FCHO1 Is a Prognostic Biomarker of Colon Adenocarcinoma and Associated with Immune Infiltration and Glycolysis

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Abstract

Background

Colon adenocarcinoma (COAD) is a common digestive tract tumor and the molecular mechanism is very complicated. Overexpression of FCHO1 plays the role of oncogene or tumor suppressor gene in the process of tumorigenesis and development. However, possible mechanisms of FCHO1 in COAD remains unknown.

Methods

Online database Oncomine, TIMER and TCGA were used to clarify the expression level of FCHO1 in COAD. Using receiver operating characteristic (ROC) curve and GEIPA database to evaluate the prognostic value of FCHO1 in COAD. Then, STRING and GeneMANIA database were used to construct the protein-protein interaction network. The GO/KEGG enrichment analysis were performed by Using Funrich. Co-expression genes of FCHO1 were acquired by Linkedomics database, GSEA analysis was used to explore the possible pathway in co-expression genes of FCHO1. The correlation between FCHO1 expression and hypoxia-related genes, glycolysis-related genes and immune infiltrates was analyzed using the TIMER, Starbase and TCGA cohort.

Results

Our results revealed that high expression level of FCHO1 was significantly increased in COAD tissues than normal tissue. High expression of FCHO1 in COAD predicted worse survival, including OS (HR = 1.8 p = 0.0022), DFS (HR = 1.6 p = 0.043). GSEA analysis showed that co-expression genes were significantly linked with MicroRNAs in cancer, Oxidative phosphorylation, Cell cycle, Notch signaling pathway, VEGF signaling pathway and p53 signaling pathway. Further, the result revealed that hypoxia-related genes (NFKB1, VEGFB) was simultaneously positively correlated with FCHO1 expression in TIMER, Starbase database. Glycolysis-related genes (HK1, G6PD and SLC2A1) was positively associated with FCHO1 expression in TIMER, Starbase database. At the same time, PDCD1 and LAG3 expression, which as immune checkpoint, were positively correlated with FCHO1 expression.

Conclusion

Collectively, FCHO1 may act as a promising diagnostic and prognostic biomarker and correlated with hypoxia, glycolysis and immune infiltration in COAD.

Introduction

Colorectal cancer, one of the most common digestive tract malignant tumors, ranks the third on both malignant morbidity and cancer-related lethality worldwide, the occurrence of new cases of colorectal cancer about 1.8 million each year and about 880,000 colorectal cancer deaths occurred[1]. With the improvement of living conditions, especially in diet, the incidence and mortality of colorectal cancer in China are increasing rapidly and maintaining an upward trend[2]. Despite great advancement in diagnosis, treatment and prevention for colorectal cancer, approximately 20% of colorectal patients have distant metastasis at initial diagnosis, and the 5-year survival rate of these patients is less than 20%[3]. Colon adenocarcinoma (COAD), the most common histological subtype, is mainly treated by surgery. Unfortunately, the molecular mechanism of COAD progression is still slow although its morbidity and mortality rates have been decreased during the past decades[4]. Therefore, it is essential to understand the molecular mechanisms of COAD, which could provide more diagnostic and therapeutic biomarkers for tumor treatment.

FCH Domain Only Protein 1 (FCHO1), a member of the F-BAR protein family, is function as a key coat nucleator at the surface clathrin assembly[5]. Through the N-terminal F-BAR domain forms acrescent-shaped, antiparallel, dimeric structure that can bind to phosphatidylinositol 4,5-biphosphate (PI (4,5) P2) on inside of the cell membrane, inducing and stabilizing membrane curvature[6]. Recent studies showed F-BAR domain only protein 1 (FCHO1) deficiency was associated with immune deficiency in humans[7]. Knockdown of FCHO2 suppresses tumorigenesis and as a potential biomarker for lung cancer[8]. The silencing of FCHO1 led to zebrafish embryonic developmental defects, and its pathological features indicate that FCHO1 dysfunction may cause immune cell deficiency or developmental defects[9]. However, the potential mechanisms of FCHO1 in COAD were still unclear, especially the correlation with hypoxia, immunotherapy, glycolysis.

