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RESEARCH ARTICLE

Title: Analysis of LOXL1 Expression in Gastric Cancer and Mining of Traditional Chinese Medicine Based on Bioinformatics

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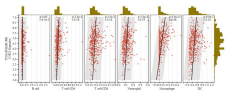

ARTICLE HISTORY

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Abstract: There is an urgent need to explore valuable therapeutic targets of gastric cancer. Here, we evaluated the expression of lysyl oxidase-like 1 (LOXL1) in gastric cancer and its prognostic value by bioinformatics approaches. Then, we analyzed the correlation of LOXL1 and its related genes with immune cell infiltration. The potential therapeutic targets of LOXL1 in gastric cancer and corresponding Chinese medicines were also explored. The results showed that LOXL1 was over-expressed in gastric cancer tissues as compared with paracancerous tissues, and patients with higher LOXL1 expression had poorer prognosis. The expression of LOXL1 was positively correlated with the infiltration of immune cells, among which the correlation between LOXL1 and macrophages was the most significant, and the expression of LOXL1 related genes was also correlated with the infiltration of immune cells. The traditional Chinese medicine orange leaves, orange kernel, green peel, tangerine peel and turmeric were found potentially to act on LOXL1 by network pharmacology analysis. Therefore, LOXL1 is a potential therapeutic target for gastric cancer. Some traditional Chinese medicine may inhibit the progression of gastric cancer by regulating LOXL1.

Keywords: LOXL1; Gastric cancer; Prognostic analysis; Immune cell infiltration; Traditional Chinese medicine

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1. Introduction

Gastric cancer (GC) is one of the most common digestive tract tumors and the third leading cause of cancer death worldwide. More than one million people worldwide are diagnosed with gastric cancer each year [1], which is a serious threat to people's lives and health. Since the early

clinical symptoms of the disease are not obvious, the patients with gastric cancer are often diagnosed at middle-late stage, and miss the best opportunity for surgical treatment. In recent years, much attention has been focused on targeted treatment of gastric cancer to improve the 5-year survival rate of patients [2]. The discovery of novel valuable therapeutic targets is the key to improve the survival rate and prognosis of gastric cancer patients. Extracellular matrix (ECM), a complex three-dimensional acellular macromolecular network, is composed of collagen, proteoglycan, glycosaminoglycan, elastin, fibronectin, lamina and several other glycoproteins. These ECM molecules bind to cells through adhesion receptors and anchor them to specific locations of the three-dimensional network, which play an important role in the construction and function of normal tissue morphology. Transmission of external signals from ECM into cells through surface receptors dynamically regulates cell survival, growth, migration, differentiation, senescence and death, which is very important to maintain the normal homeostasis of cells [3]. Abnormal changes in the composition and structure of ECM are closely related to the occurrence of various diseases, including fibrosis [4], cancer, diabetes [5] and myopathy [6-7]. In recent years, the role of ECM dysregulation in the initiation and progression of malignant tumors has attracted increasing attention. Tissue fibrosis, interstitial stiffness and remodeling of ECM are related to tumor migration, invasion and low survival rate of patients [8]. In breast cancer, the mechanism of ECM stiffness has been described as collagen cross-linking caused by tumor-associated macrophages [9]. LOXL1, a copper-dependent monoamine oxidase, is involved in regulating the cross-linking between collagen and elastin in ECM, thus affecting the stability of ECM network [10]. Previous studies have shown that the abnormal expression of LOXL1 in patients with glioma and ovarian cancer promotes the proliferation and invasion but inhibits the apoptosis of tumor cells, and is closely related to the poor prognosis of patients [11]. Studies have shown that LOXL1 is up-regulated in colorectal cancer (CRC), and promotes the immune escape, proliferation and metastasis of CRC cells. Moreover, it also deteriorates CRC patient prognosis by limiting the infiltration of CD8+T cells [12]. However, there is few systematic analysis of its role in the progression of gastric cancer. The potential function and mechanism of LOXL1 in gastric cancer need to be further studied. This study investigated the expression of LOXL1 in gastric cancer and its relationship with the prognosis of patients using bioinformatics methods. We also screened LOXL1-related genes, analyzed the correlation of the expression of these genes with patient prognosis and immune cell infiltration, so as to explore the potential therapeutic value of LOXL1 in gastric cancer. Finally, we searched for the traditional Chinese medicine components potentially intervening gastric cancer by targeting LOXL1.

