1 Antisense RNA 1) was initially identified with the lncRNA class, which has been reported to associate with Exfoliation Syndrome and modulate tumor progression in several types of cancer [6-8]. Expression of LOXL1-AS1 modulates the proliferation and migration of human choriocarcinoma cells through miR-515-5p and NF- κ B signaling pathways [9]. Expression of LOXL1-AS1 modulates facilitates the proliferation and inflammation of chondrocytes through miR-423-5p-mediated KDM5C signaling pathways. However, the expression and role of LOXL1-AS1 in cholangiocarcinoma remain investigated.

In this study, we compared the LOXL1-AS1 mRNA expression between CHOL tissues and normal tissues. Our data was collected from The Cancer Genome Atlas (TCGA), the R and Gene Expression Profiling Interactive Analysis (GEPIA) identified the correlation of LOXL1-AS1 with CHOL.

3. Materials and Methods

3.1. Data Source and Preprocessing: CHOL patient datasets, with the mRNA levels of LOXL1-AS1 in several cancers including CHOL and clinical information from STAD projects, were collected from the publicly available TCGA. The exclusion criteria were STAD samples which excluded the cases with missing data on age, overall survival time. Then, in order to calculate the influences of LOXL1-AS1 expression on survival curve, tumor tissues were divided into 2 groups which were LOXL1-AS1-lower-expression group and LOXL1-AS1-higher-expression group according to the expression of LOXL1-AS1 in further study.

3.2. LOXL1-AS1 Gene Expression Analysis and Survival Analysis by GEPIA: The mRNA levels of LOXL1-AS1 in several cancers including CHOL were collected from Gene Expression Profiling Interactive Analysis (GEPIA) (<http://gepia.cancer-pku.cn/index.html>), using disease state (tumor or normal) as the variable, were generated to study differential expression of LOXL1-AS1.

HOXL1-AS1-lower-expression group and LOXL1-AS1-higher-expression group were distinguished according to LOXL1-AS1 expression above or below the median value to calculate the influences of LOXL1-AS1 expression on survival curve.

3.3. Tiics (Tumor-Infiltrating Immune Cells) Analysis by TIMER

TIMER database, which produced an inference on the number of TIICs (tumor-infiltrating immune cells) was used to analyze the TIICs [10]. We analyzed the influences of LOXL1-AS1 expression on the abundance of infiltrating immune cells, including T cells, B cells, TAMs, monocytes, M1 macrophages, M2 macrophages, natural killer (NK) cells, neutrophils, dendritic cells (DCs), T-helper (Th) cells, T-helper 17 (Th17) cells, follicular helper T (Tfh) cells, exhausted T cells, Tem, and Tregs.

3.4. Clinical Statistical Analysis

Clinical information were collected from the publicly available TCGA. All the clinical information were analyzed by R package, using the P-values, fold changes, and ranks. To study the influence of HOXL1-AS1 expression and other clinicopathological factors (age, race and gender) on survival, we used Multivariate Cox analysis, and we set up P-value < 0.05 as the cut-off criterion.

3.5. Gene Set Enrichment Analysis

GSEA is a computational method that determines the statistical significance of a priori defined set of genes and the existence of concordant differences between two biological states [11]. We generated an initial list on the classification of the genes according to their correlation with the LOXL1-AS1 expression by GSEA. Gene sets with a discovery rate (FDR) < 0.05 were considered to be significantly enriched.

4. Results

4.1. The Expression Levels of LOXL1-AS1 Mrna in CHOL and Other Cancers

Firstly, by using the TCGA database, gene expression analyses and the corresponding normal samples of TCGA by Wilcoxon rank sum test, which showed that LOXL1-AS1 mRNA levels were significantly higher in breast invasive carcinoma(BRCA), cholangiocarcinoma (CHOL), head and Neck squamous cell carcinoma(HNSC), kidney Chromophobe (KICH), liver hepatocellular carcinoma(LIHC), lung adenocarcinoma(LUAD), lung squamous cell carcinoma(LUSC), stomach adenocarcinoma(STAD) tissues compared with the corresponding normal tissues ($P < 0.05$) (Figure 1A). Although because some information was lacked or inconsistent, the expression of LOXL1-AS1 in some types of human cancers is meaningless compared with the corresponding normal tissues, these data showed that LOXL1-AS1 expression was upregulated in cancer tissues. Secondly, we compared the expression of LOXL1-AS1 in TCGA dataset. In unpaired samples we compared the expression of LOXL1-AS1 in 9 paracancerous samples and 36 CHOL samples, the expression of LOXL1-AS1 was significantly high in CHOL samples($P=8.6e-19$) (Figure 1B). In paired samples we compared the expression of LOXL1-AS1 in 9 paracancerous

samples and 9 CHOL samples, the expression of LOXL1-AS1 was also significantly high in CHOL samples ($P=2.5e-05$) (Figure 1C). These data showed that LOXL1-AS1 expression in tumor samples

was obviously higher than in normal samples, especially cholangiocarcinoma. LOXL1-AS1 was highly expressed in CHOL.

