Translational Oncology 55 (2025) 102344

Contents lists available at ScienceDirect

Translational Oncology

journal homepage: www.elsevier.com/locate/tranon

Original Research

APOA1 promotes tumor proliferation and migration and may be a potential pan-cancer biomarker and immunotherapy target

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ARTICLE INFO

Keywords:
APOA1
Pan-cancer
GC
Immune
Prognostic marker

ABSTRACT

Introduction: Aberrant expression of APOA1 has been reported in various cancers. However, a comprehensive investigation into its role in cancer is currently lacking.

Methods: Online websites and databases such as TIMER2.0, GEPIA2, UALCAN and GSCA were used to investigate the relationship between APOA1 expression and prognostic value, immune infiltration, gene mutations, and drug sensitivity. In addition, in vitro CCK-8 and transwell migration and invasion assays were performed to determine the biological functions of APOA1 in gastric cancer (GC) cells.

Results: The pan-cancer analysis showed that APOA1 is differentially expressed in different cancer types and significantly correlated with tumor stages. A survival analysis revealed that APOA1 predicted a poor prognosis in ACC, KIRC, STAD, and a good prognosis in BRCA, OV, and UCEC. We also found that the most common genetic alteration type of APOA1 was deep deletion, and the DNA methylation level of APOA1 decreased in various cancers. Furthermore, APOA1 expression negatively correlated with immune cells infiltration in cancers, including CD4+ T, CD8+ T, and myeloid dendritic cells. For STAD, GO/KEGG enrichment analysis revealed the possible involvement of APOA1 in cholesterol metabolism and PPAR signaling pathway. Finally, we further performed in vitro experiments to verify that overexpression of APOA1 could promote the proliferation, migration and invasion of GC cells.

Conclusion: The results of this study indicate that APOA1 is a potential tumor prognostic biomarker and immunotherapy target. In addition, APOA1 plays an essential role in the proliferation, migration, and invasion of GC cells by vitro experiments.

Introduction

According to the Global Cancer Research Center (GLOBOCAN), in 2022, there were nearly 20 million new cases of cancer globally, including nonmelanoma skin cancers (NMSC), with 9.7 million cancer-related deaths, including those from NMSC [1]. This highlights a significant public health challenge. Despite continuous advancements in therapeutic approaches, patient prognosis and survival rates remain unsatisfactory [2]. Therefore, identifying new tumor markers for cancer diagnosis and treatment is of critical importance.

Several tumor biomarkers have been identified, each with its unique advantages and limitations. Circulating tumor DNA (ctDNA) has emerged as a promising biomarker for predicting recurrence and survival in non-small cell lung cancer (NSCLC). Studies have shown that ctDNA levels can detect minimal residual disease (MRD) nearly 5 months before clinical or radiological progression, thereby guiding treatment adjustments. However, the sensitivity and specificity of ctDNA detection methods vary, and standardization is still required [3]. Circulating tumor cells (CTCs) offer unique opportunities for in-depth analysis, including RNA/DNA sequencing and protein expression profiling. Despite their potential, the low concentration of CTCs in blood makes them difficult to capture and analyze. Recent advancements, such as the use of graphene oxide microfluidic chips, have shown promise in improving CTC detection and progression-free survival (PFS) prediction. Nevertheless, the high cost and technical complexity of CTC isolation and analysis remain significant barriers to widespread clinical

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https://doi.org/10.1016/j.tranon.2025.102344

Received 4 November 2024; Received in revised form 26 January 2025; Accepted 2 March 2025

application [4]. The combination of multiple biomarkers is also gaining attention as a means to enhance diagnostic and prognostic accuracy. For instance, a study by the Chinese Academy of Medical Sciences demonstrated that the combination of dynamic tumor mutation burden (TMB) and ctDNA significantly improved survival prediction in locally advanced NSCLC patients treated with concurrent chemoradiotherapy (cCRT) and immune checkpoint inhibitors (ICIs). However, integrating multiple biomarkers requires sophisticated analytical methods and a deeper understanding of their interactions, which are still under investigation [5].

In tumor biology, dysregulation of lipid metabolism has been observed to promote the growth and metastasis of malignant cells [6–8], suggesting that targeting lipid metabolism could be a potential anti-tumor strategy. APOA1, located at 11q23.3, encodes apolipoprotein A1 (ApoA1) [9], which plays a key role in regulating cholesterol transport and maintaining cellular cholesterol homeostasis. APOA1 has been identified as a potential biomarker for hepatocellular carcinoma (HCC) [10], bladder cancer [11], and kidney renal clear cell carcinoma (KIRC) [12]. For HCC, studies have mainly relied on retrospective analysis of clinical data to investigate the correlation between ApoA1 expression levels and patient prognosis, as well as the potential of ApoA1 as a prognostic and therapeutic biomarker in combination with hypoxia and cellular landscape markers. For bladder cancer, research has focused on detecting APOA1 protein levels in urine samples to study its expression patterns and correlation with disease progression. For KIRC, a study involving 91 KIRC patients and 93 healthy controls was conducted for clinical validation, not only measuring APOA1 mRNA levels in KIRC tissues but also the preoperative serum concentration of ApoA1 protein in KIRC patients, and analyzing its relationship with patient prognosis, ultimately summarizing its association with overall survival (OS) and disease-free survival (DFS). However, these studies were based on clinical sample analysis and did not conduct further in-depth research through other in vivo or in vitro experiments. Moreover, previous studies have primarily focused on specific cancer types, and the broader implications of APOA1 in pan-cancer tumorigenesis remain unclear. For example, a study by Ping Lu et al. investigated the prognostic value of lipid metabolism-related genes and serum ApoA1 in NSCLC patients. Using the TIMER and GEPIA tools, they analyzed the differential expression of lipid metabolism-related genes in cancer tissues and found that CD36, LDLR, ACSS2, FASN, and SCD were downregulated in lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC), while ACLY was significantly upregulated. This study provided valuable insights into the role of lipid metabolism in NSCLC but did not explore its broader significance across multiple cancer types [14]. Similarly, Wang et al. demonstrated the effectiveness of an immunometabolic therapeutic strategy targeting ApoA1 using oncolytic viruses in an intracranial glioblastoma multiforme (GBM) animal model. This strategy can restore the phagocytic function and anti-tumor immunity of tumor-associated macrophages (TAMs), thereby improving GBM treatment outcomes. Metabolomic analysis revealed that its mechanism of action involves restoring TAM function through the regulation of cholesterol efflux. However, this study did not address the broader role of APOA1 in other cancer types or the potential mechanisms by which ApoA1 promotes tumorigenesis across different cancers [13].

This study was designed to fill the existing knowledge gaps concerning the role of APOA1 and lipid metabolism dysregulation in various cancer types. Through a comprehensive analysis employing multiple public databases, we examined molecular and clinical characteristics, such as gene and protein expression, clinical associations, survival status, DNA methylation, genetic alterations, immune infiltration, related pathways, and drug sensitivity. Notably, APOA1 has been identified as a diagnostic and prognostic biomarker in GC, although the underlying mechanisms have remained unclear [15]. To elucidate the carcinogenic effects of APOA1, we conducted in vitro experiments by introducing the APOA1 gene into various GC cell lines and confirmed its role in promoting GC cell proliferation, migration, and invasion through CCK-8, migration, and invasion assays. Our analysis indicated that APOA1 expression is differentially associated with tumor stages and prognosis across different cancer types, with specific genetic alterations observed. Furthermore, we found that APOA1 expression negatively correlates with immune cell infiltration, including CD4+ T cells, CD8+ T cells, and myeloid dendritic cells. Enrichment analysis for stomach adenocarcinoma (STAD) suggested the involvement of APOA1 in cholesterol metabolism and the PPAR signaling pathway. In conclusion, APOA1 not only serves as a marker of immune infiltration and poor prognosis but also represents a promising therapeutic target for cancer treatment, emphasizing the critical need for a more comprehensive understanding of its role in pan-cancer tumorigenesis.

Materials and methods

Pan-cancer expression and clinical correlation analysis of APOA1

The analysis of APOA1 expression differences in pan-cancer was conducted using TIMER2.0 [16–18] (http://timer.cistrome.org/), GEPIA2 [19] (http://gepia2.cancer-pku.cn/), UALCAN [20,21] (http://ualcan.path.uab.edu/), the Human Protein Atlas (HPA) database [22] (https://www.proteinatlas.org/). and starBase or ENCORI [23,24] (https://starbase.sysu.edu.cn/) database. Furthermore, the CPTAC dataset from UALCAN was employed for the purpose of analyzing APOA1 protein expression. To explore the potential correlation between APOA1 and tumor stage and grade, we utilized online resources, specifically the UALCAN, GSCA [25] (https://guolab.wchscu.cn/GSCA/), and GEPIA2 platforms.

Survival prognosis analysis

GEPIA2 was used to analyze the correlation between APOA1 expression and OS and DFS in 33 cancer types. The log-rank test was used to estimate the significance of APOA1. To examine the relationship between APOA1 expression and patient prognosis, univariate Cox regression analysis of APOA1 for OS, disease-specific survival (DSS), disease-free interval (DFI), and progression-free interval (PFI) was performed using the Sangerbox tool [26,27] (http://www.sangerbox.com/tool).

Genetic alteration analysis

The cBioPortal [28–30] (https://www.cbioportal.org/) was utilized to compile genetic mutation data pertaining to APOA1 based on TCGA Pan-Cancer Atlas data, including alteration frequency, mutation type, and copy number alteration (CNA). We further explored the epigenetic methylation of APOA1 between tumour and normal tissues by UALCAN and MethSurv database [31] (https://biit.cs.ut.ee/methsurv/).

Immune infiltration analysis

The TIMER 2.0 database employed TIMER, EPIC, TIDE and other algorithms to evaluate the correlation between immune cell infiltration and APOA1 expression in pan-cancer. The expression level of APOA1 was correlated with immune score, ESTIMATE score, and stromal score based on tumor samples from the TCGA database using the online website Sangerbox.

To further examine the relationship between cell types and APOA1 in the microenvironment of specific types of cancer cells, we conducted an analysis of APOA1 mRNA expression levels on immune cells in various cancer types using TISCH2 [32] (http://tisch.comp-genomics.org/).

The TISIDB website [33] (http://cis.hku.hk/TISIDB/) was utilized to examine the correlation between APOA1 expression and a range of immunomodulatory factors, including immuno-inhibitors, immuno-stimulators, chemokines and chemokine receptors.

TMB and MSI analysis

We investigated the relationships between APOA1 gene expression and several genomic instability parameters, including TMB and microsatellite instability (MSI) in the context of multiple cancer types using Sangerbox.

Enrichment analysis of APOA1-related genes

To identify APOA1 co-expression genes in STAD, the LinkedOmics portal [34] (http://linkedomics.org/login.php) was employed for this analysis. Subsequently, we conducted a further investigation of APOA1 co-expression genes using Gene Set Enrichment Analysis (GSEA) to explore their associations with biological processes, cellular components, molecular functions, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways.