2. Materials and methods

2.1 Research data

Transcriptome data (RNA-seq data) of gastric cancer and the clinical information of the corresponding patients were downloaded from the Cancer Genome Map (the cancer genome atlas, TCGA) website. The sangerbox (http://sangerbox.com/) database was used to standardize the RNA-seq data of gastric cancer. The transcriptome data of other types of tumors were downloaded from TCGA and Genotype-Tissue Expression (GTEx) database for gene differential expression and survival analysis.

2.2 Analysis of differential expression of genes

The expression of LOXL1 in different types of tumors and human tissues was analyzed by Gene Expression Profiling Interactive Analysis(GEPIA)and sangerbox database. The method selected for GEPIA database was analysis of variance (ANOVA). The expression values of the samples in sangerbox database were transformed by log2 (x transformation 1). The expression differences between normal and tumor samples in each tumor were calculated by R software (version 3.6.4), and the differences were analyzed by unpaired Student's t-Test. Based on the two databases, the cancer types with up-regulation and down-regulation of LOXL1 were screened.

2.3 Prognostic analysis

Kaplan-Meier plotter database (https://kmplot.com/analysis) can be used for online survival analysis. The sangerbox database can be used to analyze the prognosis of genes. The prognostic analyses of LOXL1 in different tumors were performed by sangerbox database, and verified by Kaplan-Meier plotter survival analysis, with evaluating risk ratio (HR), 95% confidence interval (CI) and Log-rank *P* value.

2.4 Screening for the differentially expressed genes related to LOXL1

Based on the TCGA-STAD dataset, Linked Omics was used to analyze the genes that were positively and negatively correlated with LOXL1 expression, which were shown on volcano map and heat map respectively. Pearson correlation analysis was selected in the statistical method, and the correlation was measured by correlation coefficient and *P* value.

2.5 Functional enrichment of the differentially expressed genes related to LOXL1

The top 50 genes with the highest positive and negative correlation with LOXL1 were annotated by gene ontology (GO) and enriched by Kyoto Encyclopedia of Genes and Genome (KEGG) using Metascape database (http://metascape.org/gp/index.html#/main/step1).

2.6 Construction of protein interaction network

Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) is a database for the study of protein-protein interactions. The PPI of related differentially expressed genes is constructed by STRING database. Visual analysis of PPI is carried out by Cytoscape software. Using the cytoHubba plug-in of

Cytoscape software, the top five positively correlated and negatively correlated genes were obtained through the detail point column coding minutia cylinder-code (MCC) algorithm of the plug-in.

2.7 Prognostic analysis of LOXL1-related genes

The prognosis and survival of the top five positively and negatively related genes of LOXL1 in gastric cancer were analyzed by Kaplan-Meier plotter database.

2.8 Analysis of LOXL1 and its related genes with immunocyte infiltration

Sangerbox and TIMER databases were used to analyze the correlation of LOXL1 and LOXL1 related genes with immunocyte infiltration. The immune infiltrating cells were B cells, CD8+T cells, CD4+T cells, macrophages, neutrophils and dendritic cells. STAD was selected as the tumor type to obtain the correlation between the expression of LOXL1 and its related genes in gastric cancer and immunocyte infiltration.

2.9 Mining of traditional Chinese medicine and its active ingredients maybe targeting LOXL1

The Coremine database, established by PubGene, includes drugs, traditional Chinese medicine and chemical components. LOXL1 was imported into Coremine database to search for the corresponding traditional Chinese medicine. The active components and corresponding targets of traditional Chinese medicine were screened in TCMSP database.

2.10 Statistical analysis

SPSS 25.0 software was used for statistical analysis, with independent sample t test being used for normality test, Mann-Whitney U test for non-normality test, and Spearman for correlation test. $P < 0.05$ was considered to be statistically different.

