ILDR1 Is a Prognostic Biomarker and Associated With Immune Infiltration in Gastric Cancer

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ILDR1 Is a Prognostic Biomarker and Associated with Immune Infiltration in Gastric Cancer

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ABSTRACT

Objective: To investigate the association between ILDR1 and prognosis and immune infiltration in gastric cancer.

Methods: We analyzed the RNA sequencing data of 9736 tumor tissues and 8587 normal tissues in the TCGA and GTEx databases through the GEPIA2 platform. The expression of ILDR1 in gastric cancer and normal gastric mucosa tissues with GEPIA and TIMER. Clinical subgroup analysis was made through Kaplan-Meier analysis. Analyzed the correlation between ILDR1 and VEGFA expression in gastric cancer, through the gene sequencing data of gastric cancer in TCGA. Explored the relationship between ILDR1 methylation and the prognosis of gastric cancer patients through the MethSurv database. The correlation between ILDR1 and immune cells and the correlation of copy number variation were explored through the TIMER database.

Results: ILDR1-high GC patients had a lower PFS and OS. High ILDR1 expression was significantly correlated with tumor grade. There was a negative correlation between the ILDR1 expression and the abundances of CD8+ T, Macrophages and DC and etc. The methylation level of ILDR1 is associated with a good prognosis of gastric cancer. ILDR1 copy number variation was correlated with immune cells, IDLR1 arm-loss was associated with the infiltration of B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells, and arm-duplication was associated with the infiltration of B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils and dendritic cells.

Conclusion： The increased expression of ILDR1 is associated with poor prognosis in patients with gastric cancer. ILDR1 can be used as a novel predictive biomarker to provide a new therapeutic target for gastric cancer patients.

Key words: ILDR1, gastric cancer, biomarker, immune infiltration, prognosis

Key words: ILDR1, gastric cancer, biomarker, immune infiltration, prognosis， bioinformatics analysis

INTRODUCTION

Gastric cancer (GC) is a highly aggressive disease. In recent years, the incidence rate ranks fifth among all types of cancer in the world, and the mortality rate ranks second. Approximately 1,000,000 new cases of gastric cancer occur each year.[1,2]. Many patients have a 5-year survival rate of less than 5% due to late recurrence or distant metastasis[3]. Although there are various clinical treatment methods for gastric cancer, including surgery, chemotherapy, radiotherapy, targeted therapy, biological immunotherapy, etc., there is still no significant clinical effect [4,5]. At present, more and more researches believe that the occurrence and poor prognosis of gastric cancer are related to abnormal gene expression, and these genes can interact and play a role through the regulatory network. Therefore, the specific pathogenesis of gastric cancer is not clear, although it has been used to search for tumor prognosis or Great efforts have been made to diagnose biomarkers, but little use in clinical practice [6,7]. Therefore, it is particularly important to evaluate the relationship between biomarkers and diseases on the basis of genetic level, protein level and clinical factors.

ILDR1 encodes a protein containing an immunoglobulin-like domain, the encoded protein can act as a multimeric receptor on the cell surface. Diseases associated with ILDR1 include Deafness, Autosomal Recessive 42 and Autosomal Recessive Non-Syndromic Sensorineural Deafness[8-10]. In addition, the expression of this gene may be a diagnostic marker of cancer progression, and the role of ILDR1 in cancer remains unclear.

So far, no studies have investigated the function of ILDR1 in gastric cancer. This study evaluated the expression of ILDR1 in the Cancer Genome Atlas (TCGA) and Comprehensive Gene Expression (GEO) database, and studied the correlation between the prognosis of gastric cancer and the expression of ILDR1 in order to provide a basis for the clinical treatment of gastric cancer.

Methods and materials

1.1 Verification of the differential expression of ILDR1 in gastric cancer

Gene Expression Profiling Interactive Analysis (GEPIA2) [11](http://gepia2.cancer-pku.cn/) , the platform includes RNA sequencing data of 9736 tumor tissues and 8587 normal tissues from TCGA and Genotype-Tissue Expression (GTEx) databases, which can analyze the differential expression of genes in tumor and normal tissue samples. Tumor Immune Estimation Resource (TIMER) was originally used to detect the infiltration of immune cells in the sample [12], At the same time, TIMER also provides genetic analysis tools. In this study, GEPIA2 and TIMER were used to analyze the expression difference of ILDR1 in gastric cancer samples and normal ovarian samples.

1.2 Prognostic analysis and clinical subgroup analysis of ILDR1

The Kaplan Meier mapping platform can evaluate the prognostic value of more than 50,000 genes (mRNA, miRNA, protein) in 21 cancer types. The tool is mainly based on meta-analysis to explore the prognostic value of genes in tumors. In order to verify the relationship between ILDR1 and the prognosis of gastric cancer, based on the patient's overall survival (OS) and progression-free survival (PFS), we used the Kaplan Meier mapping platform to explore the relationship between ILDR1 and the prognostic value of gastric cancer. In order to further determine the independent prognostic role of ILDR1, we used the Kaplan Meier mapping platform to group all patients according to gender (male, female), HER2 status (HER2+, HER2-) and TNM stage (Stage1, Stage2, Stage3, and Stage4), and Kaplan Meier survival analysis was used to detect the prognostic value of ILDR1 in different clinical subgroups.