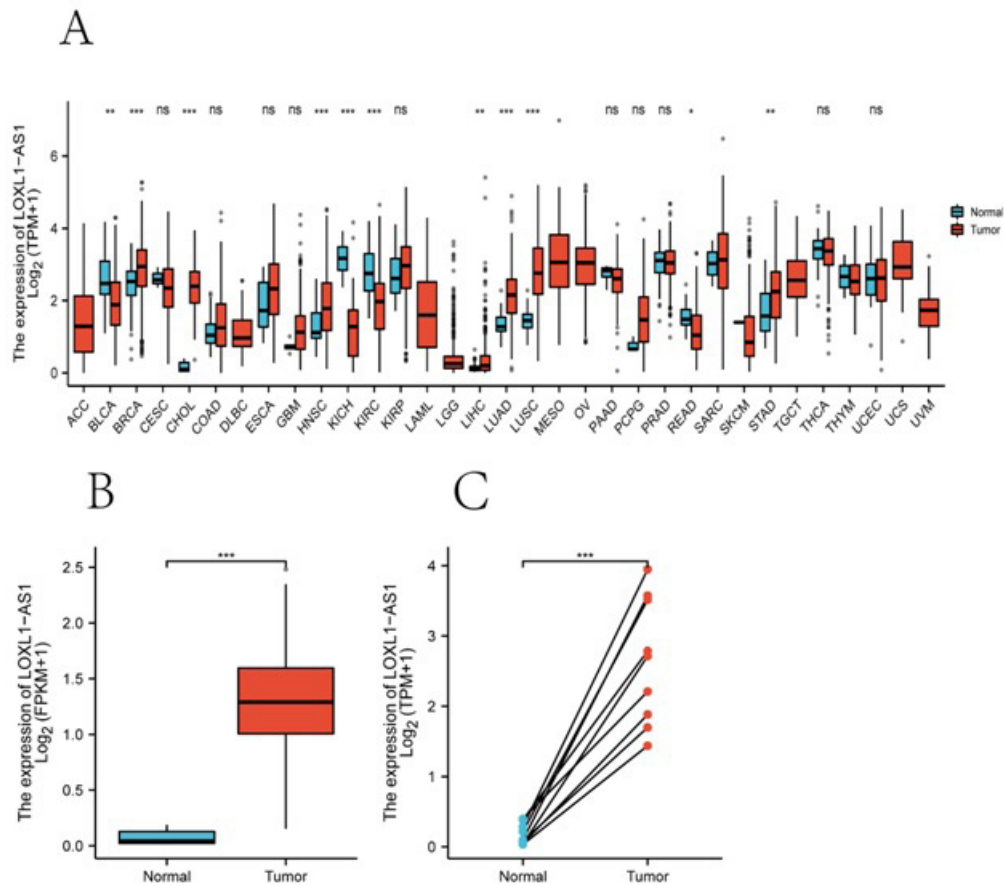


Figure 1: Differential expression levels of LOXL1-AS1 in different types of human cancers. (A) High or low expression of LOXL1-AS1 in different human cancer tissues compared with normal tissues. (B, C) Differential expression levels of LOXL1-AS1 in CHOL.

4.2. High LOXL1-AS1 Expression Was Associated with Poor Prognosis in Human Cancers

Next, we conducted survival analyses of OS, DSS, and PFI. Due to the lack of data of cholangiocarcinoma patients in Kaplan-Meier plotter database, we downloaded the data of cholangiocarcinoma patients from TCGA database and analyzed the prognostic value of LOXL1-AS1 expression in human cancers. High LOXL1-AS1 expression was associated with poorer prognosis in CHOL (OS: HR=2.93, $P=0.049$; DSS: HR=3.55, $P=0.035$; PFI: HR=2.50, $P=0.054$; Figure 2A–C); High LOXL1-AS1 expression was associated with poorer prognosis in GBM (OS: HR=1.60, $P=0.008$; DSS: HR=1.79, $P=0.002$; PFI: HR=1.73, $P=0.002$; Figure 2D–F); High LOXL1-AS1 expression was associated with poorer progn-

osis in MESO (OS: HR=2.79, $P<0.001$; DSS: HR=2.36, $P=0.007$; PFI: HR=2.30, $P=0.003$; Figure 2D–F).