3. Results

3.1 Expression of LOXL1 in pan-cancer and gastric cancer

Combining the statistical results of GEPIA database (Figure 1A, B) and sangerbox database (Figure 2), the expression of LOXL1 was significantly up-regulated in the following seven tumor tissues: glioblastoma multiforme (GBM), breast invasive carcinoma (BRCA), stomach adenocarcinoma (STAD), head and neck squamous cell carcinoma (HNSC), lung squamous cell carcinoma (LUSC), pancreatic adenocarcinoma (PAAD), and uterine carcinosarcoma (UCS). It was significantly down-regulated in 4 kinds of tumors: prostate adenocarcinoma (PRAD), kidney renal clear cell carcinoma (KIRC), acute lymphoblastic leukemia (ALL), and kidney chromophobe (KICH). The Expression profile and Boxplot modules of GEPIA database showed the up-regulation of LOXL1 in gastric cancer (Figure 3).

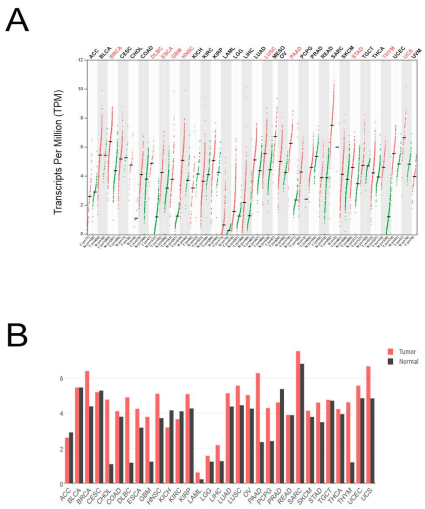

Fig. (1). GEPIA database. (A) LOXL1 gene expression levels in different tumor samples and paired normal tissues (Tumor name red indicates increased LOXL1 gene expression in this tumor. Each point represents the expression of the sample). (B) LOXL1 gene expression levels in different tumor samples and paired normal tissues (height of bars indicates median expression in certain tumor types and normal tissues).

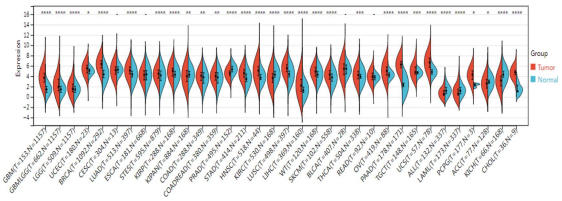

Fig. (2). Sangerbox database. Expression levels of LOXL1 in different tumor samples and paired normal tissues. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P<0.0001$.

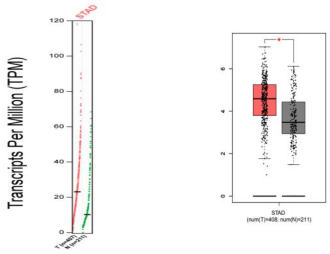

Fig. (3). GEPIA database. Expression of LOXL1 in gastric cancer and normal tissues. * $P < 0.05$.

3.2 Prognostic value of LOXL1

Since the expression of LOXL1 is abnormal in several tumor tissues, we further analyzed the relationship between LOXL1 expression and patient prognosis in these tumors using sangerbox database (Figure 4A). Kaplan-Meier plotter curve showed that gastric cancer patients with higher expression of LOXL1 had shorter overall survival (OS) (Figure 4B). There was no correlation between LOXL1 expression and the prognosis of patients with UCS (Figure 4C), BRCA (Figure 4D), HNSC (Figure 4E), LUSC (Figure 4F) , and PAAD (Figure 4G).

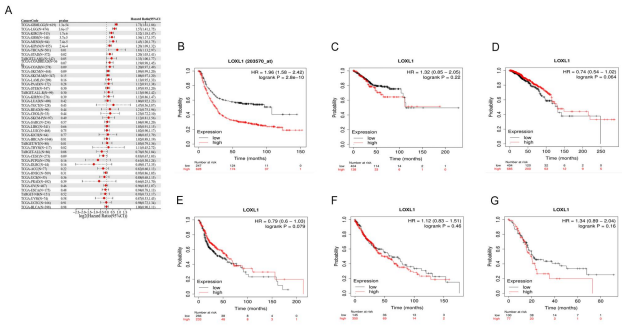

Fig. (4). Prognostic analysis of LOXL1 in different tumor tissues.