Drug sensitivity analysis

A drug sensitivity analysis was conducted using the GSCA database, which integrated the gene expression profile and drug sensitivity information from the Genomics of Drug Sensitivity in Cancer (GDSC) dataset for investigative purposes. The correlations between APOA1 expression and drug IC50 were assessed by Pearson correlation analysis.

Cell culture

The HGC27 and AGS cell lines (KeyGEN BioTECH, Jiangsu, China) were cultured in RMi-1640 (Gibco, Grand Island, NY, USA) or DMEM/F12 (Gibco, Grand Island, NY, USA) medium, both of which were supplemented with 10 % fetal bovine serum (FBS; Gibco, Grand Island, NY, USA) and 1 % penicillin-streptomycin (P/S; Gibco, Grand Island, NY, USA). All cells were incubated in a constant-temperature incubator (Thermo Scientific, Waltham, MA, USA) at 37 °C with 5 % CO_2.

Lentiviral transduction of GC cells

Lentivirus transduction was performed according to the manufacturer's instructions. Cells were cultured in six-well plates. After 24 h, Cells were transfected with a green fluorescent protein (GFP)-labeled overexpressed APOA1 lentivirus (KeyGEN BioTECH, Jiangsu, China). At 8 h after transfection, a complete fresh medium was replaced and observed under a fluorescence microscope. The cells were incubated for a period of 72 h prior to being utilized in vitro experiments.

Western blot assay

The cells were lysed using RIPA protein extraction reagent (Beyotime, Beijing, China), which was supplemented with a protease inhibitor cocktail (Beyotime, Beijing, China) and phosphatase inhibitor cocktail (Beyotime, Beijing, China). The protein concentrations were measured using the BCA protein assay kit (Vazyme, Jiangsu, China). Proteins were separated by 10 % sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred to PVDF membranes. After blocking with 5 % non-fat milk for 2 h, the membranes were incubated with antibodies specific for APOA1 and β-actin overnight at 4 °C. Horseradish peroxidase conjugated goat anti-mouse was used as the secondary antibody and incubated for 1 h at room temperature. Protein bands were detected using an enhanced chemiluminescence detection system (Biosharp, Anhui, China).

Cell counting kit-8 (CCK-8) assay

CCK-8 (Beyotime, Beijing, China) was used for cell proliferation experiments. Briefly, GC cells or GC cells with APOA1 overexpression were plated into 96-well plates at 2×10^3/well. The experiments were performed according to the kit instructions, added 10 μl CCK8 solution to each well at 24 h, 48 h, 72 h, and 96 h after cell treatment, mixed and incubated in the cell incubator for 40 min. The absorbance was detected at 450 nm. Each group of experiments was repeated three times.

Cell transwell migration and invasion assays

The transwell 24-well chambers with 8.0 μm pore size (Corning, NY, USA) were used for cell migration and invasion assays. The cells dispersed in 200 μl serum-free medium (5×10^4/ well) were seeded into the upper chamber, and 500 μl medium containing 10 % FBS was added to the lower section. The upper compartment was coated with Matrix-Gel™ Basement Membrane Matrix (Beyotime, Beijing, China) for invasion assay or without for migration assay. After incubation for 48 h at 37 °C, the non-migrating cells in the upper chamber were removed with a cotton swab, and the migrated cells on the bottom surface of the filter were fixed in 4 % paraformaldehyde for 5 min at room temperature, then stained with 0.1 % crystal violet (Beyotime, Beijing, China) at room temperature for 15 min. Five visual fields were randomly selected and observed under a light microscope (200×), and images were captured for enumeration. Each experiment was conducted independently in triplicate.

Statistical analysis

The statistical analysis and graphing were conducted using Prism 10 (GraphPad). An unpaired *t*-test was employed for intergroup comparison in the in vivo experiments. All in vivo experiments were repeated at least three times, and the data are presented as mean ± standard error of the mean. The correlation coefficient between variables was determined by employing either Pearson or Spearman coefficients. A value of $p < 0.05$ was considered to indicate significance.

Results

APOA1 expression analysis in pan-cancer

Firstly, to detect the mRNA and protein expression profiles of APOA1 in human normal and cancer tissues, the APOA1 expression data for normal tissues were evaluated using the HPA database (Fig. S1A). APOA1 mRNA expression in consensus (dataset created by combining the HPA and GTEx), HPA, GTEx (Genotype-tissue expression), FANTOM5 (Function annotation of the mammalian genome 5) dataset suggested that the distribution of APOA1 is highly tissue specific, generally enriched in the liver (Fig. S1B-E). In addition, we found that APOA1 is highly expressed in cell lines derived from liver, gastric, colorectal and ovarian cancer (OV) by HPA database (Fig. S1 G). An overview of the APOA1 mRNA expression data revealed a low tissue specificity for APOA1 distribution. Secondly, we performed a pan-cancer expression analysis of APOA1 based on the TCGA database. In 33 cancers, APOA1 expression in tumor tissue was significantly lower than that in normal controls in the majority of tumors, including cholangiocarcinoma (CHOL), esophageal carcinoma (ESCA), kidney chromophobe (KICH), kidney renal papillary cell carcinoma (KIRP), liver hepatocellular carcinoma (LIHC), LUAD, LUSC, prostate adenocarcinoma (PRAD), STAD, thyroid carcinoma (THCA), and uterine corpus endometrial carcinoma (UCEC) (Fig. 1A). Since the TCGA database lacked the normal tissues of certain tumors for comparison, we extracted expression data from GTEx and discovered that APOA1 was downregulated in CHOL, LUAD, LUSC, STAD, testicular germ cell tumors (TGCT), and THCA compared to normal tissues, whereas APOA1 was upregulated in ovarian serous cystadenocarcinoma (OV) (Fig. 1B). In order to further acquire more reliable results and make the performance more representative, we proceeded to analyze the expression levels of APOA1 in pan-cancer by additional databases- ENCORI (Fig. S1 G). In terms of protein expression levels, the CPTAC database revealed that

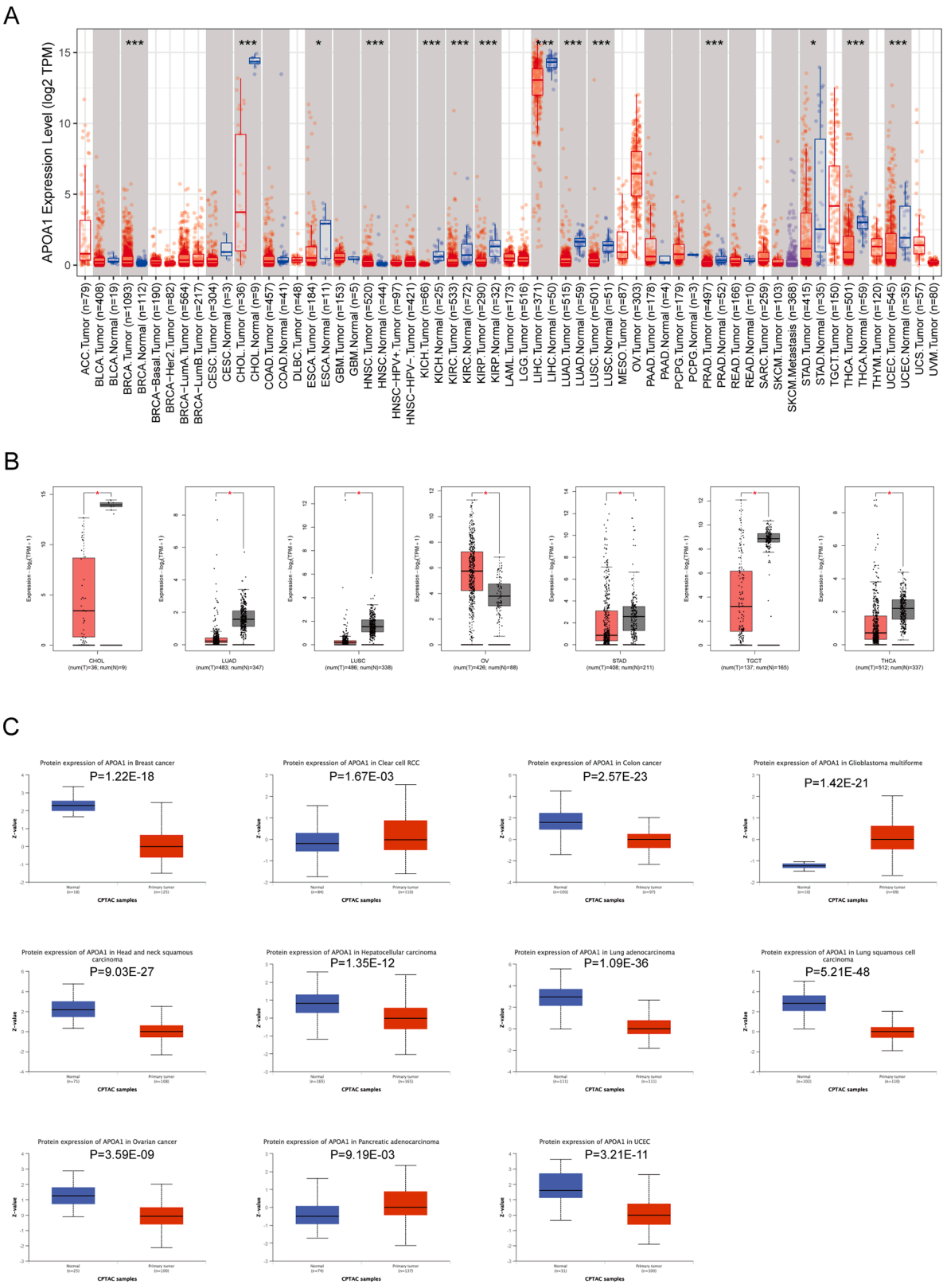

Fig. 1. The expression level of APOA1 in different types of cancer.
Differences in APOA1 expression between 33 normal and tumor tissues in TCGA from the TIMER2.0 database. B. Expression of APOA1 gene in different tumors determined by GEPIA2. C. Total protein expression of APOA1 in different cancers from the CPTAC dataset.

total APOA1 protein expression was higher in clear cell renal cell carcinoma, GBM and pancreatic adenocarcinoma (PAAD) tissues than in normal tissues. Conversely, the expression of breast cancer (BRCA) colon cancer, head and neck squamous carcinoma (HNSC), LIHC, LUAD, LUSC, and UCEC was lower than that of normal tissues (Fig. 1C).

