APOA1 mRNA and serum APOA1 protein as diagnostic and prognostic biomarkers in gastric cancer

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Background: Gastric cancer (GC) remains a formidable challenge in oncology, ranking as a leading cause of cancer mortality globally. This underscores an urgent need for innovative prognostic markers that can revolutionize patient management and outcomes. Recent insights into cancer biology have spotlighted the profound influence of lipid metabolism alterations on tumorigenesis, tumor progression, and the tumor microenvironment. These alterations not only fuel cancer cell growth and proliferation but also play a strategic role in evading immune surveillance and promoting metastasis. The intricate web of lipid metabolism in cancer cells, characterized by deregulated uptake, synthesis, and oxidation of fatty acids (FAs), opens new avenues for targeted therapeutic interventions and prognostic evaluations. Specifically, this study zeroes in on apolipoprotein A-I (*APOA1*), a key player in lipid metabolism, to unearth its prognostic value in GC. By delving into the role of lipid metabolism-related genes, particularly *APOA1*, we aim to unveil their potential as groundbreaking biomarkers for GC prognosis. This endeavor not only aims to enhance our understanding of the molecular underpinnings of GC but also to spearhead the development of lipid metabolism-based strategies for improved diagnostic, prognostic, and therapeutic outcomes.

Methods: Transcriptomic and clinical data from GC patients and healthy individuals were sourced from The Cancer Genome Atlas (TCGA) database, a comprehensive project that molecularly characterizes over 20,000 primary cancer and matched normal samples across 33 cancer types. Significantly differentially expressed lipid metabolism-related genes were identified using the "limma" package in R. Prognostic genes were selected via univariate Cox regression analysis. Differential gene enrichment analysis was performed using Metascape (http://www.metascape.org). The Human Protein Atlas (HPA, https://www.proteinatlas.org) provided information on APOA1 protein expression in GC and healthy tissues. Immune cell infiltration was analyzed using the CIBERSORT algorithm (http://cibersort.stanford.edu).

Results: Significant differences in lipid metabolism-related gene expression were observed between GC and normal tissues, closely linked to FA metabolism, oxidoreductase activity, and sphingolipid metabolism. *APOA1* emerged as a potential prognostic biomarker by intersecting prognostic and differentially expressed lipid metabolism genes. Immunohistochemical analysis confirmed *APOA1* downregulation in GC. The receiver operating characteristic (ROC) analysis demonstrated its predictive value, with the area under the curve (AUC) being 0.64 [95% confidence interval (CI): 0.52–0.76]. *APOA1* expression correlated with immune cell infiltrations. Clinical serum *APOA1* results revealed lower levels in GC patients (1.38 *vs.* 1.26; P<0.05), associated with poor prognosis (hazard ratio =1.50; P<0.001) and clinical characteristics. ROC analysis of serum *APOA1* demonstrated good diagnostic ability (AUC: 0.63, 95% CI: 0.61–0.65). Serum *APOA1* levels significantly increased after treatment.

Conclusions: This study highlights lipid metabolism reprogramming in GC and identifies *APOA1* as a potential diagnostic and prognostic biomarker, suggesting its clinical utility in managing GC.

Introduction

Gastric cancer (GC) is the fifth most common malignant tumor worldwide, with over one million new cases estimated annually. In this article, the term 'gastric cancer' specifically refers to its most prevalent histological type—adenocarcinoma. The diagnosis of GC is often made at an advanced stage, resulting in high mortality rates and making it the third leading cause of cancer-related deaths. The 5-year survival rate for advanced GC patients is as low as 18% (1,2). Thus, prognosis significantly impacts the survival and quality of life for patients. Due to the insidious onset and rapid progression of GC, most patients are already in advanced stages at the time of diagnosis. For confirmed advanced GC, chemotherapy is the standard treatment, but the median overall survival (OS) with conventional chemotherapy is only around eight months (3). Therefore, early diagnosis and treatment of GC are crucial for improving prognosis. Currently, early detection of GC

primarily relies on endoscopic examinations. However, limitations exist regarding the coverage of endoscopy in different countries, and early GC may lack characteristic features under endoscopy, leading to the possibility of missed diagnoses even by experienced physicians. Therefore, the search for simple and feasible GC-specific or prognostic biomarkers is of great importance (4,5).

With the help of bioinformatics, we have identified a series of genes that can serve as diagnostic or prognostic biomarkers and therapeutic targets for GC (6,7). However, most of these biomarkers have not been extensively validated or are associated with complex and costly detection methods, which limits their clinical applicability. Therefore, it is crucial to identify novel markers that are readily accessible in clinical settings and can accurately predict prognosis for GC patients.

In recent years, the role of lipid metabolism in cancer has been extensively explored. Tumor cells alter lipid metabolism to meet their needs for growth and metastasis (8). Specifically, alterations in lipid metabolism can promote tumor cell growth, invasion, and metastasis, and regulate the characteristics of the tumor microenvironment through intracellular signaling pathways (9,10). Changes in lipid metabolism have been confirmed to be associated with tumor growth and treatment response in various cancers, including colorectal cancer, pancreatic cancer, and prostate cancer (11-13). However, the alterations in lipid metabolism in GC and whether these changes can serve as prognostic biomarkers have not been fully investigated. In this study, we aim to analyze differentially expressed lipid metabolism-related genes between GC and normal patients by using bioinformatics analysis of The Cancer Genome Atlas (TCGA) database. We analyze the pathways to elucidate the reprogramming of lipid metabolism pathways in GC. Subsequently, we screen prognostic lipid metabolism-related genes by intersecting significantly upregulated genes and prognosis-related genes. By integrating bioinformatics analysis and clinical data collection, we validate the significance of the selected biomarkers in diagnosing and predicting prognosis in GC. Furthermore, by collecting clinical data from GC patients, we further validate the

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Highlight box

Key findings
- The study identified apolipoprotein A-I (*APOA1*) as a potential prognostic biomarker for gastric cancer (GC). *APOA1* was found to be downregulated in GC tissues and associated with poor prognosis.
- The serum *APOA1* levels were significantly lower in GC patients, and these lower levels were associated with poor prognosis and clinical characteristics. The receiver operating characteristic analysis demonstrated the diagnostic ability of serum *APOA1*.

What is known and what is new?
- GC is a significant global health concern with poor survival rates, necessitating the search for effective diagnostic and prognostic markers.
- This study utilizes bioinformatics and clinical data to identify and validate lipid metabolism-related genes *APOA1* as potential diagnostic and prognostic biomarkers for GC.

What is the implication, and what should change now?
- The protein level of *APOA1* demonstrated excellent diagnostic capability.
- Elucidate the role of *APOA1* in GC would be highly valuable.