1.3 The expression of ILDR1 in normal human tissues and organs

Human Protein Atlas [13] It aims to use various omics technologies, including antibody-based imaging, mass spectrometry-based proteomics, transcriptomics, and systems biology to map all human proteins in cells, tissues, and organs. The study used human protein maps to analyze the expression of ILDR1 mRNA and protein levels in various tissues and organs of the human body.

1.4 Correlation analysis of ILDR1 and VEGFA expression in gastric cancer

Immunotherapy has achieved good therapeutic effects in a variety of cancers, and researchers are paying more and more attention to immunotherapy targets. This study determined the correlation between ILDR1 and VEGFA expression to determine the potential of ILDR1 as an immunotherapy target. Using the gene sequencing data of gastric cancer in TCGA, the correlation analysis between ILDR1 and VEGFA was explored based on pearson correlation analysis, which was mainly realized by R language.

1.5 Prognostic value of ILDR1 methylation

DNA methylation plays a vital role in cell growth, differentiation and disease pathogenesis. Abnormal DNA methylation is generally considered to be one of the characteristics of cancer development. MethSurv database[14]. Apply multiple R language packages to analyze DNA methylation data, thereby providing prognostic-related DNA methylation biomarkers. The study used the MethSurv database to explore the relationship between ILDR1 methylation and the prognosis of patients with gastric cancer.

1.6 Analysis of the correlation between ILDR1 and immune cells

Immune cell infiltration is closely related to the prognosis of gastric cancer. With the rise of targeted therapy, the molecular mechanism of gastric cancer occurrence and development continues to deepen, and molecular targeted therapy of gastric cancer has gradually emerged. Immune checkpoint proteins play an important role in the immune response and have many interactions with immune cells. In order to further explore the potential therapeutic targets of prognostic-related genes, we explored the correlation between ILDR1 and immune cells and the correlation of copy number variation in the TIMER database.

RESULTS

2.1 Verification of ILDR1 expression in gastric cancer

The results of using limma analysis in TIMER to explore the expression of ILDR1 (Figure 1A) showed that compared with the normal control group, ILDR1 is in BRCA, CHOL, COAD, ESCA, KIRC, KIRP, LIHC, LUAD, LUSC, compared with the adjacent tissues. The expression in cancers such as STAD and STAD was significantly increased ($P < 0.05$); while its expression in KICH and PRAD was significantly decreased ($P < 0.05$). The results of ILDR1 expression analysis in each

tumor in GEPIA (Figure 1B) showed that ILDR1 was more expressed in BRCA, CESC, COAD, LUAD, LUSC, OV, PAAD, PRAD, READ, STAD, THCA and UCEC compared with adjacent tissues. High, and low expression in KIRC.