APOA1 expression and clinicopathology in pan-cancer

To better understand the significance of APOA1 in tumorigenesis and progression, we analyzed the relationship between the APOA1 mRNA expression levels and the pathological stages in multiple cancers. To study APOA1 expression levels across various tumor stages, we used the UALCAN online tool to compare APOA1 expression in tumor samples from patients at various tumor stages. We observed that APOA1 was significantly reduced in the early tumor stages of 7 cancers (Fig. 2A), including CHOL, KICH, KIRP, LIHC, LUAD, LUSC, and PAAD, suggesting that APOA1 may have vital guiding magnitude for the early diagnosis of sufferers with these kinds of tumors. We also used the GEPIA2 database to analyze the correlation between APOA1 expression and clinicopathological stages of various cancers and found that there was a statistical difference in KICH, LIHC, rectum adenocarcinoma (READ) and THCA (Fig. 2B), while most other cancers showed no statistical difference (Fig. S2). Furthermore, the relationship between APOA1 mRNA expression levels and the various tumor subtypes was examined. In breast invasive carcinoma (BRCA), APOA1 mRNA expression was lowest in Her2. The primitive type has the highest APOA1 mRNA expression in LUSC. The chromosome instability (CIN) type had the highest STAD's APOA1 mRNA expression (Fig. 2C).

Prognostic significances of APOA1 in different cancers

To determine the correlation between APOA1 expression and prognosis in human cancer patients, the GEPIA2 database was used. Analysis of the GEPIA2 database showed that higher APOA1 expression was associated with worse OS outcomes in patients with adrenocortical carcinoma (ACC) and KIRC. However, high APOA1 gene expression was associated with better OS outcomes in patients with BRCA and OV (Fig. 3A). Furthermore, high APOA1 expression correlated with worse DFS in patients with ACC and STAD (Fig. 3B).

Furthermore, to assess the prognostic value of APOA1 in tumors, we conducted univariate Cox analyses for OS, DSS, DFI, and PFI. The results of Cox regression analysis showed that APOA1 expression levels were associated with OS in 9 tumors, with DSS in 5 tumors, with DFI in 6 tumors, and with PFI in 8 tumors (Fig. 4A–D). High APOA1 expression significantly predicted poor prognosis in patients with KICH, pan-kidney cohort (KIPAN), ACC, STAD, glioma (GBMLGG) and stomach and esophageal carcinoma (STES). Conversely, high APOA1 expression was associated with a protective effect on OS in patients with thymoma (THYM), LIHC and BRCA (Fig. 4A). Similarly, the DSS analysis revealed that APOA1 had a significant impact on the prognosis of KICH, KIPAN, STAD, ACC, and STES patients, while conferring a protective effect in BRCA patients (Fig. 4B). DFI analysis confirmed that high APOA1 expression was a risk factor for TGCT, STAD, COAD, KIPAN, COAD/READ (COADREAD) and PRAD (Fig. 4C). In addition, PFI analysis indicated that APOA1 played a risk role in patients with ACC, KIPAN, STAD, GBMLGG, TGCT, KICH, and KIRP, and a protective role in patients with uveal melanoma (UVM) (Fig. 4D). These results showed that APOA1 expression could predict patient survival in several cancer types. In conclusion, these data demonstrate that APOA1 is a potential prognostic biomarker in multiple cancer types.

APOA1 genetic alteration and DNA methylation in pan-cancer

The results from the cBioPortal database showed that the APOA1 gene is altered in several cancers, and "deep deletion" had the highest percentage. The top 3 alteration frequencies were 5.00 % in UVM, 3.86 % in STAD, and 3.51 % in uterine carcinosarcoma, respectively. Then, we found that in UVM and TGCT, the APOA1 gene alteration were all "deep deletion". In addition, no genetic alteration was found in some types of tumors, including ACC, CHOL, PAAD, pheochromocytoma and paraganglioma (PCPG), THYM and THCA (Fig. 5A). We analyzed the correlation between the mRNA obtained by APOA1 gene transcription in pan-cancer and the variation of CNA. CNAs in APOA1 were most commonly observed as shallow deletion, diploid, and gain (Fig. 5B). Furthermore, analysis of the cBioPortal database indicated that missense mutations were the predominant type of APOA1 gene mutation in cancers (Fig. 5C).

DNA methylation is one of the most important epigenetic mechanisms to regulate gene expression, and aberrant DNA methylation patterns are strongly associated with human diseases including cancer [35]. Using the UALCAN web portal, the methylation status of the APOA1 promoter region may be examined. The level of methylation in the APOA1 promoter was markedly elevated in CHOL and KIRP specimens relative to that observed in normal tissues. The APOA1 promoter methylation level was markedly lower in bladder urothelial carcinoma (BLCA), BRCA, COAD, HNSC, KIRC, LIHC, LUAD, LUSC, PAAD, READ, TGCT, and THCA than in normal tissues (Fig. 5D). As APOA1 levels are mainly expressed in the gastrointestinal tract and liver, we further analysed the effect of APOA1 methylation levels on survival/prognosis of patients with digestive system tumours using the MethSurv database. High methylation levels of APOA1 resulted in poor survival/prognosis in cancer patients with ESCA, and READ (Fig. S3). Further research is required to ascertain the precise impact of DNA methylation on APOA1 expression levels in the aforementioned malignancies. Nevertheless, a correlation may exist between methylation levels and APOA1 expression levels.

Interactions between APOA1 and immune infiltration in cancers

Tumor-infiltrating immune cells (TIICs) play a critical role in tumor microenvironment (TME) which is closely related to immune escape of tumor cells, thus influence tumor progress [36]. Therefore, we investigated the relationship between APOA1 expression status and TIICs. The results showed that in most TCGA cancers, APOA1 expression was negatively correlated with infiltration levels of CD4+ T cells, CD8+ T cells, myeloid dendritic cells, monocytes, and M1 macrophages, but APOA1 expression was positively correlated with M2 macrophages and myeloid-derived suppressor cells (MDSCs) (Fig. 6).

To further explore the association of cell types and APOA1 in the microenvironment of specific types of cancer cells for study, we analyzed the APOA1 mRNA expression levels on immune cells in various cancer types using TISCH2, 26 single-cell datasets showed relatively higher levels of APOA1 mRNA. APOA1 was highly expressed in malignant cells in CHOL, ESCA, hepatoblastoma (HB), LIHC, and OV. APOA1 was also highly expressed in the CD8+ T and mono/macro cells in CHOL, LIHC, and OV (Fig. 7A). APOA1 was highly expressed in epithelial cells in LIHC, OV and STAD. Next, we investigated the relationship between APOA1 and TME-associated cells in STAD. In GSE134520 dataset, APOA1 was mainly expressed in pit mucosal cells. In GSE167297 dataset, APOA1 was highly expressed in epithelial cell (Fig. 7B).

In addition, we examined the correlations between APOA1 and immune, stromal and ESTIMATE scores. First, the correlation between APOA1 expression status and immune score was examined. We found that APOA1 expression levels were significantly associated with 9 of the 33 cancer types. Among them, a negative correlation was found in ACC, cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), OV, STAD, TGCT, and THCA. A positive correlation was found in KIPAN, PRAD and THYM (Fig. S4). Stromal scores were inversely correlated with APOA1 expression in 7 cancers and positively correlated in 5 cancers, as shown in Figure(Fig. S5). Furthermore, the expression status of APOA1 in ACC, CESC, LUAD, OV, STAD, and THCA was

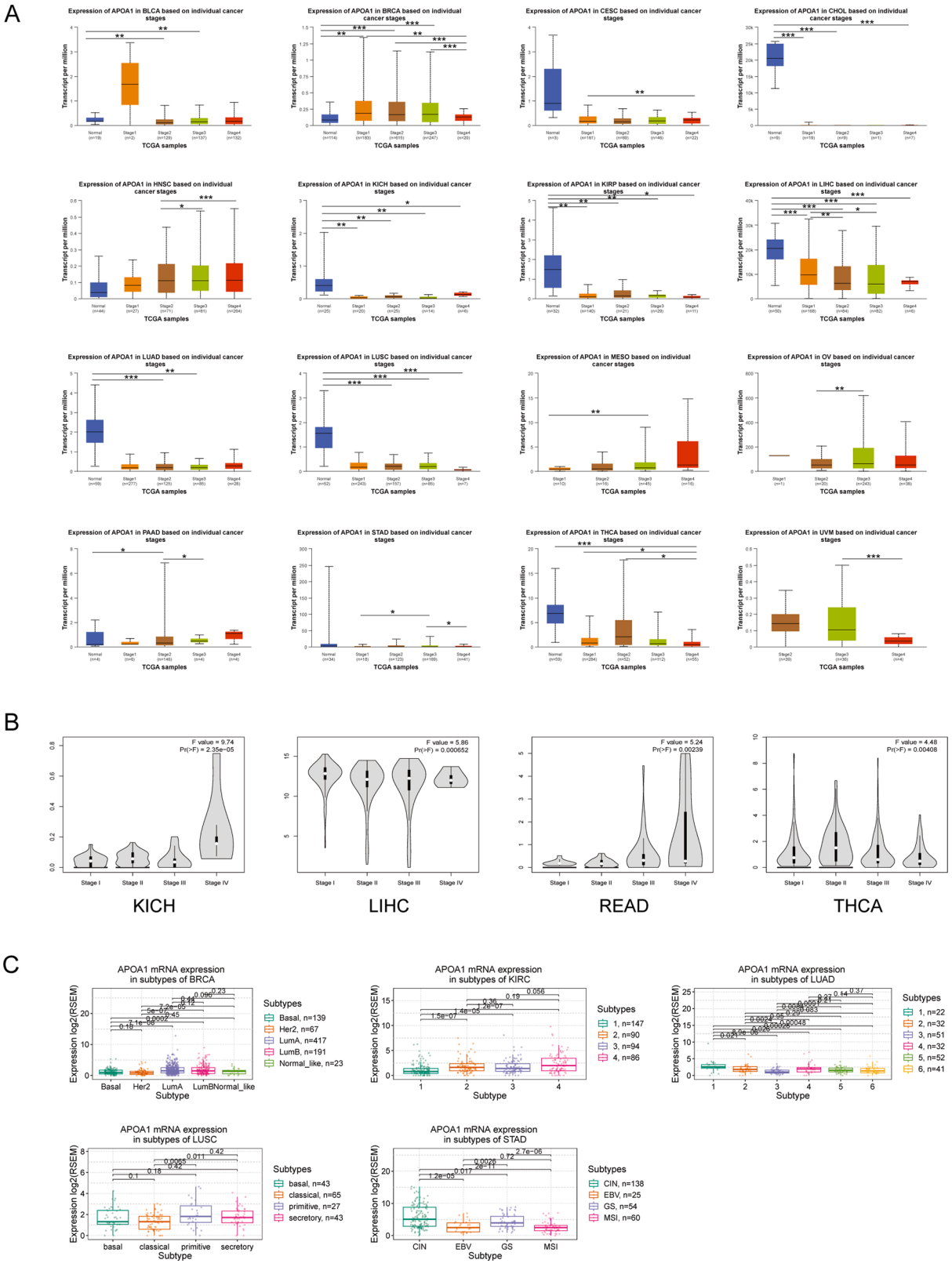

Fig. 2. The expression of APOA1 in different pathological stages of pan-cancer.
A. Correlations between the APOA1 expression and tumor stage. B. The APOA1 expression in different pathological stages of KICH, LIHC, READ and THCA using GEPIA2. C. Evaluate the correlation between the expression of APOA1 and subtypes of BRCA, KIRC, LUAD, LUSC, and STAD by using GSCA.