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diagnostic and prognostic predictive power of the selected biomarkers. Through the integration of bioinformatics and clinical data, our study aims to provide a deeper understanding for early diagnosis and prognosis prediction in GC. We present this article in accordance with the REMARK reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-1966/rc).

Methods

Data collection and processing

TCGA (https://portal.gdc.cancer.gov/) is currently the largest cancer genomics database (14). We downloaded transcriptomic and corresponding clinical data from 375 GC patients and 32 healthy controls from the TCGA database.

Lipid metabolism-related gene acquisition

We obtained a dataset containing lipid metabolism-related genes from the Gene Set Enrichment Analysis (GSEA) website (15) (https://www.gsea-msigdb.org/). The datasets downloaded included HALLMARK_FATTY_ACID_METABOLISM, REACTOME_METABOLISM_OF_LIPIDS, and Kyoto Encyclopedia of Genes and Genomes (KEGG) GLYCEROPHOSPHOLIPID_META-BOLISM. By taking the intersection of these datasets, a total of 861 lipid metabolism-related genes were collected.

Differential gene analysis

We performed differential gene expression analysis using the R language and the "limma" package. This analysis helped us identify statistically significant differentially expressed genes.

Gene functional enrichment analysis

We imported the differentially expressed genes into the Metascape database (https://metascape.org/) for gene enrichment analysis, including molecular functions (MF), biological processes (BP), cellular components (CC), and KEGG pathway analysis (16,17).

Immune infiltration analysis

We evaluated the immune infiltration status in GC based on the immune infiltration data available in the TCGA database. We utilized the CIBERSORT tool (18) to analyze the immune infiltration status. Specifically, we compared and analyzed the differences in immune cell infiltration between the *APOA1* low-expression group and the high-expression group.

Protein expression analysis

We utilized the Human Protein Atlas (HPA) database (https://www.proteinatlas.org/), an open-source database containing information on human proteins discovered in normal and cancer tissues (19). In our study, we employed the protein expression module of HPA to examine the protein levels of *APOA1* in normal gastric tissue and GC tissue. Specifically, we assessed and compared the expression levels of APOA1 protein in both types of tissues.

Clinical data collection

We collected clinical data from 2,741 GC patients treated at The Second Hospital of Dalian Medical University between 2012 and 2023. Additionally, clinical data from 813 patients with chronic gastritis were also included. Under the criteria that included: patients diagnosed with GC or chronic gastritis, with no restrictions on age or gender, who had not undergone chemotherapy or radiotherapy, had no history of other tumors, had complete clinical records. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of The Second Hospital of Dalian Medical University (No. 2023-045). Informed consent was taken from all the patients. Patients were excluded if they had a history of chemotherapy or radiotherapy, were diagnosed with fatty liver or hyperlipidemia, had severe cardiovascular diseases, cirrhosis, or other malignancies, or if their clinical data were incomplete. The data were obtained from electronic medical records and included information such as gender, age at diagnosis, tumor grade, tumor location, and lipid profile results. Additionally, the data encompassed the TNM classification of malignant tumors, where "T" stands for the size and extent of the main tumor, "N" represents the number of nearby lymph nodes that have cancer, and "M" indicates whether cancer has metastasized. This classification system, known as the TNM staging system, provides a detailed overview of cancer progression.

APOA1 testing

On the morning after admission, fasting venous blood

samples of 10 mL were collected from the patients and transferred into anticoagulant tubes. The blood lipid profile, including triglycerides, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), apolipoprotein A-I (*APOA1*), and apolipoprotein B (*APOB*), was measured. The blood lipid analysis was performed using the SIEMENS ADVIA2400 instrument (Siemens Healthcare, Erlangen, Germany).

Statistical analysis

Independent sample *t*-tests were conducted to analyze quantitative variables. The receiver operating characteristic (ROC) curve analysis and Kaplan-Meier survival analysis were used to assess the effectiveness of R software in predicting survival outcomes. Cox proportional hazards models were employed to examine the associations among prognostic classifications, survival outcomes, and other clinical parameters. Results with a P value less than 0.05 were considered statistically significant.

Results

Analysis of lipid metabolism-related gene expression and functional enrichment in GC and control groups

In this study, we extracted data from 375 GC patients and 32 healthy control individuals from the TCGA database. By referring to the GSEA database, we identified 861 lipid metabolism-related genes expressed in patients with stomach adenocarcinoma (STAD) as recorded in TCGA. Using the limma package in R, we analyzed the differential expression of lipid metabolism genes between GC and control patients. Compared to the control group, we identified 357 upregulated lipid metabolism genes and 185 downregulated lipid metabolism genes (P<0.05) (*Figure 1A,1B*). Subsequently, we performed functional enrichment analysis of the differentially expressed genes using the Metascape website. The enrichment analysis revealed that the upregulated genes were associated with glycerophospholipid metabolism, oxidoreductase activity acting on the carbon-hydrogen (CH-CH) double bond group of donors, and sphingolipid metabolic process (*Figure 1C-1E*), while the downregulated genes were associated with lipid catabolic process and lipid modification (*Figure 1F-1H*).

Identification of prognosis-associated lipid metabolism genes

By performing univariate Cox regression analysis, a total

of 58 lipid metabolism genes were identified as prognostic markers for GC (*Figure 2A*). Intersection analysis with significantly differentially expressed lipid metabolism genes [|log fold change| >2, P<0.05] led to the selection of one significantly prognostic lipid metabolism gene, *APOA1* (*Figure 2B*). Validation of *APOA1* gene expression levels in GC and control patients was performed using the GEPIA database, which showed a downregulation of *APOA1* gene expression in GC samples compared to normal samples (P<0.05, *Figure 2C*). Analysis of APOA1 protein expression in GC and normal tissues was conducted using the HPA website, revealing weak positive staining of *APOA1* in normal tissues (HPA046715 antibody) and negative staining in GC tissues (*Figure 2D,2E*). Subsequently, TCGA-GC patients were divided into low-expression and high-expression groups based on *APOA1* expression levels, using the optimal cutoff value. Kaplan-Meier analysis consistently demonstrated worse prognosis in the *APOA1* high-expression group (*Figure 2F*). Furthermore, the predictive ability for 1-year survival was assessed using ROC analysis. The model showed a good area under the curve (AUC) value of 0.64 (P<0.001) for 1-year survival based on TCGA data (*Figure 2G*). These findings indicate that *APOA1* serves as a prognostic lipid metabolism gene marker and can be used to predict the occurrence and prognosis of GC.

Expression and prognostic value of APOA1 in pan-cancer

Figure 3 illustrates the expression pattern of *APOA1* gene in pan-cancer tissues. *APOA1* gene exhibits decreased expression in lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), STAD, testicular germ cell tumors (TGCT), and thyroid carcinoma (THCA), while it shows increased expression in ovarian serous cystadenocarcinoma (OV). These findings suggest that *APOA1* has strong tumor specificity and can serve as a highly specific tumor biomarker.