3.3 LOXL1-related genes in gastric cancer

Based on the TCGA-STAD dataset, the genes related to LOXL1 expression in gastric cancer were analyzed by Linked Omics database, and the results were represented by volcanic map (Figure 5A). A total of 12256 related genes were screened by $P<0.05$, and the top 50 positively (Figure 5B) and negatively (Figure 5C) correlated genes with the highest Pearson correlation coefficient were represented by heat map.

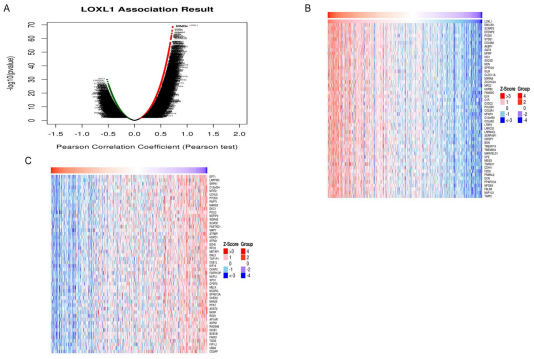

Fig. (5). Linked Omics database. Analysis of LOXL1 related genes in gastric cancer. (A) Volcano map of LOXL1 related genes. (B) Heat map of the top 50 genes positively correlated with LOXL1. (C) Heat map of the top 50 genes negatively correlated with LOXL1.

3.4 Functional enrichment analysis of LOXL1 related genes

GO analysis showed that LOXL1 positively related genes were mainly involved in such molecular functions as collagen adhesion, extracellular matrix adhesion and calcium binding, and involved in such biological processes as cell response to growth factors, cell-cell receptor signal pathway, negative regulation of cell proliferation, ossification, regulation of muscle cell differentiation, and cell endocytosis, and involved in the composition of extracellular matrix, collagen, elastofibrin, basement membrane (Figure 6A, B). LOXL1 negatively related genes are mainly involved in such biological processes as cell invasion, mRNA metabolism, mitochondrial gene expression, DNA structural changes and metabolism, and cell cycle, as well as the composition of mitochondria, microtubules and fibrous tissue (Figure 6C, D). KEGG pathway analysis showed that LOXL1 positively related genes were involved in protein digestion and absorption, and proteoglycan cancer pathway (Figure 6E, F), while LOXL1 negatively related genes were involved in eukaryotic ribosomal pathway, nuclear-cytoplasmic transport pathway and human T cell leukemia virus type 1 infection pathway (Figure 6G, H).

Fig. (6). GO and KEGG enrichment analysis of LOXL1-related genes.

3.5 PPI network analysis

In order to study the biological function and regulation of LOXL1-related genes in gastric cancer and para-cancerous tissues at protein level, we constructed the interaction network of proteins encoded by these related genes. The PPI maps of the top 50 positively and negatively correlated genes were constructed by STRING database. The PPI map was visually analyzed by Cytoscape software, and the results are shown in figures 7A and 7B. Using the cytoHubba plug-in of Cytoscape software, through the detail point column coding MCC algorithm of this plug-in, BGN, MFAP4, FBLN5, EFEMP2 and ELN were identified as the top five positively correlated genes (Figure 7C), and KIF15, BUB1B, FANCI, NCAPG and ASPM as the top five negatively correlated genes (Figure 7D).

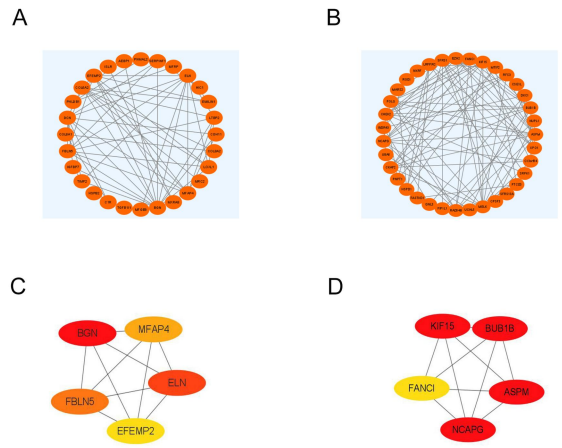

Fig. (7). (A) LOXL1 positive correlation gene PPI map. (B) LOXL1 negative correlation gene PPI map. (C) PPI map of the top 5 LOXL1 positively correlated genes. (D) PPI map of the top 5 LOXL1 negatively correlated genes.