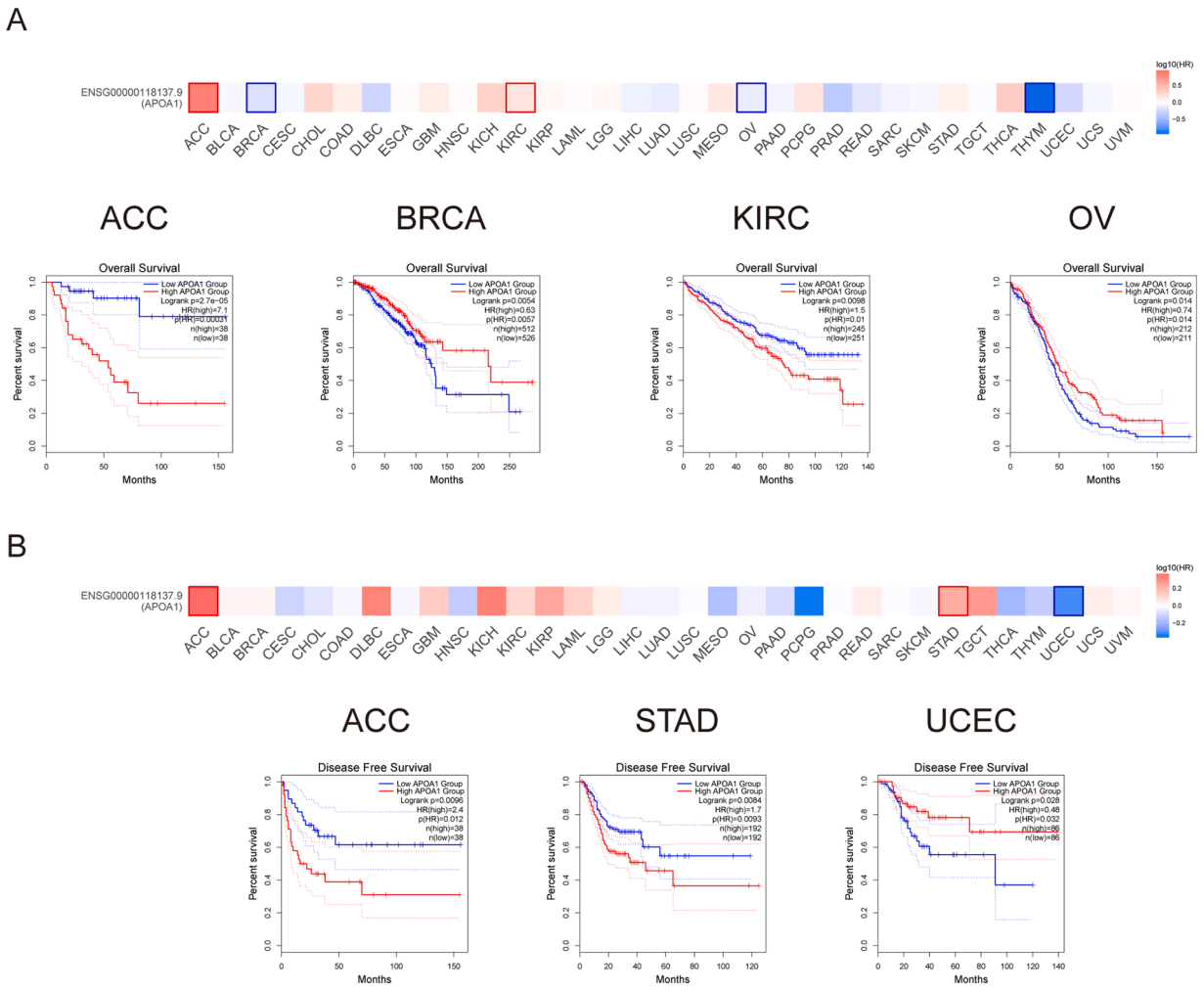

Fig. 3. Correlation between APOA1 gene expression and survival prognosis of cancers.
Correlation between APOA1 gene expression and survival prognosis of cancers. A. OS. B. DFS.

significantly negatively correlated with ESTIMATE score, while it was positively correlated in PIKAN and PRAD (Fig. S6).

Furthermore, we investigated the relationship between the expression of APOA1 and chemokine, chemokine receptor, immune stimulator and immune inhibitor in the TISIDB database (Fig. 8). In the context of STAD, a remarkable correlation was observed between APOA1 and chemokine, including CXCL14 ($r = 0.343$ and $p = 9.45e\text{-}13$), CXCL17 ($r = 0.288$ and $p = 2.71e\text{-}09$), and CCL25 ($r = 0.242$ and $p = 6.64e\text{-}07$) in Fig. 8A. Furthermore, there was a strong association between APOA1 and chemokine receptors, specifically CCR6 ($r = 0.192$ and $p = 8.31e\text{-}05$), CCR5 ($r = -0.154$ and $p = 0.00162$), and CXCR3 ($r = 0.153$ and $p = 0.00178$), as shown in Fig. 8B. APOA1 showed a strong correlation with immune stimulators such as TNFSF9 ($r = -0.344$ and $p = 8.31e\text{-}13$), HHLA2 ($r = 0.282$ and $p = 6.16e\text{-}09$), and TNFSF13B ($r = -0.226$ and $p = 3.35e\text{-}06$) (Fig. 8C). A significant association was found between APOA1 and immune inhibitors such as CD274 ($r = -0.348$ and $p = 4.13e\text{-}13$), IDO1 ($r = -0.223$ and $p = 4.62e\text{-}06$), and PDCD1LG2 ($r = -0.223$ and $p = 4.65e\text{-}06$) (Fig. 8D).

Correlation of APOA1 mRNA expression with TMB and MSI analysis

TMB and MSI are closely associated with anti-tumor immune responses and may serve as predictive markers of tumor immunotherapy efficacy [37]. Therefore, we examined the correlation between TMB, MSI and APOA1 expression. The results showed that APOA1 expression had a significant positive correlation with TMB in LUSC, while it had a negative correlation in acute myeloid leukemia (LAML), STES, STAD and THYM (Fig. S7A). MSI is a significant clinical marker in oncology, frequently linked to deficiencies in DNA mismatch repair (MMR) [38]. Our analysis revealed a notable positive correlation between APOA1 expression and MSI in specific cancers, including BRCA and TGCT. Conversely, an inverse correlation was observed in GBMLGG, STES, KIPAN, and STAD (Fig. S7B).

APOA1 serves as a biomarker for gastric cancer

The above studies identified that APOA1 was significantly overexpressed in STAD and that survival analysis demonstrated that APOA1 expression levels were strongly correlated with OS ($p = 0.01$), DSS ($p = 1.3e\text{-}3$), DFI ($p = 0.01$), and PFI ($p = 2.7e\text{-}3$) in STAD. For this reason, we next explored the possible biological functions and mechanisms of APOA1 in the context of STAD. Initially, we further evaluated the prognostic value of APOA1 in STAD using univariate and multivariate Cox regression models, both of which resulted in APOA1 being considered as an independent prognostic standard (Fig. 9A,B). In addition, we developed a nomogram based on APOA1 expression and pathological stage to facilitate more accurate prognostic assessment in clinical practice (Fig. 9C). Furthermore, the calibration curves were used to evaluate the accuracy of the current model in assessing the prognosis of STAD patients at 1, 3 and 5 years, and the results are shown in (Fig. 9D), indicating a favorable assessment performance.

We further explore the biological processes and molecular

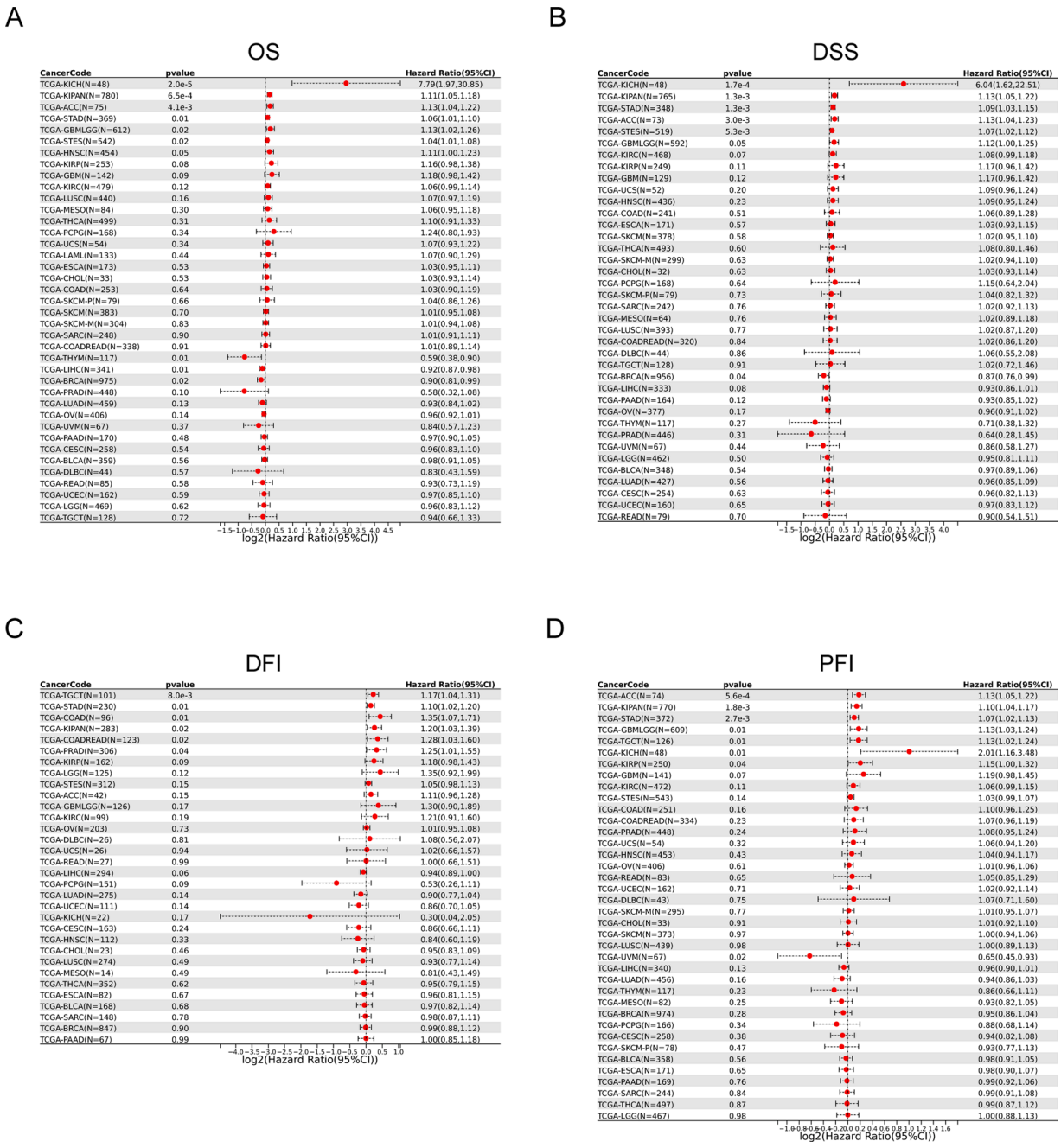

Fig. 4. The forest maps of APOA1 expression level with survival in different types of cancers.
Association between APOA1 expression level and patients' OS (A), DSS (B), DFI (C), and PFI (D).