Correlation of APOA1 expression with clinical features

Our study results showed that the expression of *APOA1* mRNA did not differ across different age groups (*Figure 4A*), genders (*Figure 4B*), and TNM stages (*Figure 4C-4E*). However, in terms of tumor grade, patients with G2 grade exhibited higher *APOA1* expression compared to those with G3 grade (*Figure 4F*, P<0.01). This suggests a possible correlation between *APOA1* expression and the malignancy grade of GC.
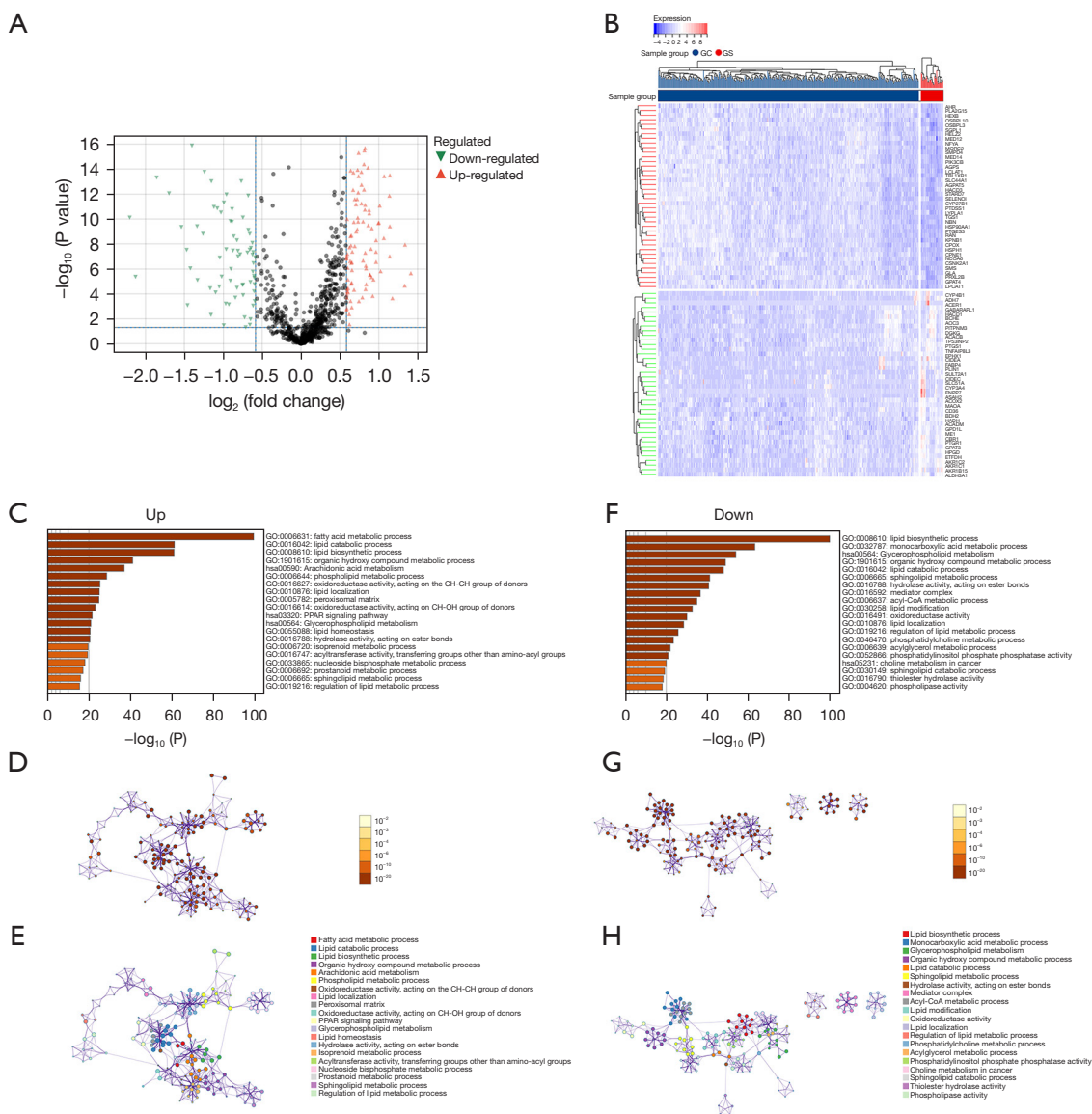
Figure 1 Identification of lipid metabolism-related genes in TCGA-GC. (A) Volcano plot showing the differential expression of lipid metabolism-related genes. Black dots, not significant. (B) Heatmap depicting the differential expression of lipid metabolism-related genes between GC tumors (n=375) and normal tissues (n=32). (C) Functional enrichment analysis of upregulated lipid metabolism genes. (D) Functional clustering network of upregulated lipid metabolism genes. (E) Network of enriched terms colored according to cluster ID for upregulated genes. (F) Functional enrichment analysis of downregulated lipid metabolism genes. (G) Functional clustering network of downregulated lipid metabolism genes. (H) Network of enriched terms colored according to cluster ID for downregulated genes. GC, gastric cancer; GS, gastritis stomach; GO, Gene Ontology; PPAR, peroxisome proliferator-activated receptor; TCGA-GC, The Cancer Genome Atlas focused on Gastric Cancer; ID, identity.