In addition, N stage and LOXL1-AS1 expression are independent prognostic factors. Association between the expression of LOXL1-AS1 and clinicopathological parameters of CHOL was shown in Table 1. Univariate analysis of correlation of using Cox regression revealed that N stage is significantly associated with overall survival. And as shown in Table 2, multivariate survival analysis of correlation of using Cox regression revealed that N stage and LOXL1-AS1 expression are independent prognostic factors.

According to Figure 2, Table 1 and Table 2, high LOXL1-AS1 expression was an independent predictor of tumor prognostic factors.

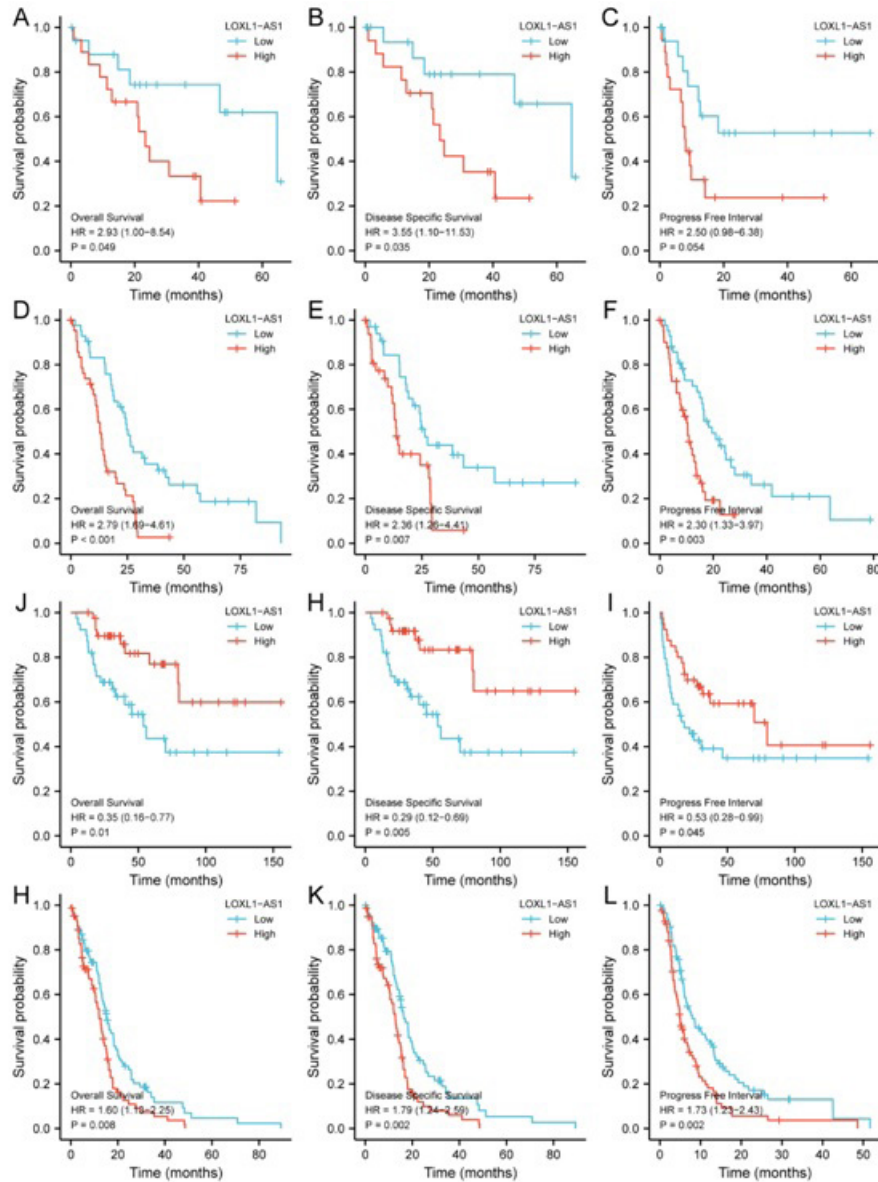


Figure 2: Kaplan-Meier survival curves comparing the high and low expression of LOXL1-AS1 in different types of human cancers using the Kaplan-Meier plotter database. (A–C) Survival curves of OS, DSS, and PFI between LOXL1-AS1-high and -low patients with CHOL. (D–F) Survival curves of OS, DSS, and PFI between LOXL1-AS1-high and -low patients with GBM. (G–I) Survival curves of OS, DSS, and PFI between LOXL1-AS1-high and -low patients with MESO. OS, overall survival; DSS, disease specific survival; PFI, progression free interval; GBM, glioblastoma multiforme; MESO, mesothelioma.

Table 1. Association between the expression of LOXL1-AS1 and clinicopathological parameters of cholangiocarcinoma.

Characteristic	Low expression	High expression	P-value
n	18	18	
T stage, n (%)			1.000
T1	9 (25%)	10 (27.8%)	
T2	6 (16.7%)	6 (16.7%)	
T3	3 (8.3%)	2 (5.6%)	
T4	0 (0%)	0 (0%)	
N stage, n (%)			0.037
N0	15 (48.4%)	9 (29.0%)	
N1	1 (3.2%)	6 (19.3%)	
M stage, n (%)			1.000

M0	14 (42.4%)	14 (42.4%)	1.000
M1	2 (6.1%)	3 (9.1%)	
Age, n (%)			
<=65	8 (22.2%)	9 (25%)	
>65	10 (27.8%)	9 (25%)	
Gender, n (%)			0.738
Female	11 (30.6%)	9 (25%)	
Male	7 (19.4%)	9 (25%)	

Table 2. Multivariate analysis of variables associated with OS survival in patients with cholangiocarcinoma.

Clinical characteristics	HR(95%CI)	p-Value
Age (>65 vs. <=65)	1.268 (0.499-3.221)	0.617
Gender (Male vs. Female)	1.387 (0.544-3.534)	0.494
Weight (>70 vs. <=70)	0.965 (0.360-2.583)	0.943