mechanisms of APOA1 in STAD via LinkedOmics database. Fig. 10A shows the genes positively and negatively associated with APOA1. The top 50 genes are shown in Fig. 10B and C. Furthermore, GSEA using the LinkedOmics online tool indicated that APOA1 co-expression genes were mainly involved in immunity-related Gene Ontology (GO) terms, including "protein-containing complex remodeling", "protein-lipid complex subunit organization", "site of DNA damage", "damaged DNA binding", and "MHC protein complex" (Fig. 10D–F). Regarding the KEGG, APOA1 co-expressed genes were involved in "fat digestion and absorption", "cholesterol metabolism", "PPAR signaling pathway", and "natural killer cell-mediated cytotoxicity" (Fig. 10G). These results suggest that APOA1 plays a critical role in STAD by participating in lipid metabolism and tumor immunity.

Drug sensitivity of APOA1

The GSCA database results showed a significant negative correlation between APOA1 expression and drug sensitivity, such as EHT 1864. A significant positive correlation was found between APOA1 expression and drug sensitivity, including YM155 (Fig. 11).

APOA1 plays an important role in the proliferation of GC

After systematically analyzing the involvement of APOA1 in human cancers, we proposed that APOA1 is a potential biomarker and therapeutic target for human cancers, including GC. Subsequently, in vitro experiments were performed to validate this hypothesis using gastric cancer as an example. We increased APOA1 expression in cells by lentivirus, and a Western blot test was used to confirm the efficacy of the

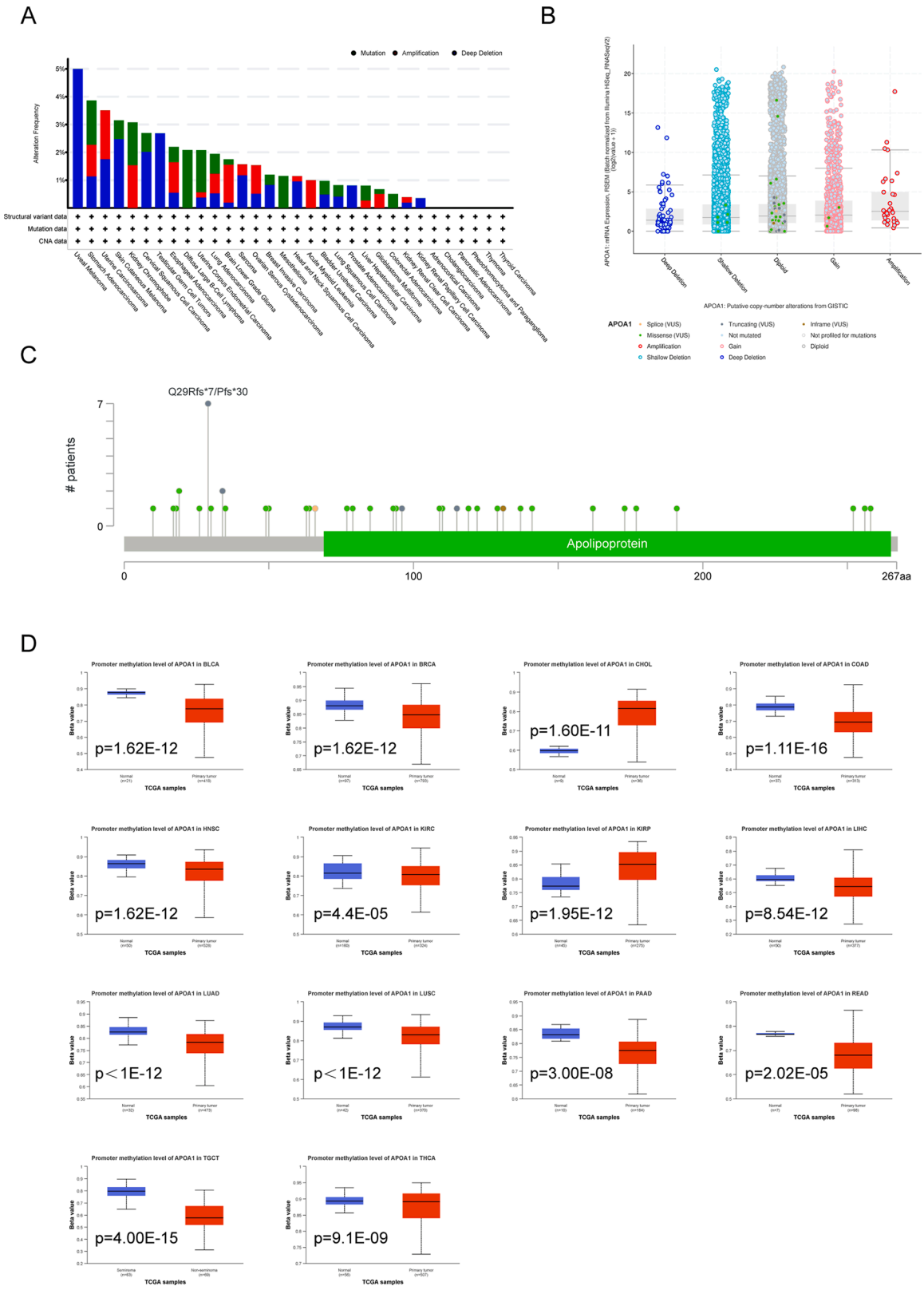

Fig. 5. The genetic and epigenetic features of APOA1 in pan-cancer.
A. The alteration frequencies and different mutation types of APOA1 in pan-cancer according to cBioPortal dataset. B.The correlation between the mRNA obtained by APOA1 gene transcription in pan-cancer and the variation of CNA. C.The visualization of APOA1 mutation sites in pan-cancer. D.The promoter methylation levels of APOA1 in different types of cancer.

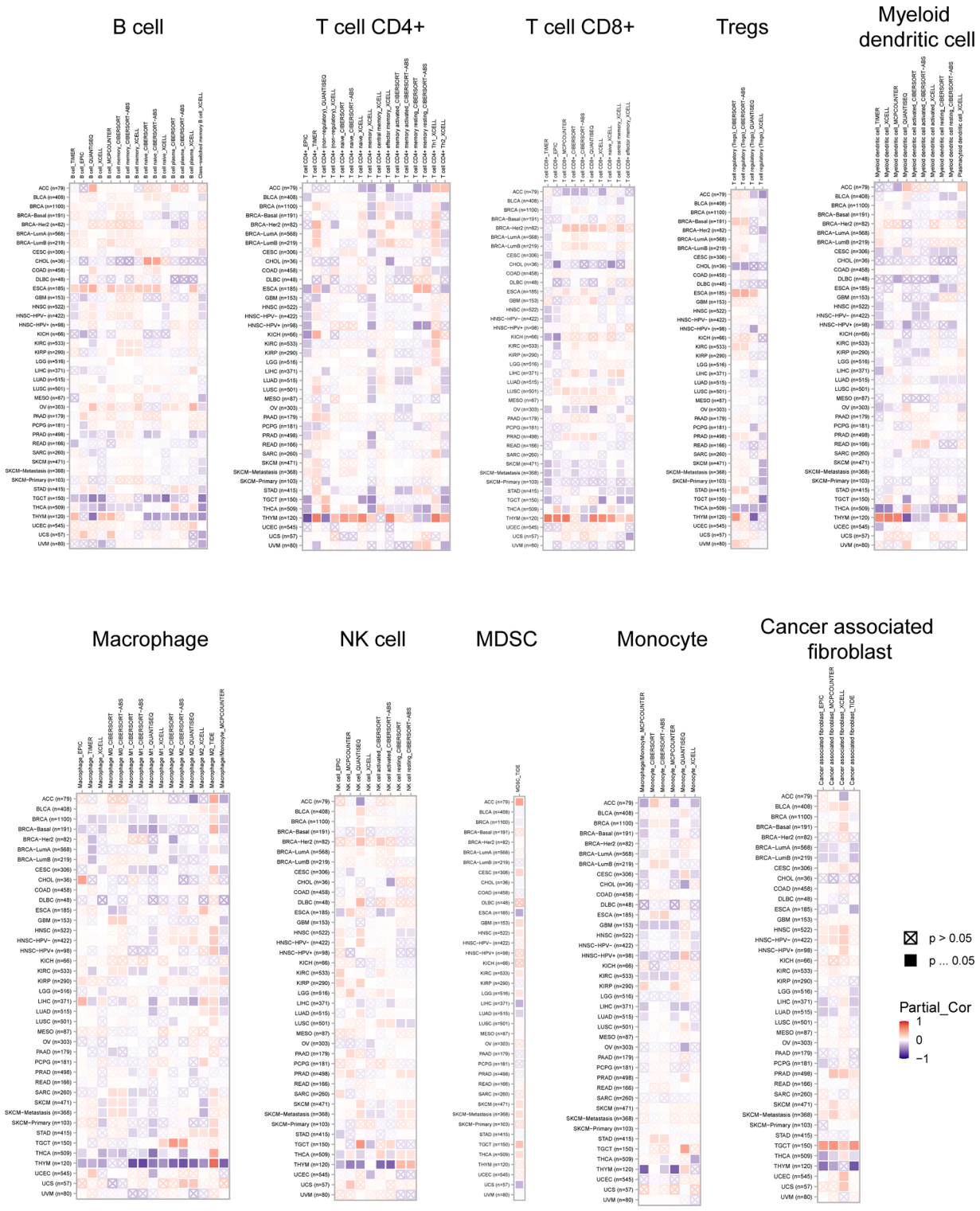

Fig. 6. Correlation between APOA1 expression and immune cells.
Correlation between APOA1 expression and immune cell infiltration. Correlation was tested by Spearman methods. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

increase (Fig. 8A, B). After verifying the overexpression of APOA1 in gastric cancer cells, the effects of APOA1 on cell proliferation, migration and invasion were evaluated. The results of the CCK-8 experiment showed that APOA1 overexpression significantly increased the activity of HGC27 and AGS cells (Fig. 12A). Furthermore, cell migration and invasion were analyzed after APOA1 overexpression. The results of Transwell assay indicated that the migrated cells and invasive cells were increased in HGC27 and AGS cells after APOA1 overexpression (Fig. 12B, C).