Correlation between APOA1 expression and immune infiltration levels in GC

The impact of *APOA1* on immune infiltration in GC was further evaluated. As shown in *Figure 5A*, the *APOA1* high-expression group exhibited higher expression of type II interferon (IFN) response, while the *APOA1* low-expression group showed higher expression of antigen-presenting cell (APC) co-inhibition, APC co-stimulation, cytolytic activity,
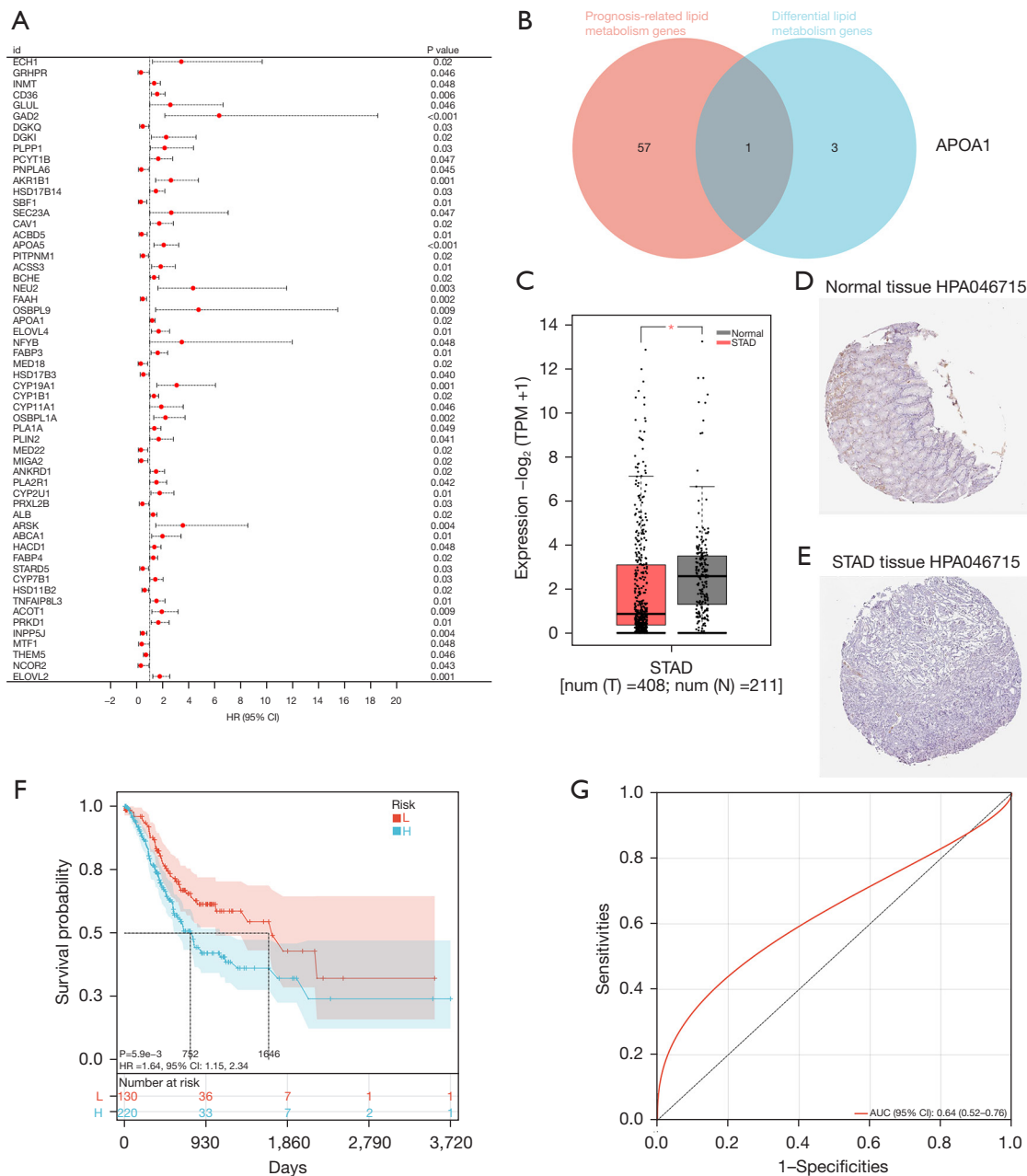
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Figure 2 Construction of the risk model in the training cohort. (A) Univariate Cox analysis of lipid metabolism genes influencing OS prognosis. (B) Venn diagram showing the intersection of significantly differentially expressed lipid metabolism genes associated with prognosis. (C) Validation of *APOA1* gene expression in healthy controls and GC using the GEPIA website. (D,E) Validation of APOA1 protein expression in GC and normal tissues using HPA database (https://www.proteinatlas.org/). The links to the normal tissues (https://www.proteinatlas.org/ENSG00000118137-APOA1/tissue/stomach). The links to the STAD tissues (https://www.proteinatlas. org/ENSG00000118137-APOA1/pathology/stomach+cancer#img). (F) GC survival analysis based on *APOA1* mRNA expression. (G) ROC curve predicting *APOA1* gene expression. *, P<0.05. HR, hazard ratio; CI, confidence interval; APOA1, apolipoprotein A-I; TPM, transcripts per million; STAD, stomach adenocarcinoma; L, low; H, high; AUC, area under the curve; OS, overall survival; GC, gastric cancer; GEPIA, Gene Expression Profiling Interactive Analysis; HPA, Human Protein Atlas; ROC, receiver operating characteristic.
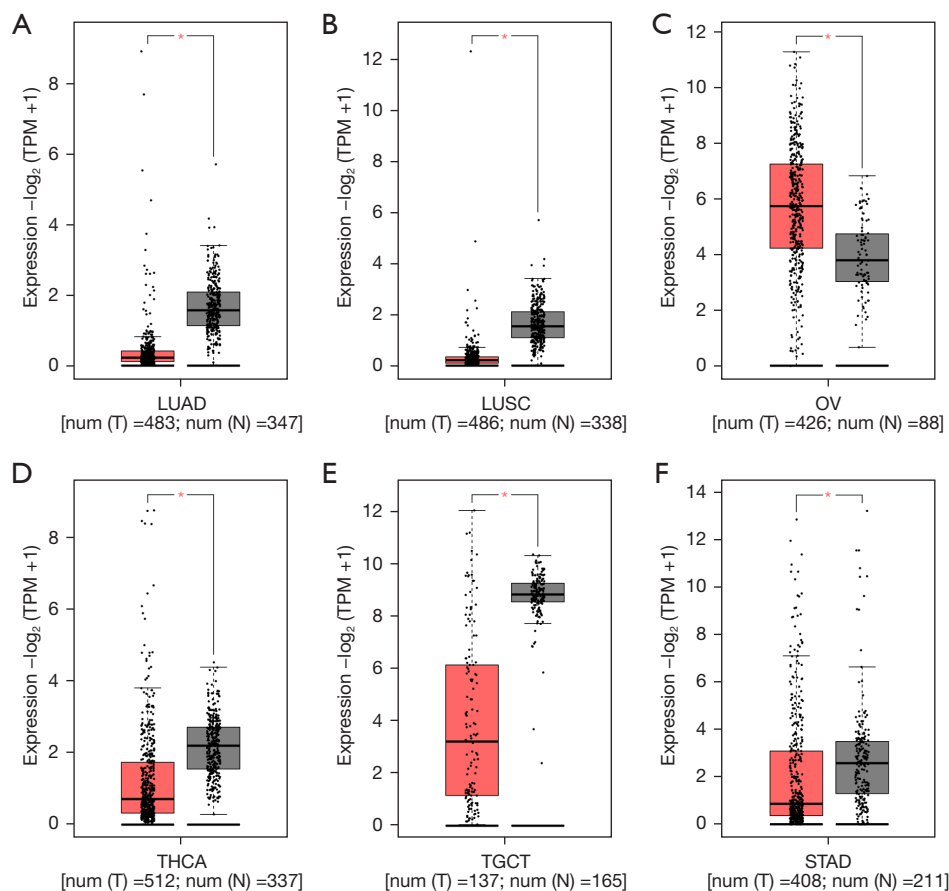
Figure 3 Expression of the *APOA1* gene in TCGA normal tissues and TCGA cancer tissues. *, P<0.05. TPM, transcripts per million; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; OV, ovarian serous cystadenocarcinoma; THCA, thyroid carcinoma; TGCT, testicular germ cell tumors; STAD, stomach adenocarcinoma; APOA1, apolipoprotein A-I; TCGA, The Cancer Genome Atlas.