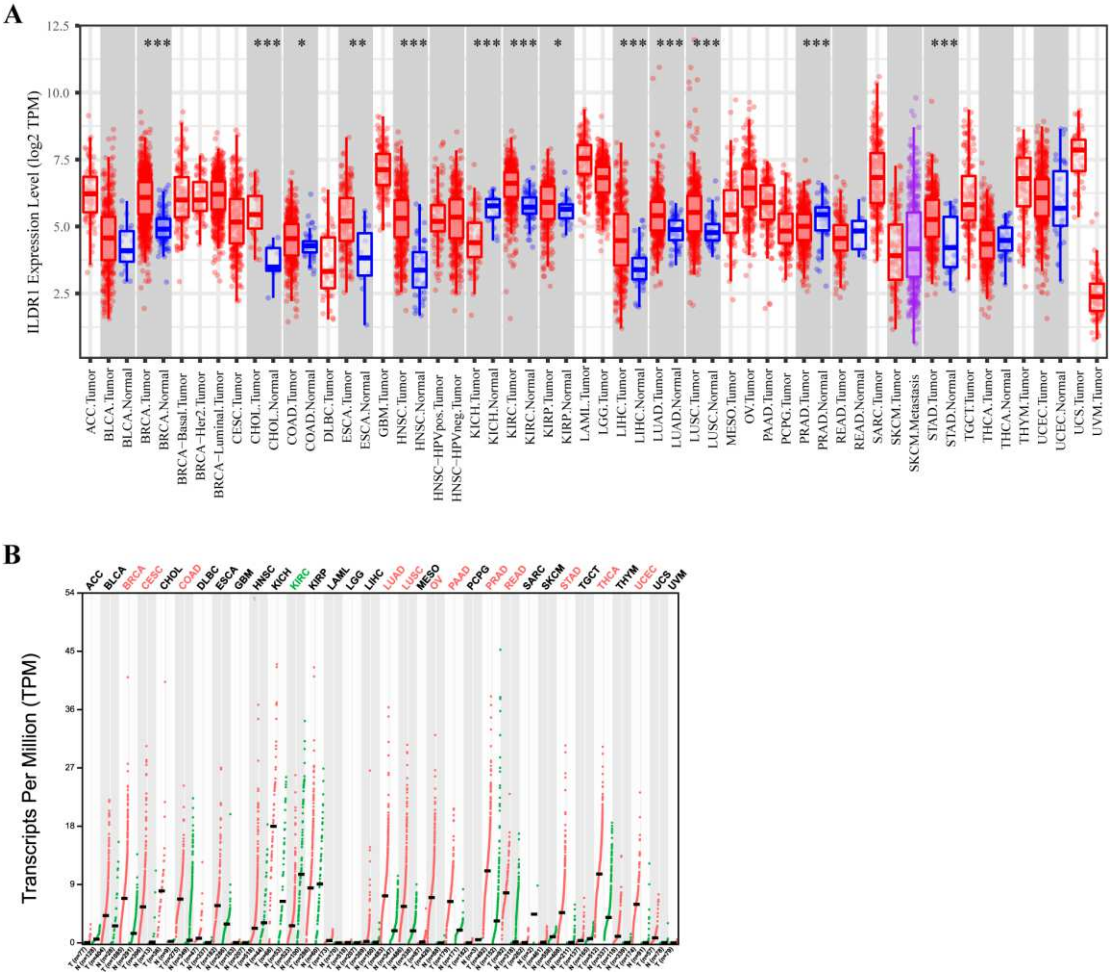

Figure 1 The differential expression of ILDR1 in various cancers including gastric cancer

2.2 The relationship between ILDR1 and the prognosis of gastric cancer and other tumors

In the Kaplan Meier mapping platform, Kaplan-Meier survival analysis was used to explore the prognostic value of ILDR1, and the results showed that whether it is based on overall survival (OS) or progression-free survival (PFS) for analysis The prognosis of gastric cancer patients with high ILDR1 expression was positively correlated (OS: Log-rank p = 7e-04, PFS: Log-rank p = 0.0051, Figure 2A, B).

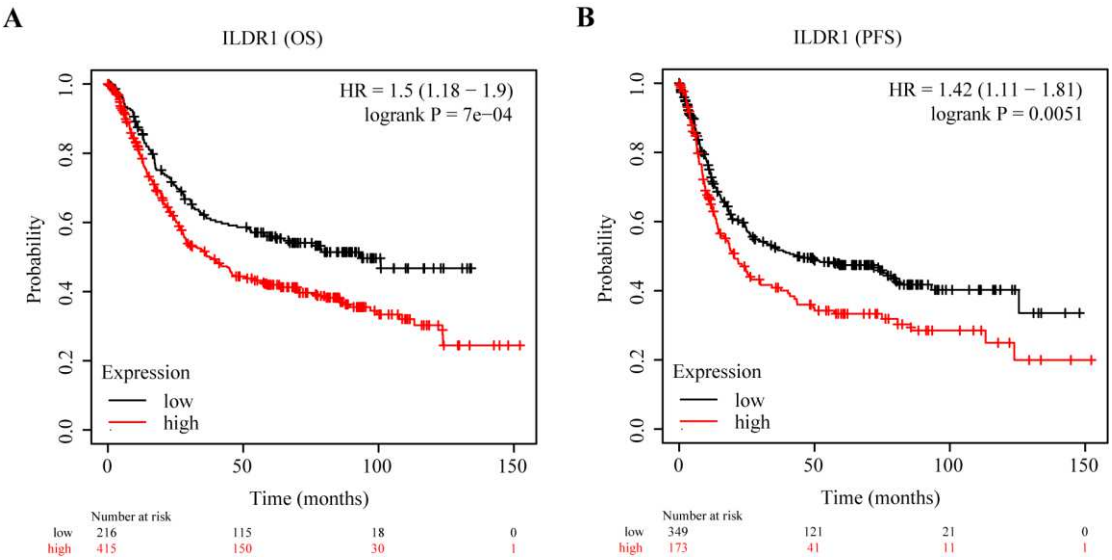

Figure 2 The relationship between ILDR1 and the prognosis of gastric cancer and other tumors

2.3 Kaplan-Meier survival analysis results of ILDR1 in different subgroups

In order to determine that ILDR1 is an independent prognostic factor for gastric cancer, we used Kaplan-Meier survival analysis to explore the prognostic value of ILDR1 in gastric cancer. The results showed (Figure 3) that ILDR1 is in male patients (HR = 1.50, logrank P = 0.0086), and female patients Patients (HR = 1.55, logrank P = 0.036), HER2+ patients (HR = 1.57, logrank P = 0.033), HER2- patients (HR = 1.83, logrank P = 0.0015), Stage 1 patients (HR = 4.04, logrank P = 0.022), Stage 2 patients (HR = 1.99, logrank P = 0.027), Stage 3 patients (HR = 1.57, logrank P = 0.027) and Stage 4 patients (HR = 1.55, logrank P = 0.029). The prognosis of the patient is poor. Therefore, ILDR1 can be regarded as an independent prognostic factor for patients with gastric cancer.