Discussion

Apolipoprotein A-I (ApoA1), encoded by the APOA1 gene, serves as a pivotal anti-inflammatory agent implicated in lipid transportation and

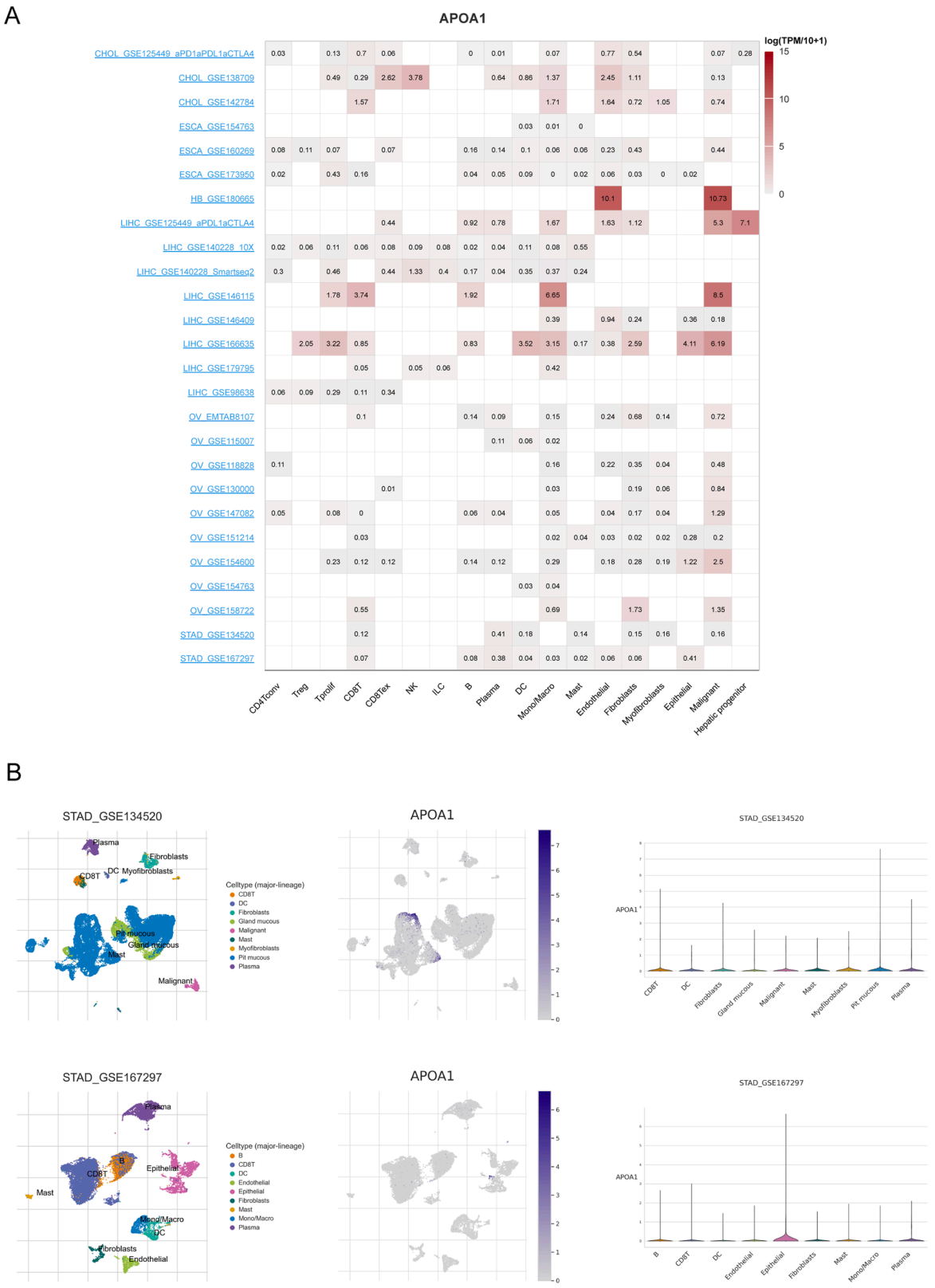

Fig. 7. Co-expression of APOA1 in immune and stromal cells by Single-cell sequencing analysis
A. The expression levels of APOA1 mRNA in some cancer cells. B. APOA1-associated single-cell analysis in the STAD_GSE134520 and STAD_GSE169297.

metabolism, and is deemed a potential biomarker for a spectrum of cancers [11–13]. Furthermore, Bing Li et al. propose a refined molecular classification scheme for GC using integrated optimal algorithms and multi-omics data, APOA 1 is the driver gene for subtype 3 (CDH1 type) [39]. However, the nuanced role of APOA1 across diverse tumor types, especially its prognostic implications and clinical relevance, remains largely uncharted. Our investigation leveraged TIMER2.0, GEPIA2, and UALCAN databases to scrutinize APOA1 expression and its prognostic

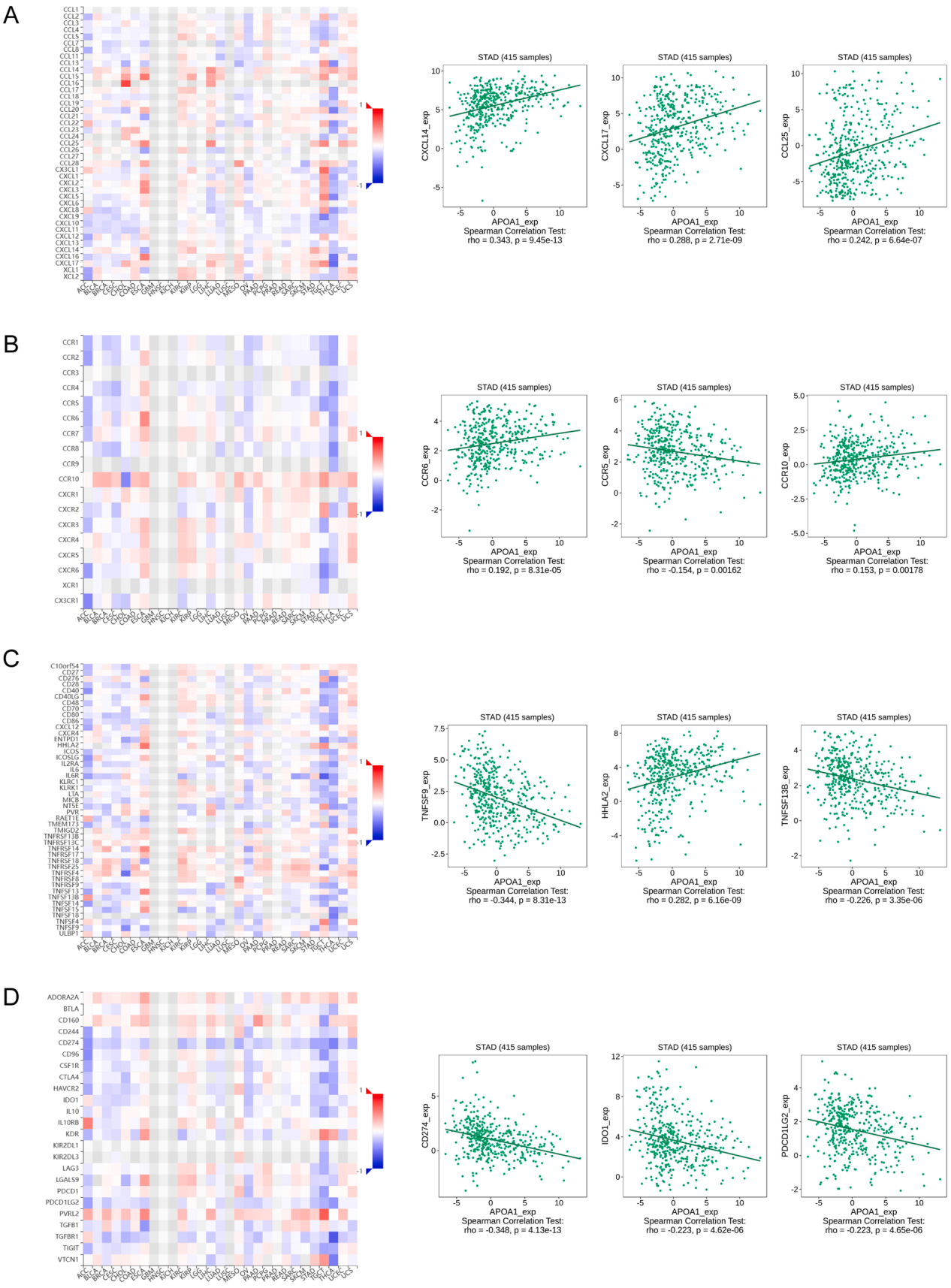

Fig. 8. Correlation between APOA1 expression and immune infiltration.
Correlations between the expression of APOA1 and (A) chemokine, (B) chemokine receptor, (C) immune stimulator, (D) immune inhibitor. Red and blue represent positive and negative correlations, respectively.