major histocompatibility complex (MHC) class I, and T cell co-inhibition. Additionally, we investigated the mRNA expression levels of *APOA1* in 375 cancer tissues using the CIBERSORT database. The *APOA1* high-expression group demonstrated higher expression in B cells naive, plasma cells, T cells CD4 memory resting, monocytes, and mast cells resting, while the *APOA1* low-expression group exhibited higher expression in T cells CD4 memory activated and natural killer (NK) cells resting (*Figure 5B*).

Study of serum APOA1 protein levels in GC patients

We collected clinical data from 2,741 GC patients and 813 gastritis control patients at The Second Hospital of Dalian Medical University to investigate the expression and prognostic significance of serum APOA1 protein levels in GC patients. The study results revealed that compared to gastritis patients, GC patients had significantly decreased levels of serum APOA1 protein (1.38 *vs.* 1.26, P<0.05) (*Figure 6A*), and it showed diagnostic potential for predicting GC occurrence [AUC: 0.63, 95% confidence interval (CI): 0.61–0.65] (*Figure 6B*). Furthermore, Kaplan-Meier analysis demonstrated that among the 2,741 GC patients, those with high serum APOA1 expression had a worse prognosis [hazard ratio (HR) =1.50, P<0.001] (*Figure 6C*). Additionally, ROC analysis of serum APOA1 protein levels for predicting 1-, 3-, and 5-year survival rates in GC patients showed good diagnostic performance: 1-year (AUC: 0.59, 95% CI: 0.56–0.63), 3-year (AUC: 0.59, 95% CI: 0.56–0.62), and 5-year (AUC: 0.60, 95% CI: 0.57–0.63) (*Figure 6D*). Furthermore, serum APOA1 protein levels showed a significant association with various clinical features (*Figure 7*). The older age group (>60 years), female patients, T4 stage, N3 stage, M1 stage, and G3 grade
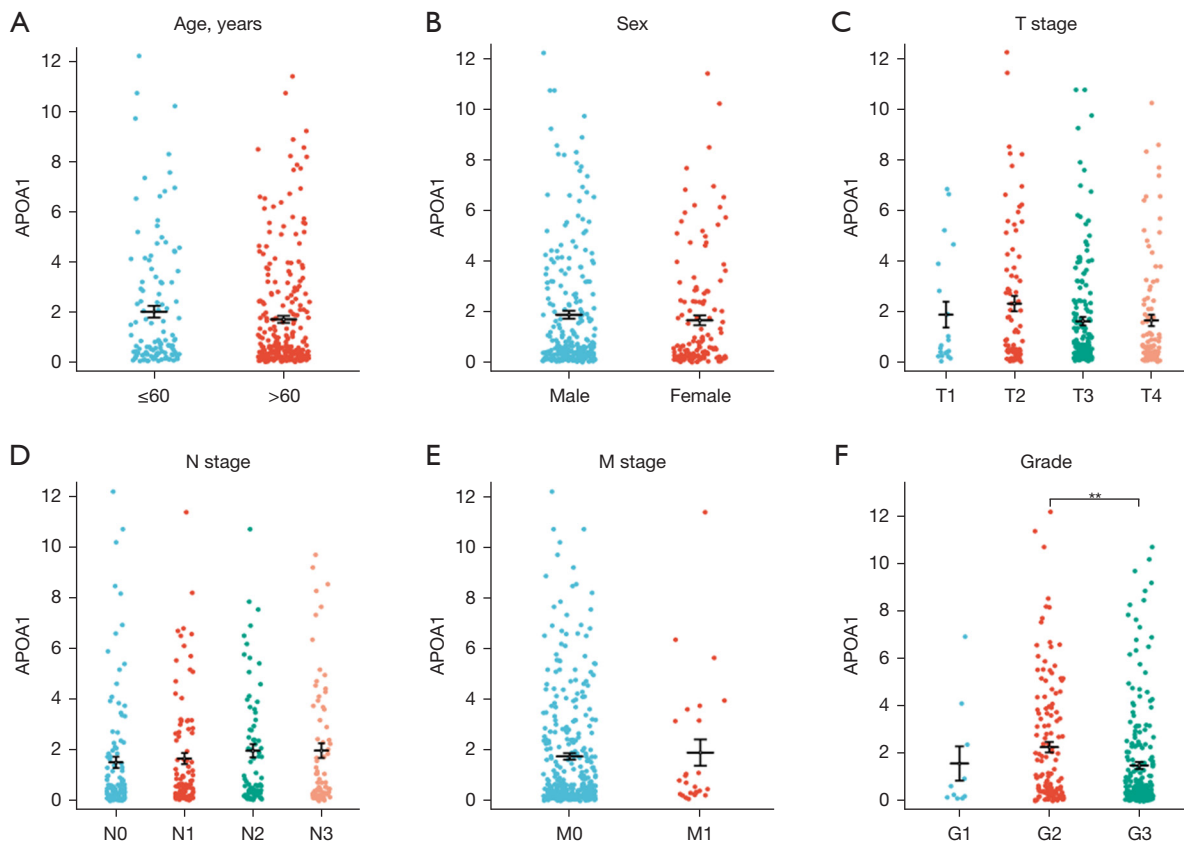
Figure 4 Analysis of the correlation between *APOA1* mRNA expression and clinical characteristics in gastric cancer patients. (A) Age. (B) Gender. (C) T stage. (D) N stage. (E) M stage. (F) Grade. **, P<0.01. APOA1, apolipoprotein A-I.

exhibited lower *APOA1* expression (P<0.05). We further validated the impact of GC treatment on serum APOA1 expression, and the results indicated a significant increase in serum APOA1 expression after treatment compared to the pre-treatment expression, further highlighting the close association between serum APOA1 expression and GC disease itself (*Figure 8*).