BMI (>25 vs. <=25)	0.515 (0.186-1.426)	0.202
N stage (N1 vs. N0)	2.289 (1.202-8.700)	0.041
LOXL1-AS1 (High vs. Low)	2.928 (1.004-8.540)	0.035

4.3. Relationship Between LOXL1-AS1 Gene Expression and CHOL Tumor Infiltrating Immune Cells

Previous analyses have shown that tumor infiltrating lymphocytes are an independent predictor of survival in CHOL patients. So we tried to study whether LOXL1-AS1 gene expression was associated with immune infiltration in CHOL. We divided 36 tumor samples into 2 parts according to LOXL1-AS1 expression. Therefore, we obtained 18 samples of high expression group and 18 samples of low expression group. We explored gene expression profiles of downloaded samples to infer the density of 22 types of immune cells by an established computational resource (CIBERSORT). Immune infiltration proportions in CHOL are not presented systematically, especially the low abundance subtypes, owing to technical limitations. The CIBERSORT algorithm applied initially to the 22 immune cell subtypes helped assess their differing concentrations in the high and low LOXL1-AS1 expression groups. Figure 3.A showed the proportion of 22 immune cell subsets clearly. Tem cells and DC cells are main immune cells effected by LOXL1-AS1 expression. Figure 3.B showed the expression level of LOXL1-AS1 was significantly positively correlated with the infiltration level of Tem cells and T helper cells. In contrast, the expression level of LOXL1-AS1 was significantly negatively correlated with the infiltration level of DC cells. This showed the

correlation of LOXL1-AS1 expression level with immune infiltration in CHOL. Therefore, a positive correlation exists between the LOXL1-AS1 expression level and infiltrating levels of Tem cells.

4.4. LOXL1-AS2 Gene Enrichment Analysis

We distinguished the signaling pathways involved in LGG between low and high LncRNA LOXL1-AS2 expression data sets by GSEA which was based on LOXL1-AS2 related signaling pathways, and there were significant differences between in enrichment of REACTOME and KEGG Collection (FDR < 0.05, NOM P-value < 0.05).