3.6 Prognostic analysis of LOXL1 related genes

The prognosis and survival of the top 5 positively and negatively related genes of LOXL1 in gastric cancer were analyzed by Kaplan-Meier plotter database. The results showed that the patients with high expression of the positively related genes BGN (Figure 8A), MFAP4 (Figure 8B), FBLN5 (Figure 8C), EFEMP2 (Figure 8D) and ELN (Figure 8E) had poor prognosis, while the patients with high expression of the negatively related genes KIF15 (Figure 8F), BUB1B (Figure 8G), FANCI (Figure 8H), NCAPG (Figure 8I) and ASPM (Figure 8J) had good prognosis.

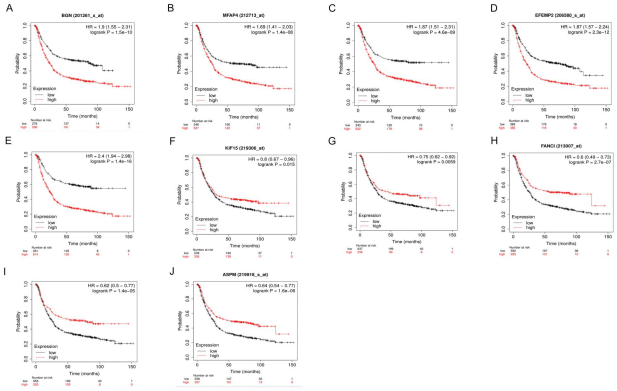

Fig. (8). Kaplan-Meier plotter database. Prognostic analysis of the top 5 LOXL1 positively and negatively related genes in gastric cancer.

3.7 The expression of LOXL1 and its related genes is associated with immunocyte infiltration in gastric cancer

We analyzed the correlation of LOXL1 expression with infiltration of 6 kinds of immune cells. The results showed that LOXL1 expression was significantly correlated with macrophages infiltration with a correlation coefficient as high as 0.46 (Figure 9). The positively correlated genes BGN, MFAP4, FBLN5, EFEMP2 and ELN, as well as the negatively correlated genes KIF15, BUB1B, FANCI, NCAPG and ASPM were submitted to the TIMER database. The correlation between the related genes and tumor purity and 6 kinds of immune cells infiltration was observed. The results showed that the expression of the positively correlated genes BGN and EFEMP2 were negatively correlated with B cells. Other positively correlated genes were positively correlated with the infiltration of CD8+T cells, CD4+T cells, macrophages, neutrophils and dendritic cells, with the most significant correlation being observed with macrophages (Figure 10A-E). The expression of LOXL1 negatively related genes was negatively correlated with the infiltration of B cells, CD8+T cells, CD4+T cells, macrophages, neutrophils and dendritic cells (Figure 10F-J). The high concentration of tumor-associated macrophages in most cancers is related to the poor prognosis of tumor patients. LOXL1 and related genes are associated with macrophage and other immune cell infiltration, suggesting that LOXL1 may participate immune-evasion of gastric cancer.

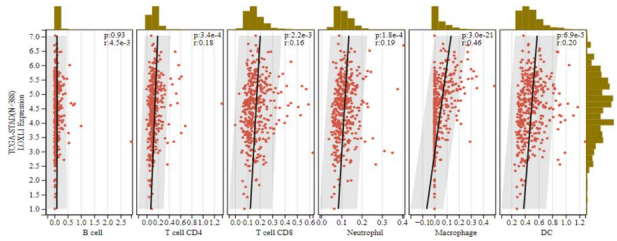

Fig. (9). Sangerbox database. Correlation between LOXL1 and immune cell infiltration in gastric cancer tissues.

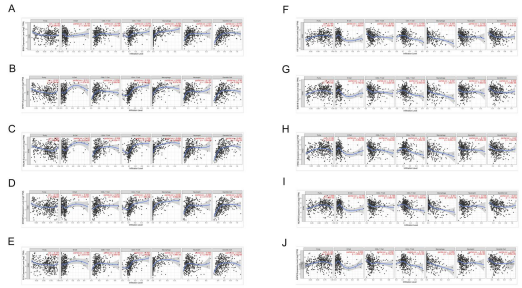

Fig. (10). TIMER database. Correlation between LOXL1-related genes and immune cell infiltration in gastric cancer tissues.