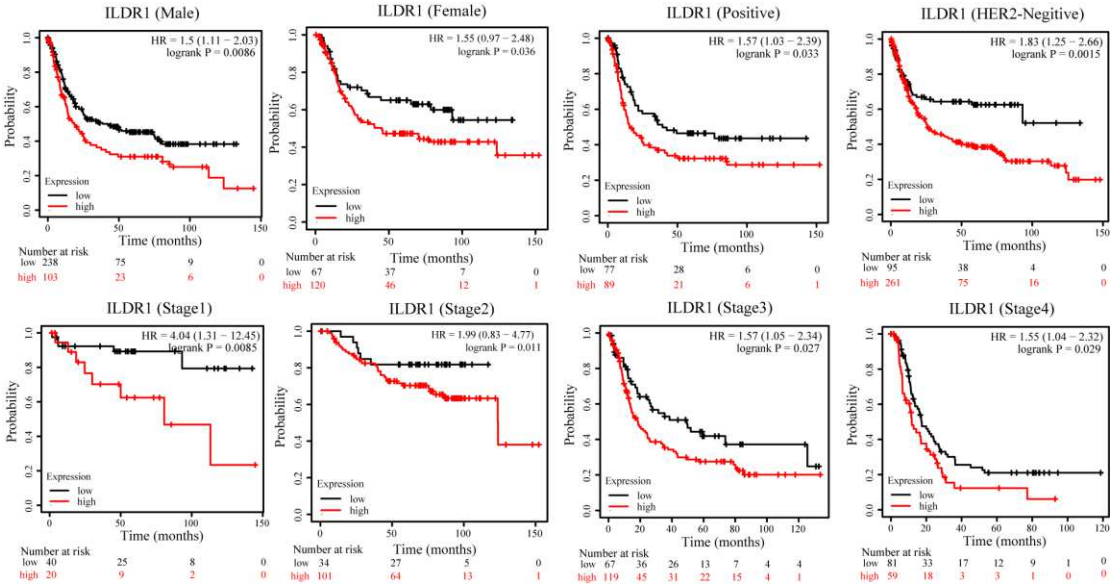

Figure 3 Survival analysis of ILDR1 in different subgroups of gastric cancer

2.4 The expression of ILDR1 in various tissues of the human body

The expression analysis of ILDR1 in various tissues of normal human body (Figure 4) shows that ILDR1 has higher mRNA levels and protein levels in endocrine tissues, male reproductive organs and female reproductive organs, and lower mRNA levels in gastric tissue. The expression is higher at the protein level.