Discussion

Lipids are essential for energy storage, membrane proliferation, and the generation of signaling molecules. Currently, alterations in lipid metabolism in cancer cells are receiving increasing attention and recognition. There is mounting evidence that cancer cells often exhibit characteristic changes in lipid metabolism, including uptake, synthesis, storage, and breakdown of lipids (20,21). Our study revealed 357 upregulated and 185 downregulated lipid metabolism genes in GC patients compared

to normal individuals, indicating lipid metabolism reprogramming in GC. Subsequent enrichment analysis of differentially expressed genes revealed that upregulated genes were associated with phospholipid metabolism, glycerophospholipid metabolism, and lipid biosynthesis, while downregulated genes were associated with lipid degradation and lipid modification. The dysregulation of the lipid metabolism pathway may constitute a significant contributing factor in the development and progression of GC. Similar findings have been observed in studies on other tumors, where FA oxidation-related pathways, such as phospholipids and glycerolipids, showed significant upregulation, while FA β-oxidation and FA metabolism exhibited significant downregulation in tumor tissues (21). This is consistent with our research findings. Chi *et al.* found that GC patients had upregulated expression of FA synthase (FASN), downregulation of lipid acidification and β-oxidation-related enzymes, leading to increased lipid synthesis and accumulation of lipid droplets in GC cells.
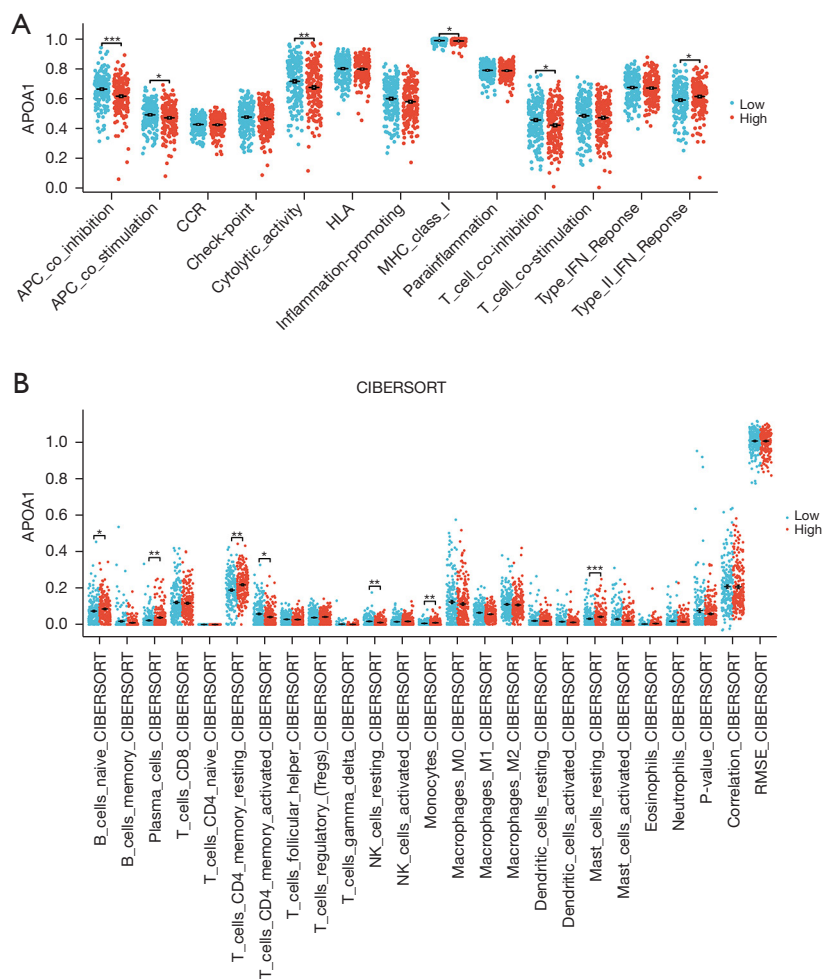
Figure 5 Analysis of the correlation between *APOA1* expression and immune cell infiltration using CIBERSORT. (A) Correlation of *APOA1* expression with proportions of 13 immune cell subtypes. (B) *APOA1* mRNA expression levels in different cell types. *, P<0.05; **, P<0.01; ***, P<0.001. APOA1, apolipoprotein A-I; APC, antigen-presenting cell; CCR, C-C chemokine receptor; HLA, human leukocyte antigen; MHC, major histocompatibility complex; IFN, interferon; NK, natural killer.

These changes were shown to promote proliferation and metastasis of GC cells (22).

Recent findings underscore the crucial role of *APOA1* in lipid metabolism and its significant impact on GC progression. Exploiting drugs to boost *APOA1* synthesis emerges as a strategic approach to positively adjust lipid profiles, potentially modulating the tumor microenvironment to slow or halt cancer progression. This approach exemplifies the synergy between pharmacological advances and dietary interventions, aiming to enhance the effectiveness of GC treatments. This strategy not only addresses individual patient needs but also paves the way for more personalized and effective therapies (23,24).