3.8 Exploration of potential traditional Chinese medicine

LOXL1 was imported into Coremine database to search for corresponding traditional Chinese medicine. The Chinese medicines corresponding to LOXL1 were orange leaf, orange kernel, green peel, tangerine peel and turmeric. The effective components from the Chinese medicine were screened in the TCMSP database, and the screening conditions were bioavailability (OB%) greater than 30% and drug-like properties (DL) greater than 0.18 as shown in Table 1. The above results show that LOXL1 is a potential target of traditional Chinese medicine in the treatment of gastric cancer.

Table 1. Some effective components and targets of traditional Chinese medicines

ID	Traditional Chinese medicines	Target point	Effective components
MOL005100	Orange leaves	LOXL1	5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one
MOL000131	Orange kernel	LOXL1	EIC
MOL000675	Orange kernel	LOXL1	oleic acid
MOL001798	green peel	LOXL1	neohesperidin_qt
MOL001803	green peel	LOXL1	Sinensetin
MOL004328	green peel	LOXL1	naringenin
MOL005100	green peel	LOXL1	5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one
MOL005828	green peel	LOXL1	nobiletin
MOL000359	tangerine peel	LOXL1	sitosterol
MOL004328	tangerine peel	LOXL1	naringenin
MOL005100	tangerine peel	LOXL1	5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one
MOL005815	tangerine peel	LOXL1	Citromitin
MOL005828	tangerine peel	LOXL1	nobiletin
MOL000449	turmeric	LOXL1	Stigmasterol
MOL000493	turmeric	LOXL1	campesterol
MOL000953	turmeric	LOXL1	CLR

4. Discussion

The formation of gastric cancer is a complex biological process, which includes the inactivation of tumor suppressor genes and the activation of various tumor-promoting genes [13]. At present, there is an urgent need to reveal the mechanism of the initiation and progression of gastric cancer and to find the key molecules that can be targeted in the treatment and prognosis of gastric cancer. The rapid development of high-throughput technology has produced a large amount of omics data. The use of various bioinformatics tools to mine and integrate this data is of great significance for identifying key molecules involved in the initiation and progression of gastric cancer.

In this study, the patient data were obtained from TCGA database, and both GEPIA and sangerbox databases showed high expression of LOXL1 in gastric cancer. Then, the relationship between LOXL1 expression in gastric cancer and prognosis was further analyzed. It was found that the prognosis of gastric cancer patients with high expression of LOXL1 was poor. LOXL1-related genes were screened and the prognosis of these genes was analyzed. The results showed that the expression of the correlated genes was related to the prognosis of gastric cancer patients. The prognosis of the patients with high expression of positively related genes was poor, and the prognosis of the patients with high expression of negatively related genes was good.

LOXL1 participates in the early and late stages of collagen and elastin polymerization in the extracellular matrix, thus providing most of the tensile strength and structural integrity for collagen and elastic fibers. Abnormal expression or activity of LOXL1 leads to connective tissue diseases and fibrotic diseases. LOX family oxidases accelerate tumorigenesis and metastasis through active remodeling of tumor microenvironment. Low expression of LOXL1 leads to insufficient ECM and poor stability. Studies have shown that the loss of LOXL1 function leads to pelvic organ prolapse [14] and exfoliation syndrome glaucoma [15], while high expression of LOXL1 leads to collagen and elastin deposition, and ECM stiffness, thus promoting fibrosis and cancer.