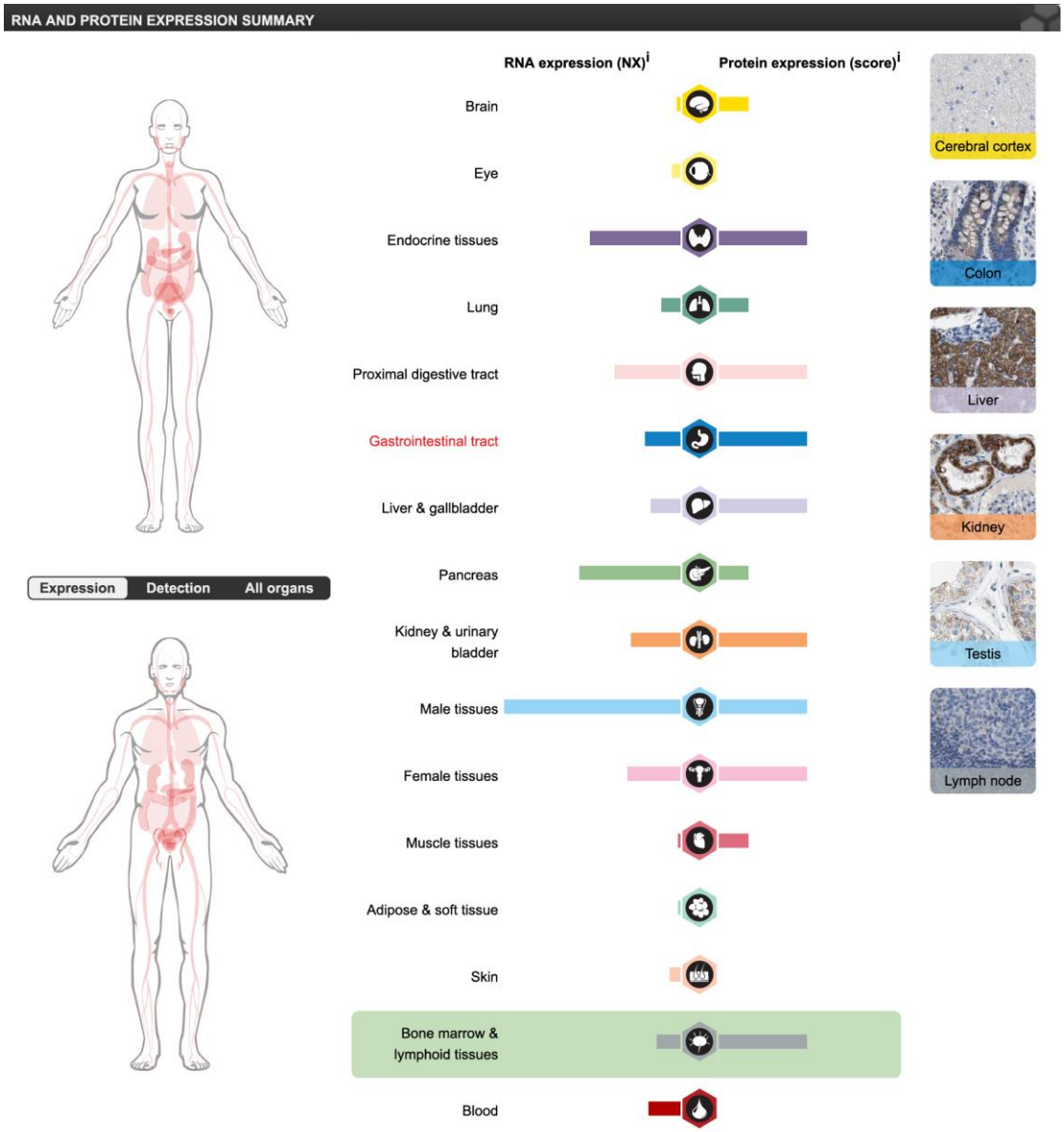

Figure 4 The expression of ILDR1 in various tissues of the human body

2.5 Prognostic value of ILDR1 methylation in gastric cancer

The expression of ILDR1 methylation in gastric cancer, and the corresponding distribution of age, gender, race, and survival status are shown in the cluster heat map (Figure 5). The methylation of ILDR1 and the Kaplan-Meier survival results of gastric cancer patients showed (Figure 4), ERO1L- ILDR1−TSS1500−Open_Sea−cg05657709(HR=0.645,Log-rankP=0.027)and ILDR1−3'UTR−Open_Sea− cg00694560 (HR=0.624, Log-rank P = 0.017) suggests that patients with

cervical cancer have a better prognosis.