In this study, COAD date sets downloaded from The Cancer Genome Atlas (TCGA) and was performed using R software package to analyzed the expression level of FCHO1 in COAD. The online databases were used to investigate FCHO1 mRNA expression in different cancer, prognostic value, protein expression and methylation level. The biological functions and signal pathways of FCHO1 co-expression genes were performed using Funrich and EGSEA. Finally, we discussed the relationship between FCHO1 with hypoxia, glycolysis, immune infiltration in COAD.

Materials And Methods

Expression of FCHO1 in COAD

ONCOMINE database (www.oncomine.org)[10] is an online cancer microarray database for RNA sequence analysis, which helps to discover from the analysis of whole gene expression[11]. The mRNA expression of FCHO1 in various cancer tissues and adjacent normal control samples using ONCOMINE database. On the other hand, TCGA (https://portal.gdc.cancer.gov/)[12] is a project jointly launched by the National Cancer Institute (NCI) and the National Human Genome Research Institute (NHGRI) in 2006. It includes clinical data, mRNA expression and other data on various human cancers. The expression

level of FCHO1 downloaded from TCGA. Use R package for statistical analysis and visualization, the prognostic value of FCHO1 in COAD was assessed by Cox model and ROC curve.

Mining of FCHO1 and clinical relevance

UALCAN (http://ualcan.path.uab/)[13] is a premier public resource to further explore TCGA gene expression data, which can provide data analysis on selected gene information and clinically relevant characteristics. Also, the survival significance of high and low expression of FCHO1 in COAD can be obtained from GEPIA(http://gepia.cancer-pku.cn/). GEPIA is a newly developed interactive web server for analyzing the RNA sequencing expression data of 9,736 tumors and 8,587 normal samples from the TCGA and the GTEx projects, using a standard processing pipeline. The LinkedOmics (http://www.linkedomics.org/) is a web server for analyzing cancer-associated multidimensional datasets of 32 cancer types and 11,158 patients.co-expression genes of FCHO1 were downloaded from database. Through the above online database to study the relationship between FCHO1 and different clinical types and stages, and then find out how FCHO1 plays a role in the development of colon cancer.

Functional Enrichment Analysis

Protein-protein interaction across ABI2-related proteins were analyzed using STRING (https://www.string-db.org/) and GeneMANIA (http://genemania.org/). Gene ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis for FCHO1-related proteins were performed using Funrich tool. Accordingly, in order to further understand the potential mechanism of FCHO1, we divided the samples from TCGA-COAD data set into two parts and performed GSEA according to the median expression level of NPM1 and NPM1 to study whether the genes in the two groups are rich in meaningful biological processes. Gene Set Enrichment Analysis (GSEA) for these co-expression genes associated with ABI2 were conducted with GSEA[14] as the reference gene set. FDR<0.2 and P<0.05 were considered statistically significant.

Correlation Between FCHO1 and Tumor Immune Function

TIMER (https://cistrome.shinyapps.io/timer/)[15] is a comprehensive resource for systematic analysis of the immune infiltration of different cancer types. To study the role of FCHO1 in immunity, TIMER database showed that FCHO1 expression level in various cancer tissues and adjacent normal control samples in COAD were obtained. P < 0.01 was considered statistically significant. Immune infiltrating cells include B cells, neutrophils, CD4+, macrophages, CD8+ T cells and dendritic cells. In addition, we analyzed the association between FCHO1 and immune cell marker genes in COAD samples using TIMER, GEPIA and TCGA database. Immune cell markers are selected from the website (www.rndsystems.com/cn/resources/cell-markers/).