High expression of LOXL1 endows anti-apoptosis and promotes glioma development by stabilizing BAG2 [16]. High expression of LOXL1 also promotes the development of intrahepatic cholangiocarcinoma (ICC) [17]. Studies have shown that the promotion of the development of gastric cancer by the high expression of LOXL1 is related to the process of epithelial-mesenchymal transformation (EMT) [18]. The expression of LOXL1 is positively correlated with the EMT gene set in GSEA. The high expression of LOXL1 may promote the metastasis and dissemination of gastric cancer cells by inducing EMT, and thus affect the prognosis of patients [19]. At the same time, the high expression of LOXL1 leads to the deposition of excessive ECM, which may in turn stimulate the expression of matrix-degrading enzymes, promoting tumor invasion. There is evidence that increased ECM deposition elevates the production and the activity of ECM-degrading enzymes, such as metalloproteinase, which promotes cancer progression [20]. Overexpression or increased activity of MMP2/9 leads to the degradation of ECM and basement membrane, allowing

tumor cells to invade other tissues and metastasize to distant organs [21]. MMPs are also related to tumor immune microenvironment. The high expression of MMP2/9 inhibits the cytotoxicity of CD8 T cells and their ability to eliminate tumor cells [22]. These are the ways that LOXL1 promotes the initiation and progression of gastric cancer. In this study, bioinformatics analysis showed that LOXL1 was positively correlated with the infiltration of 6 kinds of immune cells, among which the correlation with macrophages was the most significant. LOXL1 may regulate tumor immune microenvironment to induce macrophage polarization from M1 to M2 phenotype, promoting the development of gastric cancer [23].

In this study, through the correlation analysis of immune infiltration, we found that LOXL1 and related genes affect the infiltration of immune cells, thus changing the tumor immune microenvironment to promote the initiation and progression of cancer, which has not been clarified in previous studies. BGN, an important part of ECM, is an important member of the small proteoglycan family rich in leucine. Our bioinformatics analysis showed that BGN was negatively correlated with the infiltration level of B cells, and positively correlated with the infiltration level of macrophages with the most significant correlation. Studies have shown that BGN promotes the development of gastric cancer [24]. BGN is a regulator of immune microenvironment in gastric cancer. The overexpression of BGN is related to poor prognosis and leads to the increased infiltration of macrophages, Th17 cells and Th2 cells in gastric cancer [24]. Here, we found that LOXL1 and its related genes promote macrophage infiltration and impede B cell infiltration, which may be a new mechanism of gastric cancer progression. High expression of LOXL1 can induce ECM remodeling and hardening. Research has found that ECM sclerosis in breast tumors promotes focal adhesion, enhances PI3 kinase (PI3K) activity, and induces epithelial invasion induced by oncogenes [25]. At the same time, the high expression of LOXL1 induces collagen deposition in ECM, which inhibits T cell function and promotes angiogenesis to promote tumor progression [26].

High expression of LOXL1 changes the direction of collagen arrangement, which may facilitate the movement and infiltration of macrophages to promote tumor progression [27]. Studies have shown that macrophages typically play a role in promoting tumors. In primary tumors, macrophages can stimulate angiogenesis, and enhance tumor cell invasion, movement and infiltration, and produce immunosuppressive effects. Under the influence of IL-4, macrophages promote tumor cell invasion by producing WNT7b. This invasion is mediated by paracrine rings involving CSF1 synthesized by tumors and EGF produced by macrophages, which drive tumor cells and macrophages to migrate synchronously along collagen fibers that act as highways to blood vessels. This flow of tumor cells causes them to accumulate on blood vessels, and macrophages promote their internal invasion through a structure called metastatic tumor microenvironment (TMEN) [28]. The level of macrophage infiltration is related to the 10-year survival rate of STAD patients. The higher the level of macrophage infiltration, the worse the prognosis of the patients [29]. Th17 cells secrete cytokines such as IL-22, IL-17, TNF- α, IL-21 and IL-6 to produce inflammatory conditions and promote cancer progression [30]. Th17 lymphocytes release characteristic IL-17 to induce EMT in lung cancer cells and promote migration, metastasis and diffusion. Th17 cells are characterized by IL-17A production through IL-17F, IL-21 and GM-CSF [31]. Th2 cells produce type 2 cytokines such as IL-4, IL-5 and IL-13, which induce M2 phenotype of macrophages and promote the progression of cancer [32].

The expression level of EFEMP2 is significantly correlated with immune response. Studies have shown that macrophages, as a malignant feature of glioblastoma, show obvious assembly in gliomas with high levels of EFEMP2. It reveals the role of EFEMP2 as a potential characteristic marker of malignant gliomas, which are rich in M0-like macrophages [33].