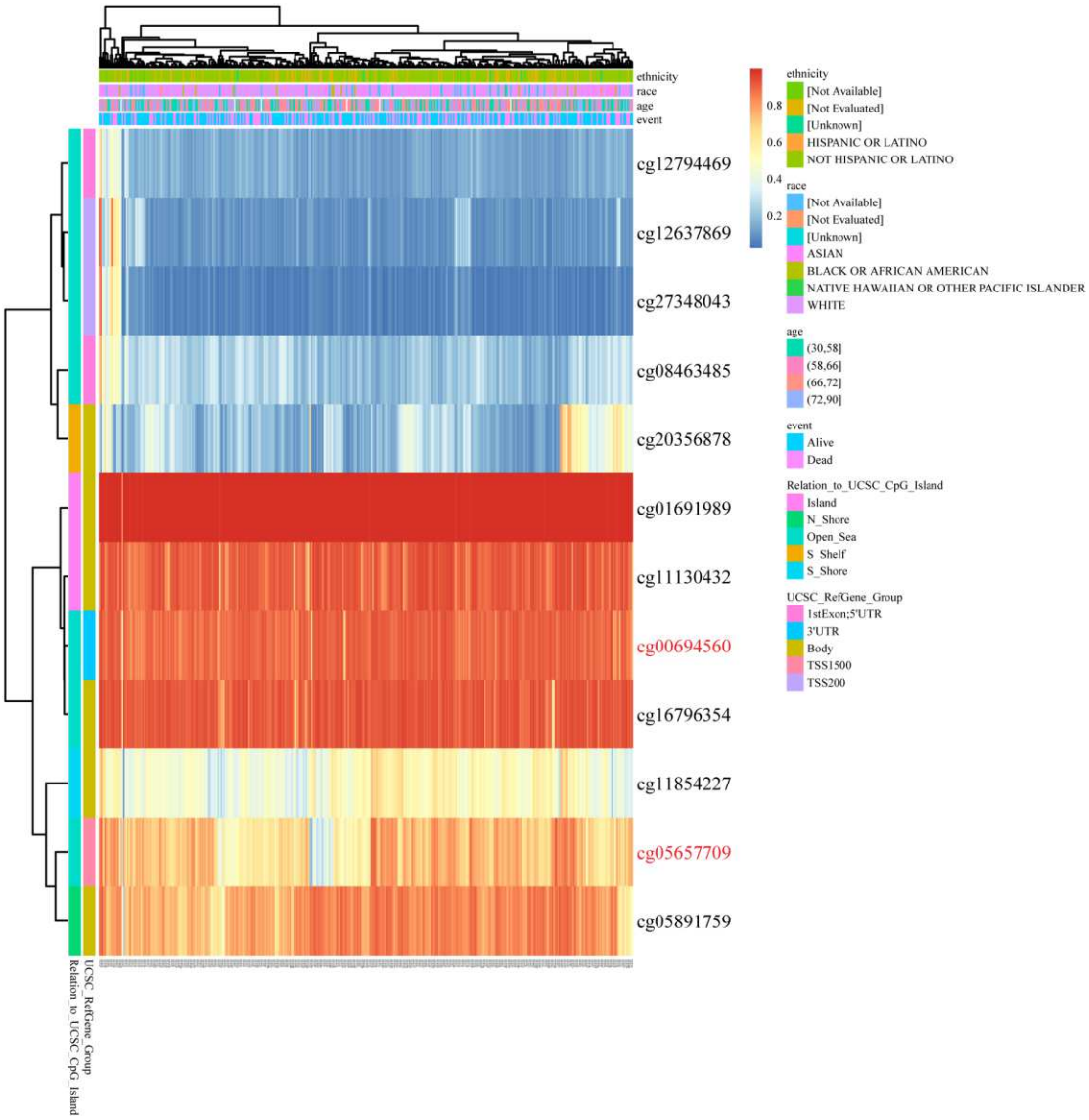

Figure 5. ILDR1 methylation expression in gastric cancer

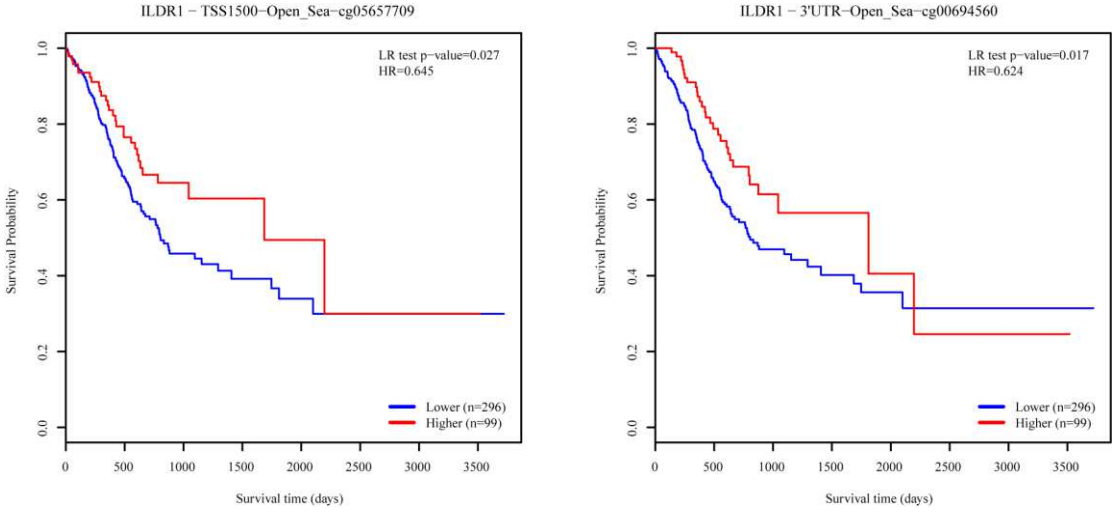

Figure 6. The prognostic value of ILDR1 methylation

2.6 Analysis of the correlation between ILDR1 and VEGFA

The Pearson correlation analysis of ILDR1 and VEGFA in all tumors including gastric cancer showed (Figure 7A) that ILDR1 and VEGFA were significantly positively correlated in all tumors (r = 0.29, p.value <0.001); while in gastric cancer Among them, ILDR1 and VEGFA still have a significant correlation (r = 0.27, p.value <0.001, Figure 7B). The correlation distribution map of ILDR1 and TP53 showed that the correlation between ILDR1 and VEGFA in gastric cancer was significantly higher (Figure 7C).