Prognostic biomarkers in GC have been extensively studied, and previous study has identified genes such as *c-MET*, *VEGF*, *PIK3CA*, *PTEN*, and *HER2* as potential GC markers (25). However, the diagnostic and prognostic role of lipid metabolism-related genes in GC has not been investigated. In our study, we selected significantly differentially expressed genes and prognostic-related genes in GC and identified one significantly differentially expressed prognostic-related lipid metabolism gene, *APOA1*, through the intersection of these gene sets. We found that both the *APOA1* gene and protein were downregulated in GC. Kaplan-Meier analysis revealed that patients with high *APOA1* expression had worse OS compared to those
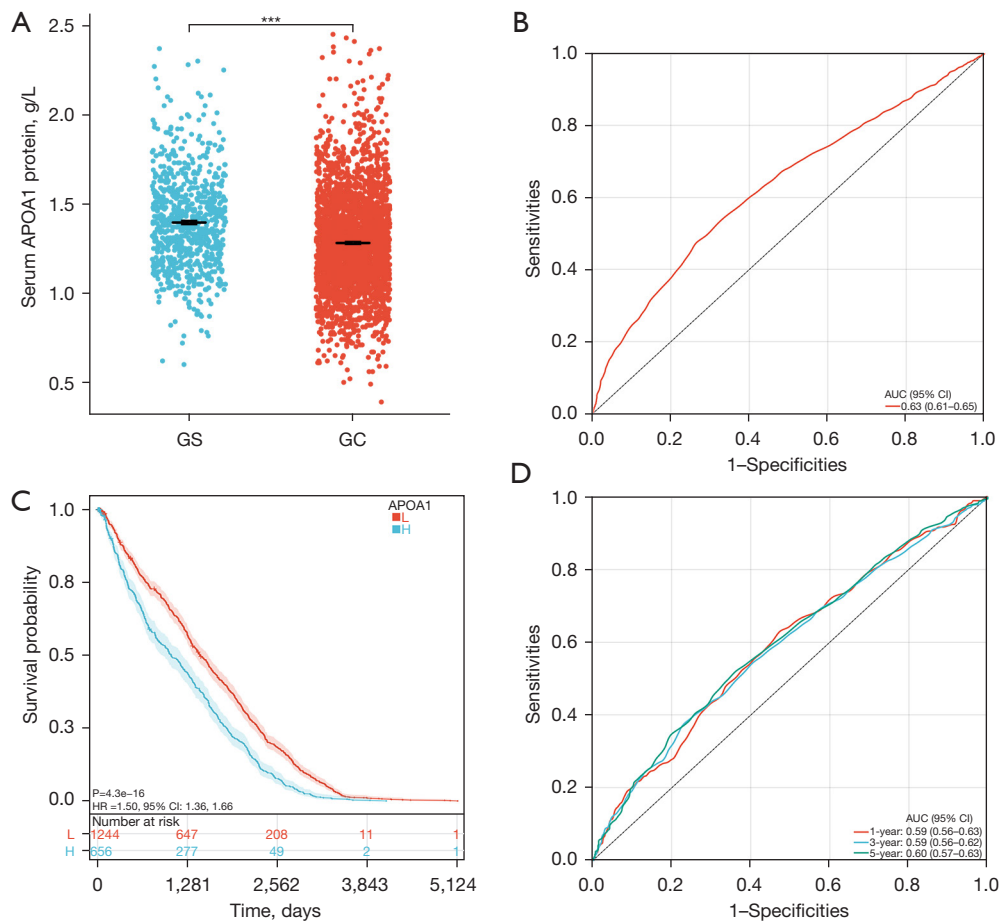
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Figure 6 Diagnostic and prognostic significance of serum APOA1 protein in GC patients. (A) Serum APOA1 protein expression in patients with GS and GC. (B) ROC curve validating the predictive power of serum APOA1 expression for GC. (C) Kaplan-Meier curve confirming the impact of *APOA1* protein expression on patient OS. (D) ROC curve assessing the diagnostic ability of serum APOA1 expression for 1-, 3-, and 5-year OS in GC patients. ***, $P<0.001$. APOA1, apolipoprotein A-I; GS, gastritis stomach; GC, gastric cancer; AUC, area under the curve; CI, confidence interval; HR, hazard ratio; ROC, receiver operating characteristic; OS, overall survival.

with low expression. Furthermore, we assessed the 1-year predictive ability based on the ROC curve, and the AUC value calculated from TCGA data for 1-year survival was 0.64. These results suggest that *APOA1* can serve as a prognostic lipid metabolism-related gene biomarker for predicting the occurrence and prognosis of GC.

APOA1 is a commonly used lipid indicator and possesses various biological activities, including anti-inflammatory, antioxidant, and anticoagulant effects, playing important roles in numerous BP (26). Previous studies (27,28) found that mice with GC exhibited lower plasma levels of *APOA1*, suggesting that the growth of malignant tumors in the stomach can lead to a decrease in plasma APOA1 protein levels in mice. The deficiency or reduction of *APOA1* can

result in the accumulation of cholesterol in tissues, and cancer cells often show increased cholesterol synthesis, possibly due to the high cellular demand for cholesterol to support rapid proliferation and cell membrane synthesis, thereby promoting tumor growth. Gumilas *et al.* (29) highlighted the potential mechanistic role of *APOA1* in liver cirrhosis and hepatocellular carcinoma (HCC), stating that the primary biological function of *APOA1* is to facilitate reverse cholesterol transport, transporting excess cholesterol from peripheral tissues to the liver for metabolism. Additionally, *APOA1* possesses various biological properties such as anti-inflammatory, antioxidant, and immune-modulatory effects. Therefore, the authors proposed that a decrease in *APOA1* levels could lead
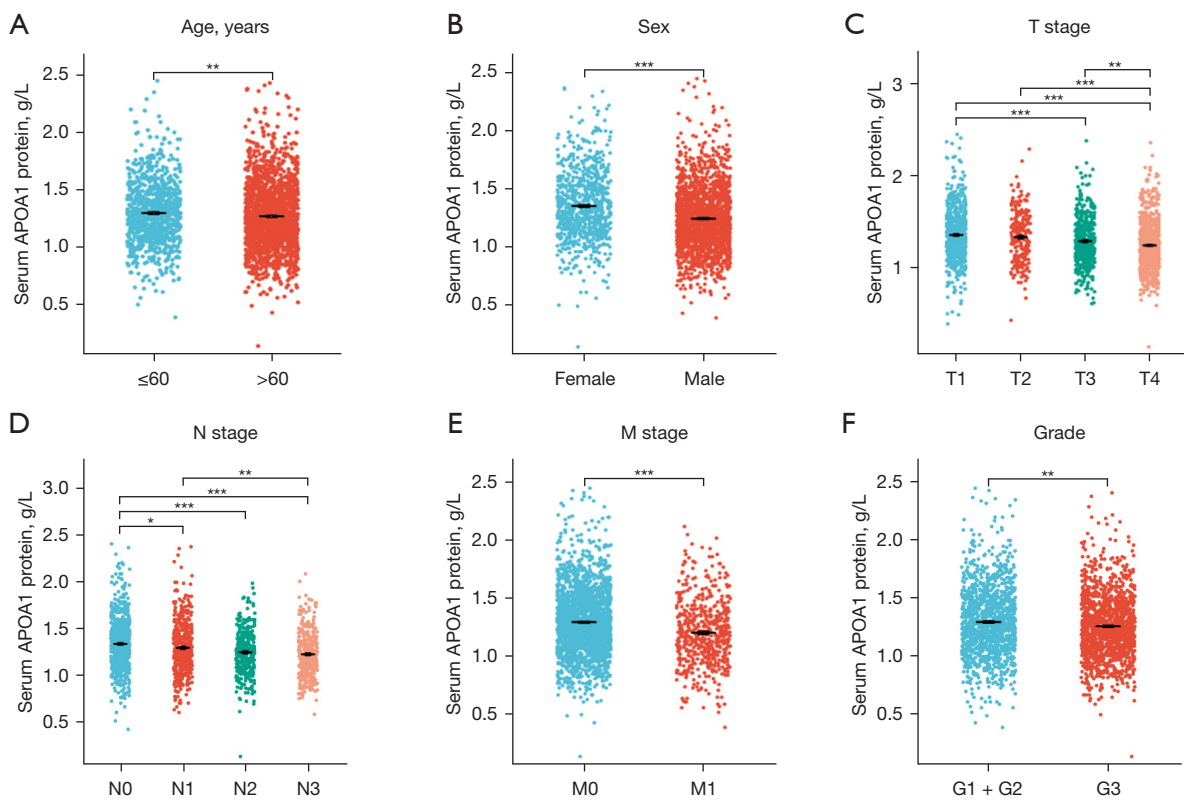
Figure 7 Relationship between serum APOA1 protein expression and clinical features of gastric cancer patients. (A) Age. (B) Gender. (C) T stage. (D) N stage. (E) M stage. (F) Grade classification. *, P<0.05; **, P<0.01; ***, P<0.001. APOA1, apolipoprotein A-I.
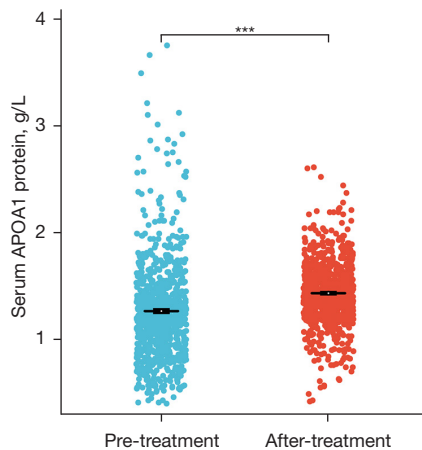