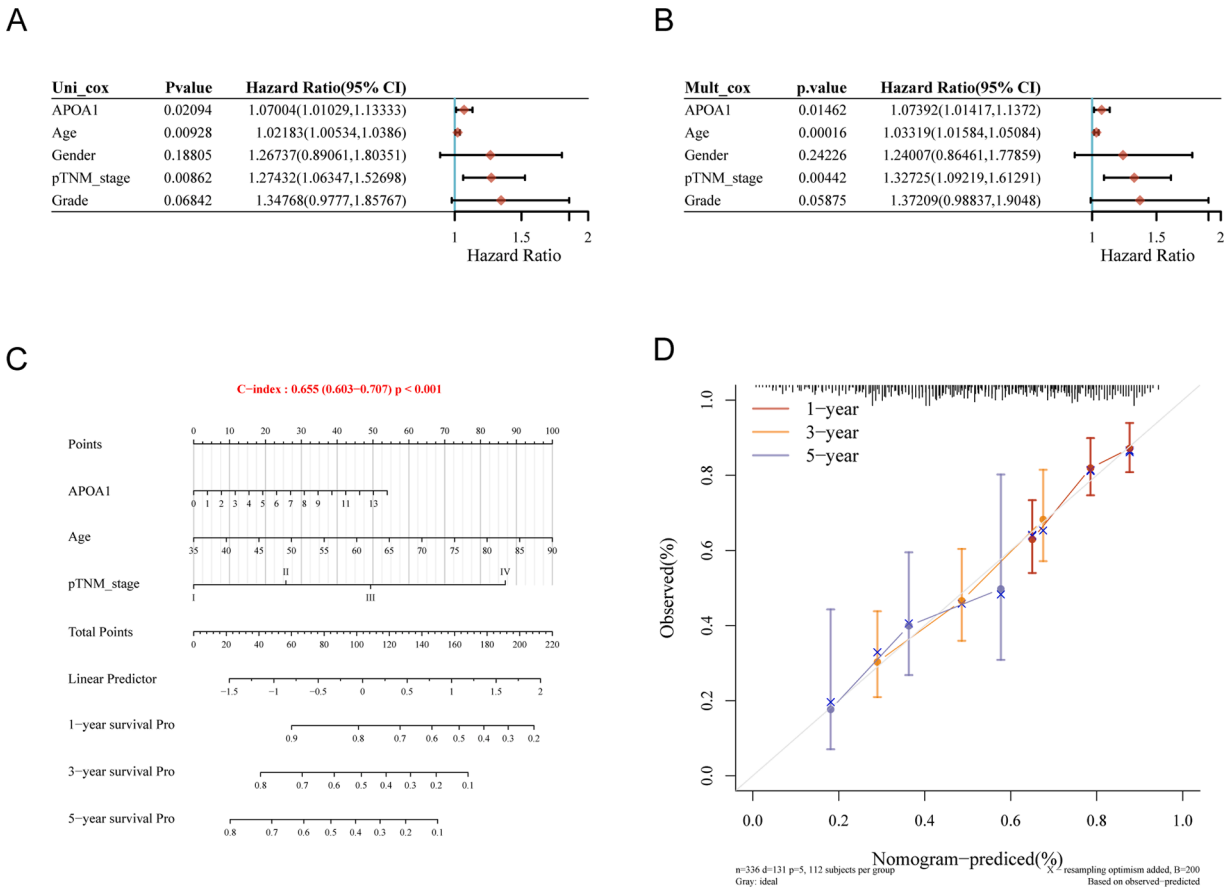

Fig. 9. Analysis of the clinical relevance of APOA1 in GC.
A-B. Prognostic significance of APOA1 in pancreatic cancer by univariate and multifactorial COX analysis. C. Nomogram based on APOA1 expression and pathological staging. D. Correction analysis diagram of the nomogram.

significance across multiple cancers. We discerned elevated APOA1 levels in BRCA and head and neck squamous cell carcinoma (HNSC), juxtaposed with a reduction in several other malignancies. Notably, diminished APOA1 expression correlated with tumor pathological stages and exhibited variability across subtypes in BRCA, KIRC, LUAD, LUSC, and STAD. These observations posit APOA1 expression as a potentially valuable diagnostic metric in clinical oncology.

Survival analyses further elucidated that heightened APOA1 expression correlated with improved prognoses in BRCA, OV, and UCEC, yet conversely indicated poorer outcomes in ACC, KIRC, and STAD. Cox regression analyses implicated high APOA1 expression as a risk factor in KICH, KIPAN, ACC, STAD, glioblastoma multiforme and lower grade glioma (GBMLGG), and esophageal cancer (ESCA), while it exerted a protective influence on OS in THYM, LIHC, and BRCA. A nomogram integrating APOA1 expression, age, and pathological stage was devised to forecast the prognosis of STAD patients. Cox regression affirmed APOA1 expression as an independent risk indicator for GC, aligning with its putative tumor-promoting function. Consequently, APOA1 emerges as a significant indicator of survival and prognosis in cancer patients, underscoring its potential as a promising biomarker.

The immune components within tumors, namely the tumor immune microenvironment (TIME), have long been recognized as closely associated with tumor development, recurrence, and metastasis [40]. With the rise of synthetic immunology and the growing understanding of the TME, immunotherapy has flourished in the field of tumor treatment and gradually emerged as a novel therapeutic approach. The advent of ICIs, which block the activity of programmed cell death protein 1 (PD-1), programmed cell death ligand 1 (PD-L1), and/or cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), has significantly transformed the landscape of cancer treatment [41]. TMB is typically defined as the number of somatic mutations per megabase in the coding regions of the cancer genome. TMB has been demonstrated to be correlated with the prognosis of immunotherapy in several types of cancers [42]. MSI, caused by defects in the mismatch repair system (MMR), serves as an indicator of tumor genetic instability. Moreover, MSI may also act as a potential biomarker for predicting the efficacy of immunotherapy [43]. Our study results indicate a significant correlation between APOA1 expression and TMB and MSI levels in various cancer types. These findings suggest that APOA1 and its encoded protein could serve as biomarkers for predicting the effectiveness of ICIs treatment in cancer patients.

Concurrently, with the widespread application of immunotherapy, immune score has been empirically verified as an indicator of cancer patients' survival, recurrence, metastasis, and drug resistance [44]. Our research discovered that in three types of cancers, APOA1 was positively correlated with immune score, while in six types of cancers, it was negatively correlated. Similarly, APOA1 was positively correlated with stromal score in five types of cancers and negatively correlated in seven types of cancers. These observations imply that APOA1 may interact with both tumor cells and immune cells within tumors. Among them, it is widely acknowledged that immune cell infiltration plays a key role in tumor progression and immunotherapy [45]. Therefore, it is crucial to discuss the relationship between APOA1 and tumor-associated immune cell infiltration. Lu et al. found that nanoparticles can target the lipid metabolism pathways within the TME, potentially disrupting the metabolic support of tumor cells and enhancing the function of immune cells. For instance, nanoparticles can deliver drugs or small molecules to inhibit enzymes involved in lipid synthesis or transport, thereby

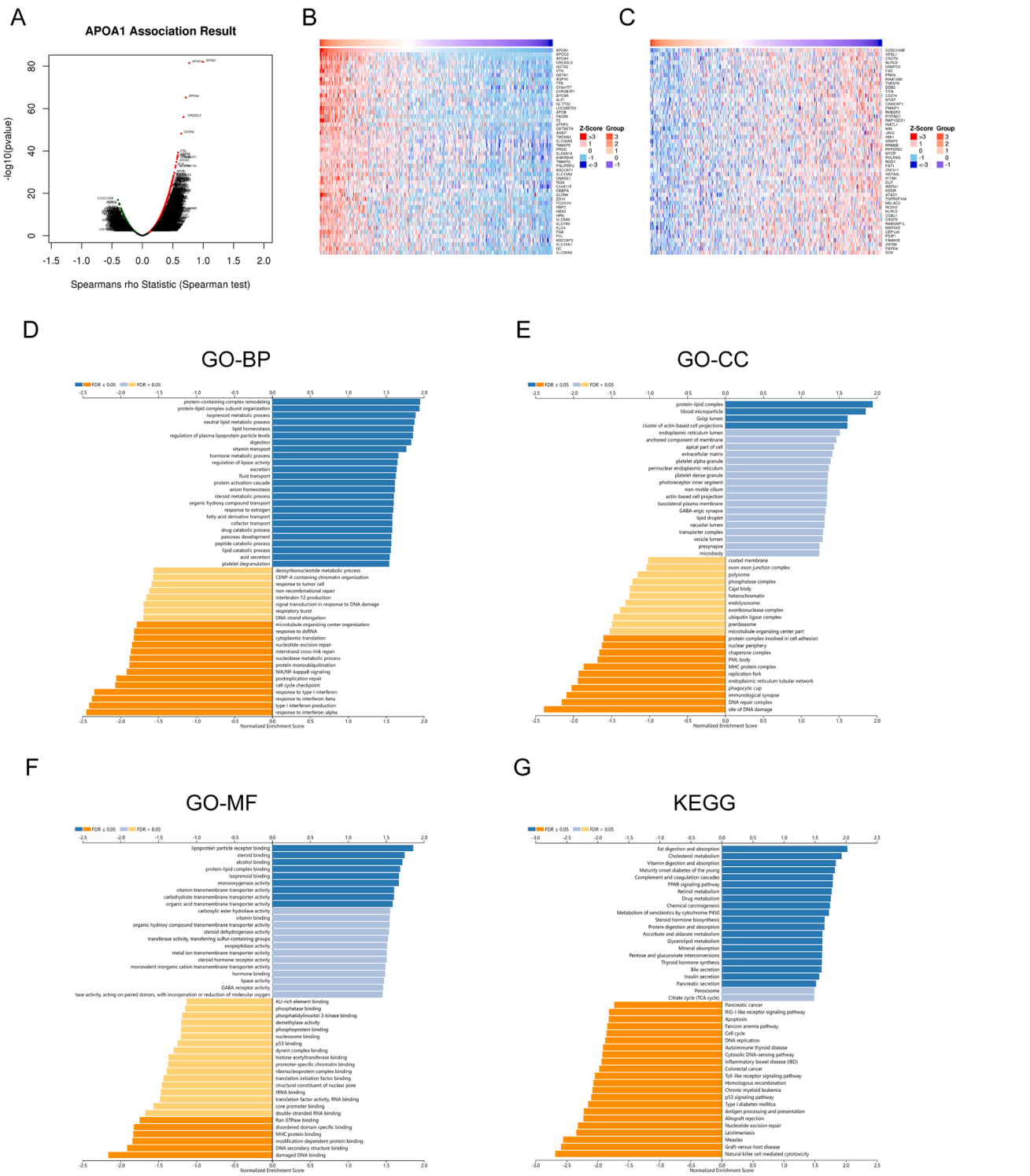

Fig. 10. The enrichment analysis of APOA1 co-expression genes in STAD.
A. The APOA1 co-expression genes in STAD. B-C The top 50 genes were positively and negatively correlated with APOA1. D-G APOA1 and its co-expression genes in STAD analyzed by GO enrichment analysis (BP, CC, MF) and KEGG pathway approaches.

depriving tumor cells of essential nutrients and weakening their ability to evade immune surveillance [46]. This is mainly due to the significant impact of lipid metabolism on tumor cells and immune cells within the TME. Tumor cells typically exhibit altered lipid metabolism to support their rapid growth and survival, while the function of immune cells is regulated by metabolic changes. This implies that APOA1 can affect the interaction between tumor cells and immune cells within the TME, thereby influencing tumor progression and treatment response.