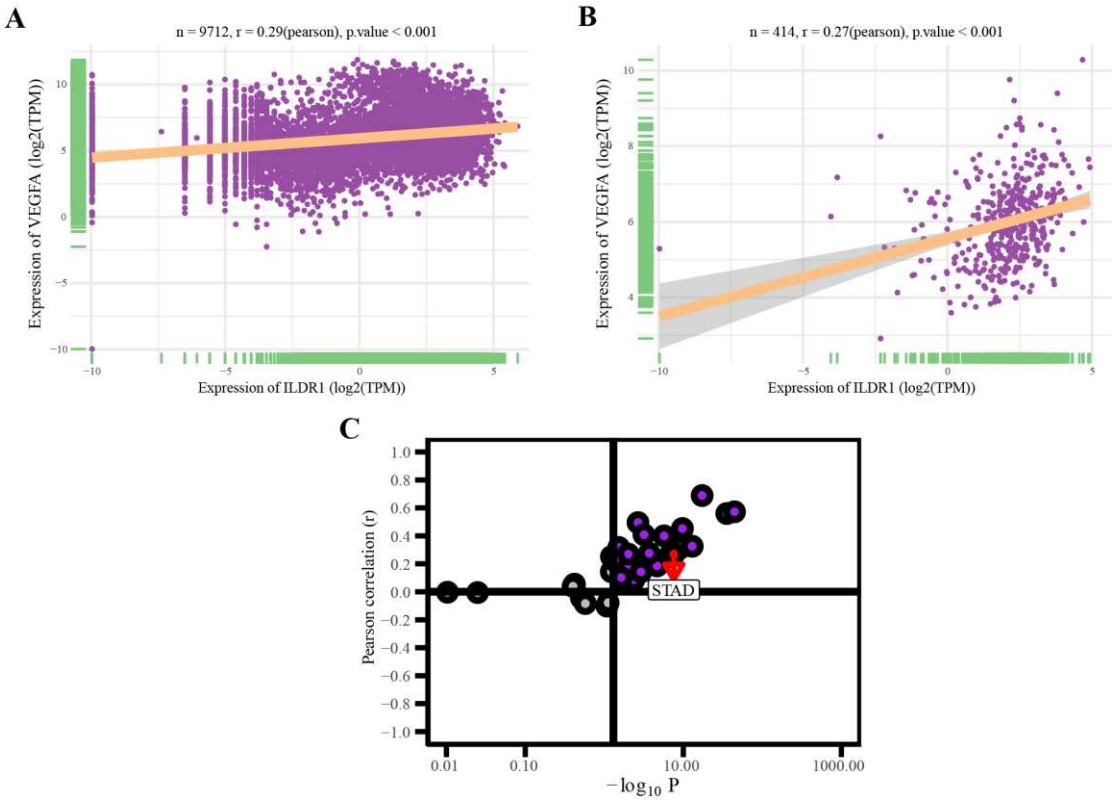

Figure 7. Correlation analysis between ILDR1 and VEGFA

2.7 The correlation between the expression of ILDR1 and ILDR2 and immune cells

The correlation analysis results of ILDR1 and ILDR2 with tumor purity and immune cells showed that ILDR1 and CD8+ T cells (cor = -0.162, P = 1.77e-03), macrophages (cor = -0.155, P = 2.73e-03)) And the infiltration of dendritic cells (cor = -1.56, P = 1.77e-02) are negatively correlated, and the correlation with the infiltration of dendritic cells is the strongest (Figure 8A); ILDR2 and B cells (cor = 0.167 , P = 1.25e-03), CD4+ cells (cor = 0.341, P = 2.01e-11), and macrophages (cor = 0.25, P = 1.13e-06) infiltration were positively correlated, and were positively correlated with neutrophils (Cor = -0.106, P = 4.06e-02) (Figure 8B).

2.8 Correlation between ILDR1, ILDR2 copy number variation and immune cells

The correlation analysis of ILDR1, ILDR2 and copy number showed that the arm loss of IDLR1 was related to the infiltration of B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils and dendritic cells (Figure 9) , And the arm duplication is related to the infiltration of B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils and dendritic cells. Therefore, the copy number variation of ILDR1 and ILDR2 is closely related to the infiltration of a variety of immune cells, and may play an opposite role.