Figure 8 Difference in serum APOA1 protein levels between pre- and post-treatment in GC patients. ***, P<0.001. APOA1, apolipoprotein A-I; GC, gastric cancer.

to chronic inflammation and metabolic abnormalities, thereby promoting tumorigenesis and tumor development. Furthermore, *APOA1* can influence tumor development

through the inhibition of inflammatory responses and regulation of cellular signaling pathways. This may involve multiple mechanisms, including the modulation of cell proliferation, apoptosis, angiogenesis, and the tumor microenvironment. *APOA1* has also been shown to be associated with tumor prognosis in other cancers such as lung cancer, colorectal cancer, prostate cancer, and kidney cancer (30-33).

Immune response plays a significant role in the development and prognosis of GC. Previous study has indicated that the infiltration of CD8+ T cells, B cells, macrophages, and dendritic cells is closely associated with the prognosis of GC patients. The infiltration of CD8+ T cells and macrophages is positively correlated with patient survival, while the infiltration of B cells and dendritic cells is negatively correlated with patient survival (34). Previous research has also demonstrated that *APOA1* can inhibit monocyte recruitment and macrophage chemotaxis in renal cancer (35). Elevated levels of *APOA1* may inhibit tumor growth and metastasis by suppressing inflammation and promoting immune function. Peng *et al.* (36) investigated

the role of *APOA1* in pancreatic cancer in mice. The study found that *APOA1* improves the tumor microenvironment by inhibiting inflammation and reducing oxidative stress, thereby suppressing tumor growth and metastasis. Additionally, other mechanisms of *APOA1* have been mentioned in the literature. For example, *APOA1* can enhance the phagocytic activity of macrophages, increasing the efficiency of tumor cell clearance. *APOA1* can also inhibit tumor cell proliferation and induce tumor cell apoptosis, thereby suppressing tumor growth and metastasis. In order to explore whether *APOA1* can affect immune infiltration in GC, we evaluated the immune infiltration in GC patients with high and low *APOA1* expression. The results showed that patients with high *APOA1* expression had higher expression of genes related to type II IFN response, while genes related to APC co-inhibition, APO co-stimulation, cytolytic activity, MHC class I, and T cell co-inhibition had lower expression. Type II IFN response is an immune response primarily regulated by IFN-γ. IFN-γ is a cytokine secreted by various immune cells such as T cells, natural killer cells, and macrophages, and it regulates multiple aspects of immune cell proliferation, differentiation, secretion, and activity. On the other hand, overexpression of genes related to APC co-inhibition and T cell co-inhibition can lead to T cell functional suppression. Cytolytic activity is involved in recognizing and killing infected cells and tumor cells. These results suggest that *APOA1* may affect the prognosis of GC patients through the infiltration of negative immune cells.

Not only in cancer tissues but also in the serum of patients, a decrease in APOA1 protein levels has been observed, such as in patients with renal cell carcinoma (35). A large meta-analysis also demonstrated a specific negative correlation between serum APOA1 levels and survival rates in breast cancer, lung cancer, colorectal cancer, ovarian cancer, nasopharyngeal carcinoma, and renal cell carcinoma (37). In this study, we analyzed the differential expression of serum APOA1 between GC patients and healthy individuals, as well as its impact on the prognosis of GC patients. We observed that serum APOA1 protein levels were significantly lower in GC patients compared to the chronic gastritis group (P<0.001). Kaplan-Meier analysis showed that patients with high serum APOA1 expression had a worse prognosis among the 2,741 GC patients (HR =1.50, P<0.001). ROC analysis of serum APOA1 protein levels for predicting 1-, 3-, and 5-year survival rates also demonstrated good diagnostic ability. Serum APOA1 expression was closely associated with clinical characteristics

of the patients, such as age over 60, female gender, T4 stage, N3 stage, M1 stage, and G3 grade group, showing lower expression levels. We further investigated the impact of GC treatment on serum APOA1 expression and found that post-treatment serum APOA1 expression significantly increased compared to its expression before treatment, providing further evidence for the close association between serum APOA1 expression and GC occurrence. Based on our findings, we speculate that *APOA1* may function as a potential tumor suppressor gene in GC. Given that APOA1 protein levels can be readily measured in plasma, changes in its expression could serve as a relevant biomarker for the diagnosis and prognosis of GC patients. A study has reported that lipid metabolism plays a significant role in GC, primarily associated with high-fat diet consumption and excessive lipid intake. Consuming a high-fat diet can lead to alterations in the gut microbiota, thereby affecting lipid metabolism. Additionally, cholesterol accumulation promotes tumor proliferation and invasion, while increased FA metabolism leads to enhanced FA synthesis and reduced FA oxidation, thereby impacting tumor growth and metastatic potential (38).

Our study has certain limitations that should be acknowledged. Firstly, we were unable to validate the clinical and prognostic significance of *APOA1* mRNA in an independent dataset due to technical constraints. Secondly, our study primarily relied on bioinformatics analysis and clinical data, without conducting biomedical experiments to investigate the underlying mechanisms of *APOA1* in GC progression and metastasis. Therefore, future investigations incorporating functional experiments to elucidate the role of APOA1 in GC would be highly valuable.

Conclusions

Our comprehensive study investigated the expression and prognostic significance of *APOA1* mRNA and protein levels in GC. The protein level of *APOA1* demonstrated excellent diagnostic capability. This finding contributes to improving the accuracy of prognosis prediction in GC patients and provides new insights for personalized treatment approaches.

Acknowledgments

Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-1966/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-1966/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of The Second Hospital of Dalian Medical University (No. 2023-045). Informed consent was taken from all the patients.

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