Result

The Expression and Clinical Features of FCHO1 in Different Database

Oncomine database was used to analyze the difference of FCHO1 mRNA expression in various tumors and our results showed that FCHO1 was significantly upregulated in colorectal cancer compared to the normal tissues (Figure 1A). Also, to explored FCHO1 expression in human cancers, we used TIMER database to analyze FCHO1 mRNA expression. The differential expression between the tumor and adjacent normal tissues for FCHO1 is shown in (Figure 1B). FCHO1 was highly-expressed in various cancers, including COAD. We identified that the expression level of FCHO1 was significantly increased in unpaired (Figure 1C) or paired (Figure 1D) COAD and normal tissues using TCGA database.

Next. we examined the correlation between FCHO1 and clinicopathological characteristic in COAD. The results of UALCAN showed that the expression of FCHO1 was significantly correlated with gender, histological subtypes, P53 mutation status, body weight, individual cancer stages, race, age and nodal metastasis status (Figure S1). In order to reflect the relationship between the accuracy of the predicted value and FCHO1 expression, The results of ROC revealed that FCHO1 had a favorable prediction accuracy for COAD, and the area under the ROC curve was 0.841 (95%CI:0.801-0.880) (figure 1E). In addition, GEIPA database was used to investigated the prognostic value in COAD, the results showed that high expression of FCHO1 in COAD predicted worse survival, including OS (HR=1.8 p=0.0022) (figureS1F), DFS (HR=1.6 p=0.043) (figureS1G). Analysis of protein of FCHO1expression level from the HPA database indicated that FCHO1 protein was obviously upregulated in COAD tissues(figureS1H). Collectively, these results indicated that FCHO1 was significantly overexpressed in COAD and may serve as a potential oncogene in COAD.

The Prognostic and Diagnostic Value of FCHO1 Expression in COAD

Clinical Characteristics and gene expression were downloaded from TCGA database. Based on the mean value of FCHO1 expression, about 454 patients with COAD were divided into high- and low-FCHO1 expression groups, but the expression of FCHO1 in COAD had no significance with clinical characteristics .Then we conducted Univariate Cox hazard regression analysis showed T stage (T3&T4), M stage(M1), Pathologic stage (Stage III and Stage IV) and N stage (N1 and N2) were significantly for COAD, Multivariate Cox hazard regression analysis showed that T stage (T3&T4), M stage(M1), Pathologic stage (Stage III and Stage IV) were significant with COAD, but the expression of FCHO1 in COAD had no significance.

Furthermore, to evaluate prognostic value of FCHO1 in clinical characteristics from TCGA database, the results showed that T stage (T3&T4), residual tumor R0 and N stage N2 were significantly related to OS for COAD (figure2A,2B,2C)but Pathologic stage (Stage III and Stage IV) and M stage (M1) had no significance (figure2D,2E). A nomogram for predicting probability of patients with 1-, 3- and 5-year overall survival (OS) was established to evaluate the prognostic value (figure 2G). Studies showed hypermethylation of the promoter CpG island silences tumor suppressor genes, such as Rb1, APC and DNA mismatch repair genes, in various cancer including CRC. Therefore, we hypothesized that hypermethylation of the promoter CpG island could inhibit gene expression, UALCAN was used to explore the promoter of FCHO1 methylation level in COAD, then we found that the methylation of FCHO1 in COAD

tissues was remarkably decreased compared with normal tissues(figure2F). Also, the promoter of FCHO1 methylation level was significantly correlated with gender, histological subtypes, P53 mutation status, body weight, individual cancer stages, race, age and nodal metastasis status (figure S2). Taken together, these findings showed that FCHO1 may act as a promising diagnostic and prognostic biomarker in COAD.

Enrichment Analysis of FCHO1Co-Expression Gene in COAD

String and GeneMANIA were used to established genes-genes network across FCHO1 and its neighboring genes. By analyzing String and GeneMANIA, we acquired 30 FCHO1-related genes (10 genes for String and 20 for GeneMANIA) (Figure3A, 3B). these neighboring genes in GO analysis demonstrates that they are mainly involved in cytoplasm, plasma membrane, exosomes, receptor signaling complex scaffold activity, GTPase activator activity, signal transconduction, cell communication and transport (Figure 3C-3E). KEGG analysis for these genes showed that integrin family cell surface interactions, S1P1 pathway and Arf6 trafficking events (Figure 3F).