5 REACTOME items including negative regulation of signaling by interleukins, leishmania infection, cell surface interactions at the vascular wall, FC epsilon receptor signaling and immunoregulatory interactions between a lymphoid and a non-lymphoid, which were significant differences between in enrichment of REACTOME Collection based on NOM P value and FDR value(Figure 4 A).The KEGG item was the negative regulation of cytokine receptor interaction(NES=-1.752, p.adj=0.044, FDR= 0.046; Figure 4 B). The REACTOME item was negative regulation of cell surface interactions at the vascular wall (NES=-1.740, p.adj=0.044, FDR= 0.046; Figure 4 C). The REACTOME item was negative regulation of cell surface interactions at the vascular wall (NES=-1.561, p.adj=0.044, FDR= 0.046; Figure 4 C).

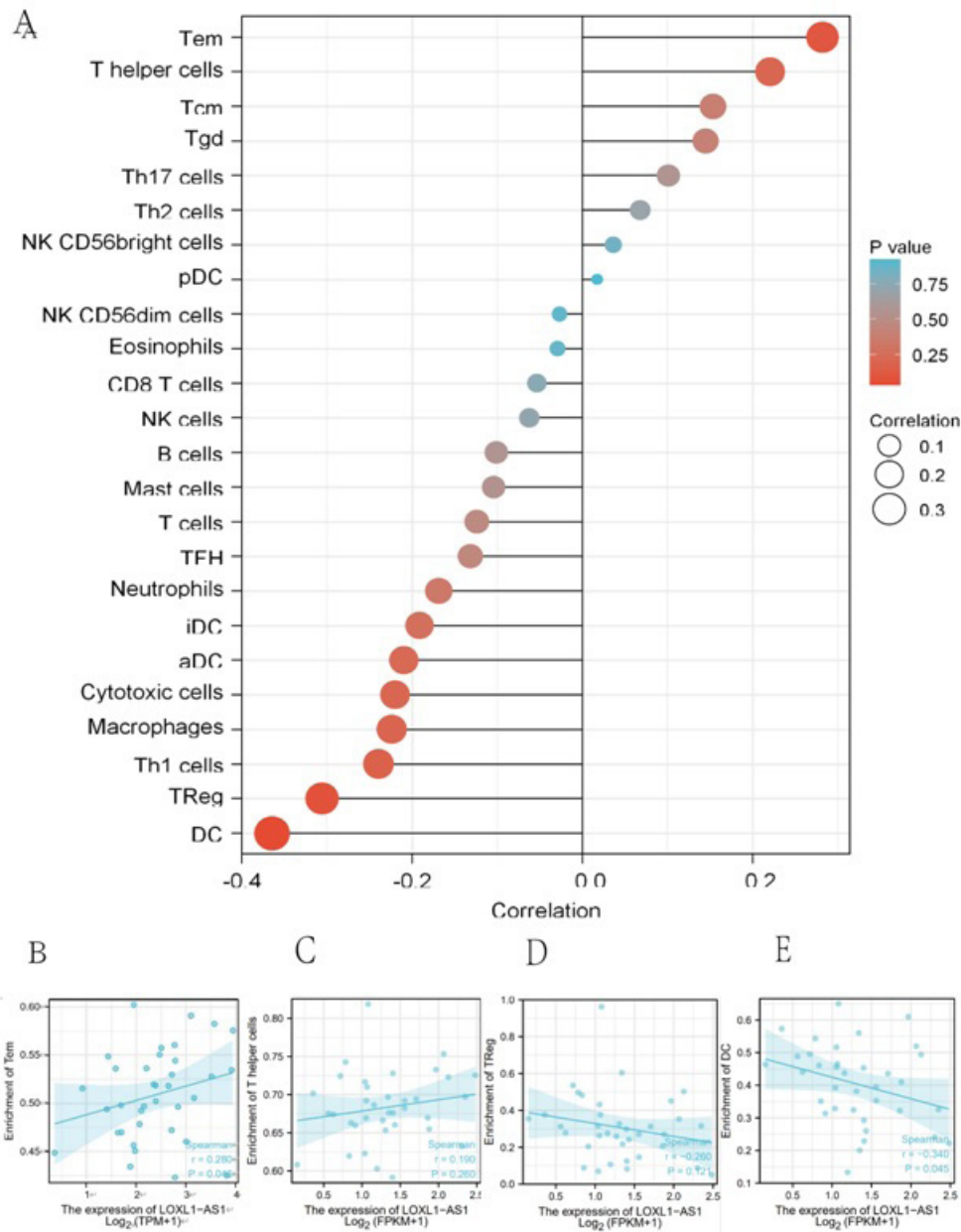


Figure 3: Analysis of LOXL1-AS1 IN 22 subpopulations of immune cells (A) The proportion of 22 subpopulations of immune cells (Tem cells and DC cells are main immune cells effected by LOXL1-AS1 expression. Among them, Tem cells ($p = 0.046$), is apparently increased in high expression group compared with low expression group. In contrast, DC cells ($p = 0.045$) are decreased in high expression group compared with low expression group. (B, C) The expression level of LOXL1-AS1 was significantly positively correlated with the infiltration level of Tem cells and T helper cells. (D, E) In contrast, the expression level of LOXL1-AS1 was significantly negatively correlated with the infiltration level of DC cells.