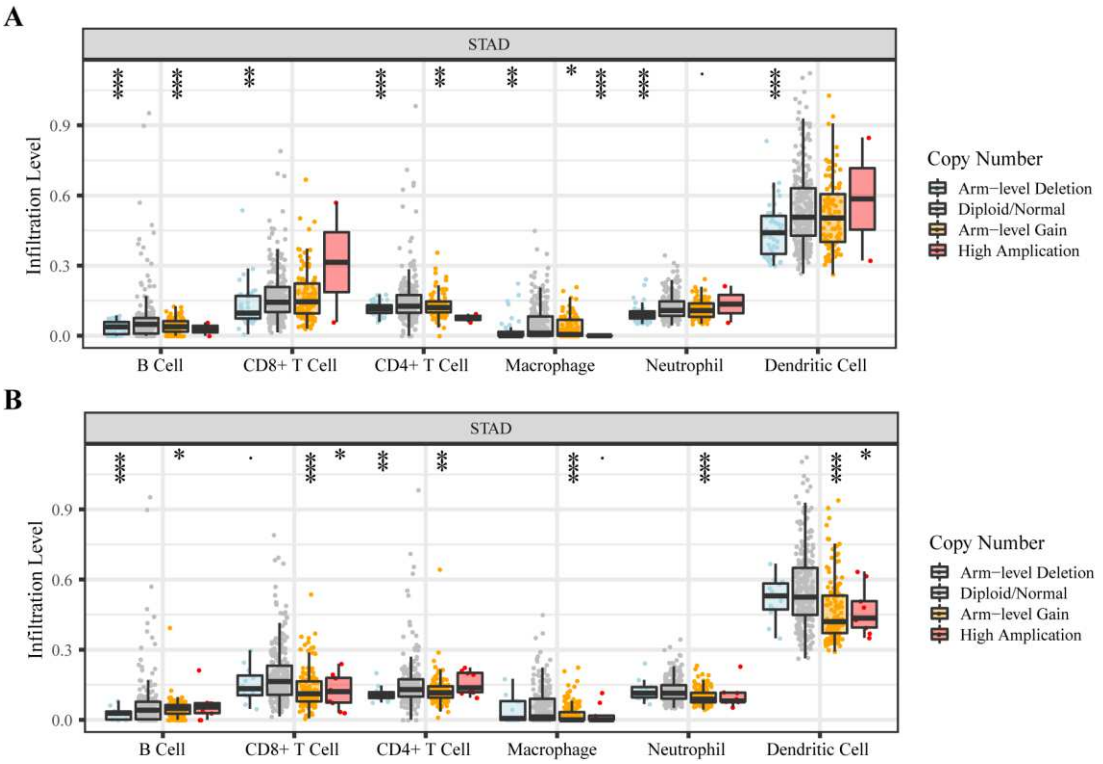

Figure 8 Correlation analysis of ILDR1 gene copy number variation

DISSCUSSION

Gastric cancer is a common gastrointestinal tumor that threatens human health. Because there are no typical and clear clinical features in the early stage, it is usually not diagnosed until the late stage, resulting in a poor prognosis of surgery and a 5-year survival rate of less than 30%[15,16]. The main cause of postoperative death is tumor metastasis or recurrence. Therefore, discovering new targets that can monitor the prognosis of patients to prolong survival is the clinical treatment goal [17].

Current research on ILDR1 focuses on auditory system diseases [18,19]. However, studies have shown that it is also related to the biological activities of tumors. The results of this study indicate that the expression level of ILDR1 in gastric cancer tissues is significantly higher than that in normal tissues. In addition, the current evaluation of the association between ILDR1 expression and the prognosis of patients with gastric cancer supports the idea that ILDR1 may play a role in gastric cancer. Kaplan-Meier database analysis showed that there is a significant correlation between the expression of ILDR1 and the prognosis of gastric cancer patients. In order to determine that ILDR1

is an independent prognostic factor for gastric cancer, we used Kaplan-Meier survival analysis to explore the prognostic value of ILDR1 in gastric cancer, and found that the high expression of ILDR1 is associated with a poor prognosis for patients. Therefore, ILDR1 can be regarded as an independent prognosis for patients with gastric cancer. factor. According to the expression of this gene in human tissues, the expression of mRNA in gastric tissue is low, while the expression of protein is high.

In addition, we also analyzed the relationship between the methylation level of ILDR1 and the prognosis of gastric cancer and found that the high methylation level of ILDR1 is beneficial to the prognosis of patients. The correlation between ILDR1 and VEGFA in gastric cancer is very large, suggesting that ILDR1 is related to tumor angiogenesis. The correlation analysis results of ILDR1 and ILDR2 with tumor purity and immune cells showed that ILDR1 was negatively correlated with the infiltration of CD8+ T cells, macrophages, and dendritic cells. Among them, the correlation with the infiltration of dendritic cells was the strongest. The copy number variation of ILDR1 is closely related to the infiltration of a variety of immune cells in gastric cancer, which may play an important role in the development of gastric cancer.

In conclusion, based on the current comprehensive analysis, the results of this study indicate that there is a strong correlation between ILDR1 expression and the prognosis of gastric cancer patients. The significant overexpression of ILDR1 in gastric cancer indicates that ILDR1 may be a promising potential prognostic marker for gastric cancer. The actual mechanism of ILDR1 in gastric cancer is still unknown, and further research is needed. The bioinformatics method used in this study is a useful tool for predicting and screening new markers, but further in vitro and in vivo verification is needed to evaluate the role of ILDR1 in gastric cancer.

Abbreviations

ILDR1：Immunoglobulin Like Domain Containing Receptor 1；GC：gastric cancer；TCGA：the Cancer Genome Atlas ；GEPIA：Gene Expression Profiling Interactive Analysis ；PFS：progression-free survival；OS：overall survival；DC：Dendritic Cells；GEO：Gene Expression omnibus；GTEx：Genotype-Tissue Expression；TIMER：Tumor Immune Estimation Resource ；HER2：human epidermalgrowth factor receptor-2；TNM：Tumor ,Lymph Node, Metastasis ；VEGFA：vascular endothelial growth factor A ；BRCA：Breast Cancer；CHOL：Cholangiocarcinoma；COAD：Colon adenocarcinoma；ESCA：Esophageal carcinoma；KIRC：Kidney renal clear cell carcinoma；KIRP：Kidney renal papillary cell carcinoma；LIHC：Liver hepatocellular carcinoma； LUAD：Lung adenocarcinoma；LUSC：Lung squamous cell carcinoma；STAD：Stomach adenocarcinoma；BRCA：Breast invasive carcinoma；CESC：Cervical squamous cell carcinoma and endocervical adenocarcinoma；OV：Ovarian serous cystadenocarcinoma；PAAD：Pancreatic adenocarcinoma；PRAD：Prostate adenocarcinoma；READ：Rectum adenocarcinoma；THCA：Thyroid carcinoma；and UCEC：Uterine Corpus Endometrial Carcinoma；

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Contributions

YLM conceived and designed the study. HBG and XML made the diagrams and tables of the article. ZYY and QL made wrote the paper. WBQ and HC revised the article. All the authors read and approved the manuscript.

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Availability of data and materials

The data used to support the findings of this study are included within the article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All the authors consent for publication.

Competing interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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