To further characterize the potential function of FCHO1 gene, we explored FCHO1-positively or negatively associated genes in COAD using LinkedOmics. The top 50 FCHO1 positively or negatively associated genes were shown (Figure 4A, 4B). Besides, we used LinkedOmics database to analyze these significant genes in GO analysis. The bar bubble graph revealed the top 10 messages for biological process (BP), cellular component (CC) and molecular function (MF) enrichment analysis, respectively. (Figure 4C-E). For BP, they were mainly enriched in metabolic process, biological regulation and response to stimulus (Figure 4C). For CC, they mainly enriched in membrane, nucleus, and membrane-enclosed lumen (Figure 4D). For MF, these genes mainly enriched in protein binding, ion binding, and nucleic acid binding (4F). Moreover, we conducted GSEA for FCHO1co-expression genes using NGSEA datebase. Results showed that they were significantly linked with MicroRNAs in cancer, Oxidative phosphorylation, Cell cycle, Notch signaling pathway, VEGF signaling pathway and p53 signaling pathway (Figure 4H-L). Collectively, studies may help us to understand the complicate molecular mechanism of FCHO1 in COAD.

The Relationship of FCHO1 with Tumor Hypoxia and Anaerobic Metabolism

Hypoxia is a common feature of tumor microenvironment and plays an important role in tumorigenesis and aggressiveness. Some studies showed hypoxia had been verified to be involved in glycolysis[16]and immune cell infiltration[17] in colorectal cancer. At the same time, Increased studies indicated that glycolysis played an important role in the progression of COAD[18, 19]. GSEA analysis of co-expressed genes suggests FCHO1 was linked with oxidative phosphorylation, which was closely related to glycolysis. Moreover, we hypothesis whether tumor hypoxia and glycolysis associated with FCHO1 expression in COAD. To analyzed TCGA-COAD date sets to investigate hypoxia-related genes and glycolysis-related genes with FCHO1 expression in COAD (Figure 5A, B). The results of correlation analysis showed FCHO1 expression was positively correlated with hypoxia-related genes (NFKB1, VEGFA and VEGFB), while FCHO1expression was negatively correlated with HIF1A and KRAS. Also, FCHO1 expression was positively correlated with glycolysis-related genes (ENO1, HK1, G6PD, PGK1, PKM and

SLC2A1), while FCHO1 expression was negatively correlated with SLC2A1. Based on TIMER, Starbase database to explore these positive relate gene whether correlated with FCHO1 expression. The result revealed that hypoxia-related genes (NFKB1, VEGFB) was simultaneously positively correlated with FCHO1 expression in TIMER, Starbase database (Figure5C-F). Also, glycolysis-related genes (HK1, G6PD and SLC2A1) was positively associated with FCHO1 expression in TIMER, Starbase database. These results suggested that FCHO1 may involve in metabolism reprogramming and hypoxic microenvironment in COAD.

Correlation between FCHO1 and immune infiltration in COAD

Previous studies indicated that immune cells infiltrationplayed an important role in the progression of COAD[17, 20]. By analyzing TIMER database to analyze the correlation between FCHO1 expression and immune infiltrating cells in COAD. we found that FCHO1 was positively associated with the expression of CD4+ T cell, while negatively correlated with the expression levels of CD8+ T cells (Figure 6A). Also, we identified that FCHO1 CNV was closely associated with the degree of infiltration of B cell, CD8+ T cell, and dendritic cell (figure 6B). We further analyzed the correlation between FCHO1and immune cell infiltration level verified by ssGSEA in TCGA-STAD using Spearman correlation. Results showed high FCHO1 expression was significantly positively correlated with NK CD56 bright cells, Th17 cells, pDC and NK cells (Figure 6C). The enrich score of NK CD56 bright cells, Th17 cells, pDC and NK cells were positively associated with high expression levels of FCHO1(Figure 6D). The correlation between FCHO1 expression and these immune cells were showed in Figure 6E-H using Spearman correlation.