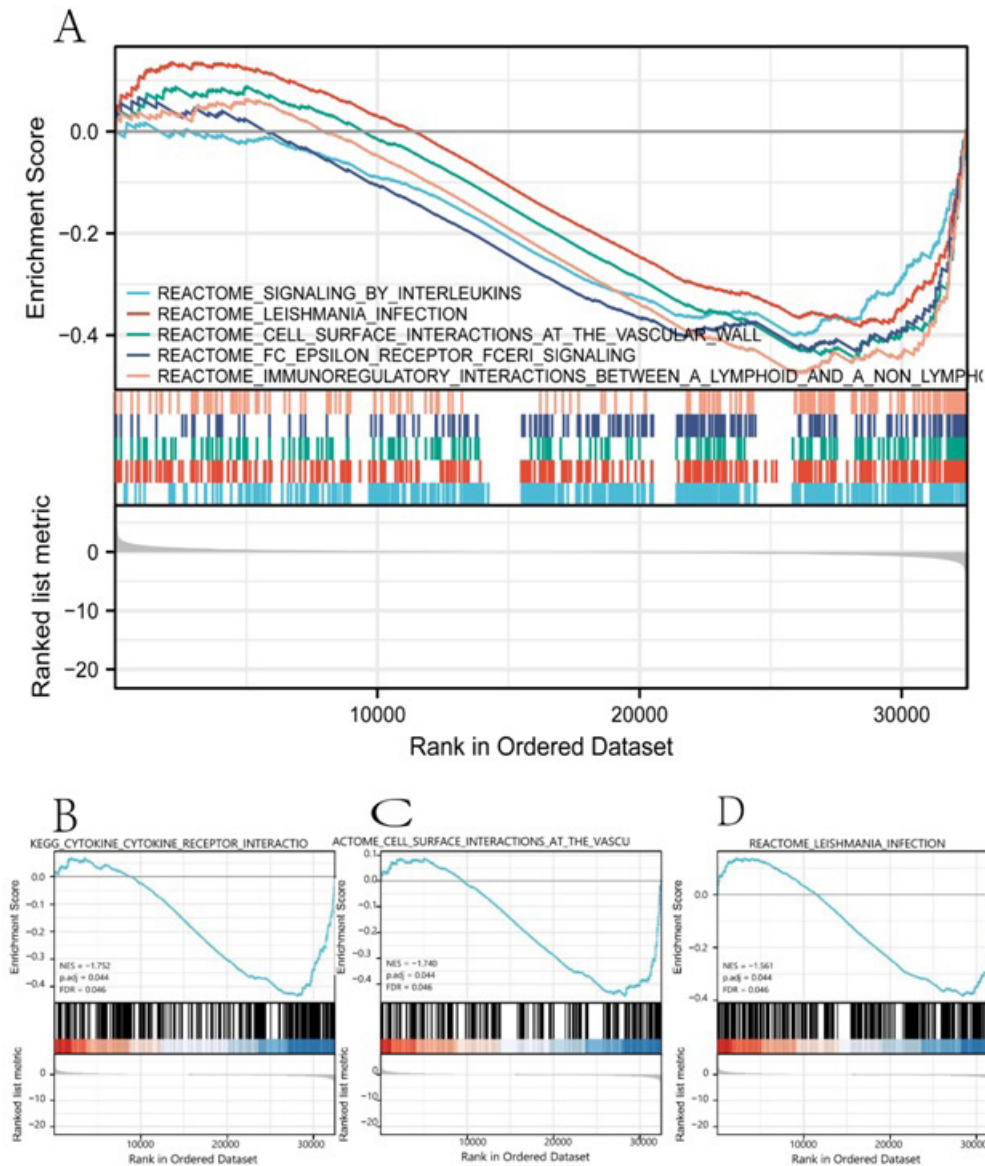


Figure 4: Gene set enrichment analysis. (A) Enrichment plots from gene set enrichment analysis (GSEA). GSEA results showing differential enrichment of genes in REACTOME with high LOXL1-AS2 expression including signaling by interleukins, leishmania infection, cell surface interactions at the vascular wall, FC epsilon receptor signaling and immunoregulatory interactions between a lymphoid and a non-lymphoid, which were significant differences between in enrichment of REACTOME Collection based on NOM P value and FDR value. (B) GSEA results showing enrichment of LOXL1-AS2 genes in KEGG, negative regulation of cytokine receptor interaction. (C) GSEA results showing enrichment of LOXL1-AS2 genes in REACTOME, negative regulation of cell surface interactions at the vascular wall. (D) GSEA results showing enrichment of LOXL1-AS2 genes in REACTOME, negative regulation of leishmania infection.

5. Discussion

LOXL1-AS1 is a gene which was reported to associate with Exfoliation Syndrome and modulate tumor progression in several types of cancer. A multitude of investigations have validated the oncogenic role of LOXL1-AS1 in diverse human cancers, including gastric cancer, breast cancer, colorectal cancer, etc [6-8, 12]. According to our knowledge, there is limited literature on the potential prognostic impact of LOXL1-AS1 in CHOL. In the present study, we found that LOXL1-AS1 expression was overtly upregulated in cholangiocarcinoma tissues. In addition, high expres-

sion of LOXL1-AS1 was closely correlated with poor prognosis of cholangiocarcinoma. We further found that the expression of LOXL1-AS1 correlated with several different immune cell subsets within tumours, thus highlighting a possible role for LOXL1-AS1 in the immunological interactions in CHOL, making it a valuable biomarker worthy of further research in this type of cancer.

In this report, we assessed the expression of LOXL1-AS1 as it related to the prognosis of many different types of cancers using GEPIA databases, revealing clear differences between tumour and normal tissue expression of LOXL1-AS1 in many cancers. In

addition, we find that variations in LOXL1-AS1 expression level relate to prognosis in CHOL. High LOXL1-AS1 expression correlates with a negative prognosis in CHOL. We download datasets from TCGA, and multivariate analysis show that an up-regulated LOXL1-AS1 expression is an independent prognostic factors for negative prognosis. Thus these results together suggest that LOXL1-AS1 may have value as a CHOL prognostic biomarker.