Single-cell analyses exposed elevated APOA1 expression in various cell types across multiple cancers, notably in CHOL, ESCA, HB, LIHC, OV, and STAD. Further examination of the TISCH2 dataset indicated predominant APOA1 expression in pit mucosa and epithelial cells in STAD. These insights suggest APOA1's potential to modulate tumor development via effects on extracellular matrix formation and tumor

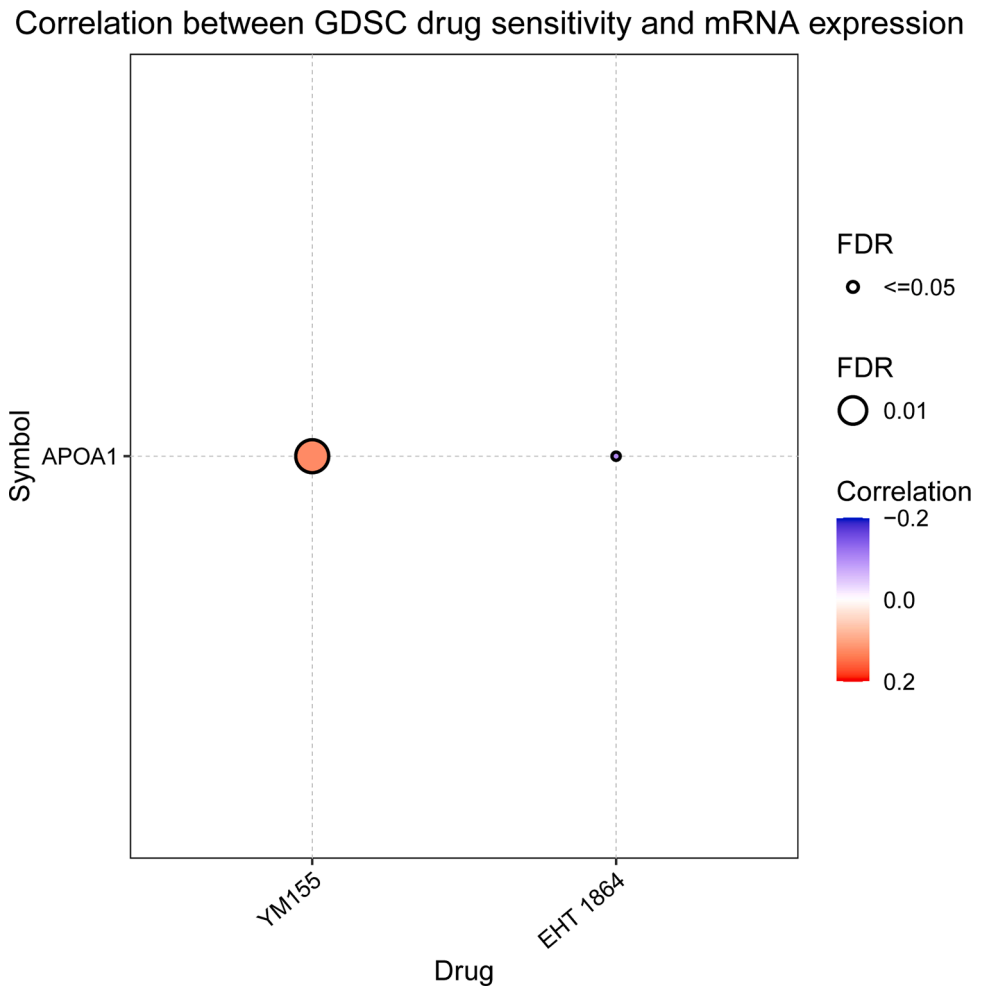

Fig. 11. Correlation of APOA1 with drug sensitivity.

cell proliferation and dissemination, thereby highlighting its role in the TIME.

Despite corroborative evidence linking APOA1 to tumor progression, experimental validation of APOA1′s role remains nascent. Consequently, we explored the nexus between APOA1 alterations and oncogenic behaviors in GC. APOA1 overexpression cell lines were established to assess the survival, migration, and invasion capabilities of GC cells. Results indicated that APOA1 overexpression markedly amplified cell activity and migration and invasion rates in GC cell lines, suggesting interactions between APOA1 and tumor and immune cells within the TME, thereby affecting tumor development and therapeutic responsiveness.

To delineate the biological functions of APOA1-related genes in STAD, an enrichment analysis was executed using the Linked Omics database. Findings implicated APOA1 in a gamut of biological processes and signaling pathways, such as protein-lipid complex subunit organization, DNA damage site interaction, damaged DNA binding, MHC protein complex involvement, cholesterol metabolism, PPAR signaling pathway modulation, and natural killer cell-mediated cytotoxicity. These results accentuate APOA1′s crucial role in STAD pathogenesis, particularly through its involvement in lipid metabolism and tumor immunity.

In summation, drug sensitivity related to APOA1 was evaluated using the GDSC databases. YM155, identified as sepantronium bromide, a small molecule initially recognized as a survivin inhibitor [47], activates the DNA damage response pathway, inducing cell cycle arrest and apoptosis, and exerting significant cytotoxic effects on ALL cells [48]. Survivin is a member of the anti-apoptotic protein family [49]. YM155′s localization to mitochondrial DNA (mtDNA) in lung cancer cells precipitates a swift decline in oxidative phosphorylation and a reduction in TCA intermediates. Moreover, YM155 and other mitochondria-targeted cancer therapeutics activate AMPK and attenuate BMP signaling [50]. Our analysis disclosed a negative correlation between APOA1 expression and sensitivity to EHT 1864, and a positive correlation with sensitivity to YM155. Thus, tumors with APOA1 upregulation may exhibit resistance to EHT 1864 yet remain vulnerable to YM155. Consequently, assessing APOA1 expression could be pivotal in guiding anti-tumor drug selection in clinical contexts, especially in combating drug-resistant tumors.

In essence, our pan-cancer analysis and cellular experiments have pinpointed APOA1 as a valuable predictor for cancer prognosis, drug resistance, and immunotherapy outcomes. Acknowledging several limitations, including potential systematic biases from database heterogeneity, the need for more exhaustive molecular mechanism investigations in GC, the restricted generalizability of single tumor cell type studies for pan-cancer research, and the necessity for further exploration of APOA1′s significance for the immune system, our study nonetheless lays a foundational framework for APOA1′s pan-cancer analysis, offering novel insights to steer future research directions.

Funding

This research was funded by the National Natural Science Foundation of China (82,171,222).

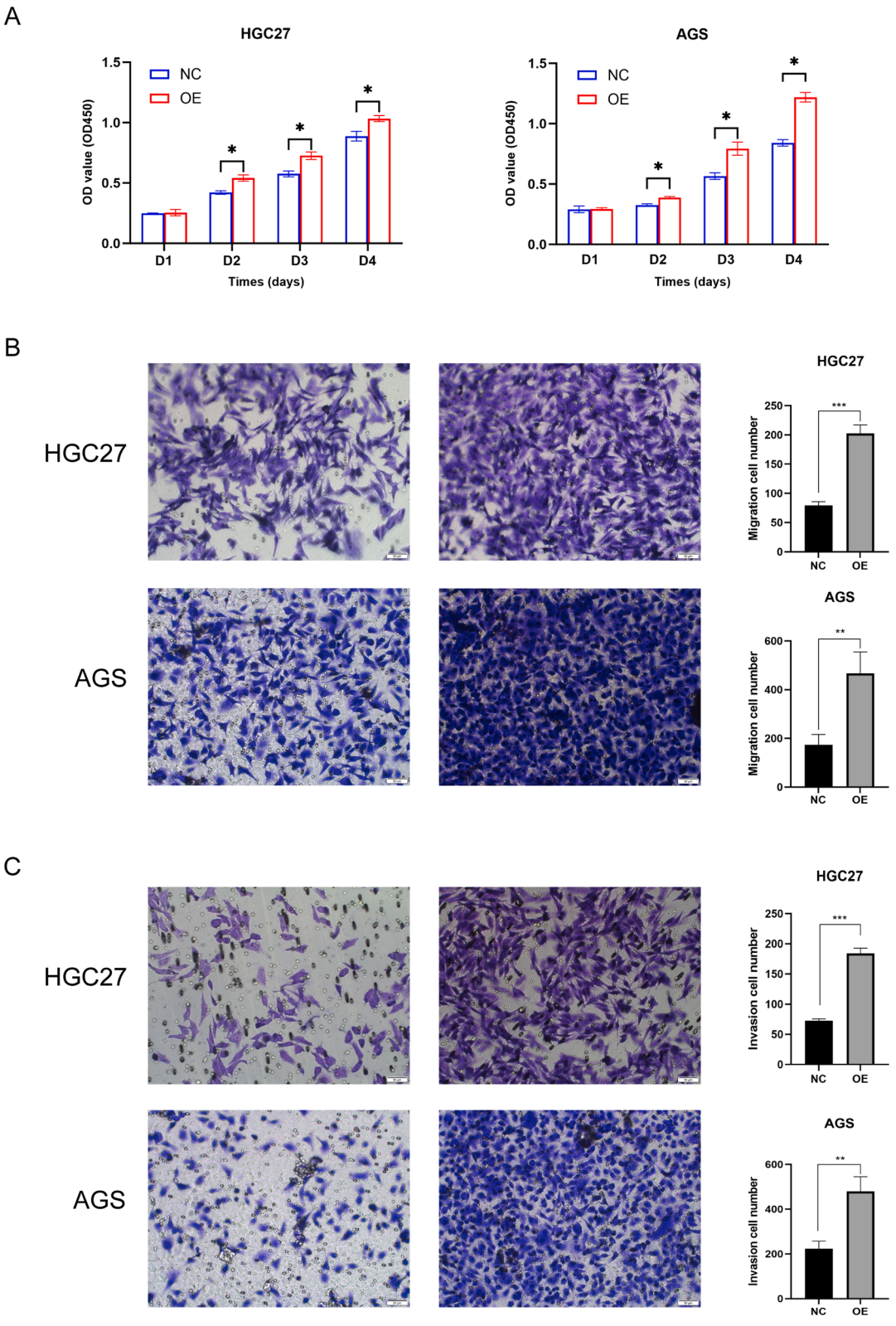

Fig. 12. Effects of APOA1 on proliferation, migration and invasion of GC cells in vitro.
A.CCK8 assay was used to assess cell proliferation in HGC27 and AGS cells ($p < 0.05$). B-C. The transwell assay was performed to assess cell migration and invasion in HGC27 and AGS cells ($p < 0.05$).

Data availability

The data sets analyzed in the current study are available in the Human Protein Atlas database (http://www.proteinatlas.org/), the UCSC Xena database (http://xena.ucsc.edu/), the SangerBox database (http://www.sangerbox.com/tool), the TISIDB database (http://cis.hku.hk/TISIDB/), and the GSCA database (https://guolab.wchscu.cn/GSCA/). Additional data can be obtained from the corresponding

author upon reasonable request.

Ethical approval

The manuscript contains no clinical trials or patient data.

Informed consent

Not applicable.

CRediT authorship contribution statement

Peiyi Xu: Writing – original draft, Visualization, Formal analysis. **Qiuyan Zhang:** Investigation. **Jing Zhai:** Investigation. **Pu Chen:** Investigation. **Xueting Deng:** Funding acquisition, Conceptualization. **Lin Miao:** Writing – review & editing. **Xiuhua Zhang:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tranon.2025.102344.

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