ELN is a key extracellular matrix protein. The expression of ELN is increased in colorectal tumors. Compared with normal colonic epithelial cells, the expression of ELN protein in cancer cells is significantly increased. ELN induces EMT to regulate tumor development and microenvironment in colorectal cancer [34]. A high level of macrophage infiltration was observed in high-risk patients with bladder cancer, who have poor prognosis. ELN protein activates macrophages to release chronic inflammatory cytokines, which change the microenvironment in local tissues and promote tumor development [35]. The high expression of LOXL1 and the remodeling and hardening of ECM may impede the infiltration of B cells and weaken the killing effect of B cells on gastric cancer cells. It is possible that B cells polarize into Breg phenotype in this environment, and inhibit anti-tumor immune response by producing immunosuppressive cytokines to regulate T cells, NK cells, etc [36].

FN1 gene is a kind of glycoprotein in extracellular matrix, which is involved in the process of cell adhesion and migration, and is considered to be related to the pathway in cancer. The high expression of FN1 is significantly related to the poor prognosis of gastric cancer [37]. FN1 gene overexpression, change ECM, regulate tumor microenvironment, promote tumor progression [38]. Studies have shown that increased ECM hardness significantly induces fibronectin messenger RNA (FN1) levels and activates integrin β3 macrophage stimulating 1 (MST1) kinase [39]. Targeted destruction of MST1 can significantly reduce the level of peripheral T cells, and lead to T cells function damage related to adhesion, homing and interstitial movement, affecting the activity of immune cells [40]. In summary, the results show that LOXL1 and related genes are involved in many regulatory changes of immune cell infiltration during the initiation and progression of gastric

cancer, which elucidates the pathogenesis of gastric cancer to a certain extent.

Traditional Chinese medicine has shown good therapeutic effects in tumor treatment. Orange leaves, orange kernel, green peel, tangerine peel and turmeric can be used for tumor prevention and treatment, and display obvious curative effect on improving the prognosis of tumor patients. Orange leaves are the leaves of citrus and its cultivated varieties of Rutaceae. Studies have found that orange leaves can inhibit the proliferation of gastric cancer cell SGC7901, liver cancer cell HepG-2, breast cancer cell MCF-7, pleomorphic malignant glioma T98, and colon cancer cell HCT-116 [41]. Tangerine seed is the dried and mature seed of tangerine and its cultivated varieties. Studies have shown that the active components of tangerine seed limonin and nomiline can activate glutathione (GSH) transferase in liver and small intestine mucosa. GSH is the inducer of detoxification enzyme, which effectively inhibits benzopyrene-induced tumors [42]. Tang showed that limonin analogues, an active component of orange nucleus, could significantly inhibit the proliferation of breast cancer cell line MCF-7 by blocking the cell in G2/M phase [43]. Green peel is the dry and immature peel of orange fruits, with flavonoids as its main chemical components. It was found that green peel could significantly inhibit the proliferation of triple negative breast cancer cells HCC1806, HCC1937 and MDA-MB-231 [44]. Tangerine peel is the dried and ripe fruit peel of oranges, which has both medicinal and edible value. Research has shown that tangerine peel can inhibit the proliferation of human lung cancer cell line A549, colon cancer cell line HCT-8, and liver cancer cell line Bel-7402 [45]. Turmeric is a perennial rhizome of the ginger family and has medicinal value. Its main active ingredient is curcumin. Curcumin inhibits the proliferation of breast cancer cells by down-regulating the expression of nuclear transcription factor-κB (NF-κB) and inhibiting the activity of ornithine decarboxylase [46]. In all, traditional Chinese medicine has great medicinal value and potential in the treatment of tumor, which enhances our confidence of using traditional Chinese medicine in gastric cancer treatment.

5. Conclusion

Therefore, our analysis found that the high expression of LOXL1 may not conducive to the infiltration of B cells but promote the infiltration of macrophages, thereby changing the tumor immune microenvironment to promote the initiation and progression of gastric cancer and affect the prognosis of gastric cancer patients. Using Chinese medicine to regulate LOXL1 may play a role in inhibiting gastric cancer and improving the prognosis of patients. The results of this study can provide new insights and ideas for the discovery of new targets for gastric cancer treatment and the development of effective anti-cancer drugs from traditional Chinese medicine.

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CONFLICT OF INTEREST

The authors declare no conflicting of interest.

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