Furthermore, the expression of LOXL1-AS1 correlated with several different immune cell subsets within tumours, such as Tem cells and DC cells, which highlighted a possible role for LOXL1-AS1 in the immunological interactions in CHOL. According to the expression of CD45RA and CCR7, CD4 + T cells and CD8 + T cells can be divided into three subsets: Tnaive, Tem and Tem cells [13-15]. According to our knowledge, several literatures have been reported that the occurrence of tumors is related to Tem cell abnormalities [13, 15]. DC cells is a type of antigen presenting cells(APC). Several literatures about initiating and regulating adaptive immune responses underpins the successful generation of anti-tumor immune responses been published [16]. In this report, we find that the expression level of LOXL1-AS1 was significantly positively correlated with the infiltration level of Tem cells and T helper cells. In contrast, the expression level of LOXL1-AS1 was significantly negatively correlated with the infiltration level of DC cells, which highlight the ability of LOXL1-AS1 to potentially regulate immune cell recruitment in CHOL, making it a valuable biomarker worthy of further research in this type of cancer.

An additional key finding in this study is that differential enrichment of genes in KEGG and REACTOME with high LOXL1-AS1 expression by gene set enrichment analysis (GSEA). 5 REACTOME items including negative regulation of signaling by interleukins, leishmania infection, cell surface interactions at the vascular wall, FC epsilon receptor signaling and immunoregulatory interactions between a lymphoid and a non-lymphoid, which were significant differences between in enrichment of REACTOME Collection based on NOM P value and FDR value. In addition, the KEGG item was the negative regulation of cytokine receptor interaction. Those results suggested that LOXL1-AS1 may serve as a potential prognostic marker of prognosis and therapeutic target in CHOL

6. Conclusion

In summary, we conclude that the poor survival in CHOL has a probable prognostic molecular marker known as the LOXL1-AS1, and LOXL1-AS1 may be an important regulator of immune cell infiltration.

7. Funding

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