To further evaluate the relationship between FCHO1 and various immune infiltrating cells of COAD. we used TIMER, StarBase, and TCGA to explore the association between FCHO1 and immune marker genes of several immune cells. The result showed that the expression of FCHO1 was positively associated with B cell, CD8+ T Cell, Tfh, Th1, Treg, T cell exhaustion, M1 Macrophage and Natural killer cell. Moreover, PDCD1 and LAG3 is immune checkpoints in tumor. By analyzing TIMER, Starbase database, PDCD1 and LAG3 expression was positively correlated with FCHO1 expression (Figure 7). These results suggested that tumor immune cells may participate in FCHO1-induced progression of COAD.

Discussion

Colon adenocarcinoma(COAD)is a common gastrointestinal tumor, but the prognosis is still unsatisfactory. At presentation, of colorectal cancer diagnoses, One-third of patients have lymph node metastases, 20–25% have distant metastases, most of which involve the liver[21]. Despite the progress of treatment in recent years, the outcome is still not unlike. Especially, its molecular mechanism is not fully clear. Illuminating the mechanisms of COAD carcinogenesis and finding biomarkers is important for researchers to provide effective therapeutic targets. We performed bioinformatics analysis of public date to explore FCHO1 expression. Then we found that FCHO1 was significantly upregulated in COAD and high expression of FCHO1 was associated with worse prognosis. These results revealed that FCHO1 may serve as a biomarker for COAD.

A study result showed silence of FCHO2 could be a meaningful therapeutic strategy in lung cancer[8]. However, the biological function of FCHO1 has not been reported in COAD. Using UALCAN database, we found that the promoter of FCHO1 methylation level was high in normal tissues compared to tumor samples. We can easily explore CpG sites on the methylation in CRC samples using Current microarray technologies, especially the Illumina Infinium Human Methylation 450 platform[22]. The hypermethylation of CpG islands in promoter regions of FCHO1 in normal tissues inhibits their transcription and suppresses the onset of tumors. Also, the promoter of FCHO1 methylation level was significantly correlated with clinical characteristics. Therefore, the methylation of promoter regions of FCHO1 may as a factor lead to FCHO1 overexpression in COAD.

Numerous evidences revealed that hypoxia plays a special role in tumor microenvironment and is strongly associated with invasion, metastasis, resistance to therapy, and poor clinical outcomes. Hypoxia plays a crucial role in triggering the epithelial-mesenchymal transition (EMT) by regulating hypoxia-inducible factors (HIFs)[23]. The results showed that FCHO1 expression was positively correlated with hypoxia-related genes (NFKB1, VEGFA and VEGFB), while FCHO1expression was negatively correlated with HIF1A and KRAS. Taken together, our findings revealed that under the hypoxia microenvironment, FCHO1 may involve in the occurrence and progression of tumors. GSEA analysis of FCHO1 co-expression genes, the results showed that they were significantly linked with MicroRNAs in cancer, Oxidative phosphorylation, Cell cycle, Notch signaling pathway, VEGF signaling pathway and p53 signaling pathway. Through mitochondrial oxidative phosphorylation, cells can obtain energy. In contrast, most cancer cells produce energy through glycolysis and lactic acid fermentation, which is widely regarded as the Warburg effect[24]. The results showed FCHO1 expression was positively correlated with glycolysis-related genes (ENO1, HK1, G6PD, PGK1, PKM and SLC2A1), while FCHO1expression was negatively correlated with SLC2A1. These suggests that FCHO1 was involved in the metabolicrep rogramming regulation of COAD in the tumor microenvironment.

The metabolic reprogramming in cancer cells can generate hostile metabolic environments which characterized as low PH, low glucose concentration and oxygen

deficit[25]. These extreme environments can hinder the antitumor response of immune cells including infiltration of immune cells and some metabolites from these procedure like kynurenine can also blunt immune cell function[26]. The T cells are known to play an important role in antitumor. Upon activation, T cells improve the glycolysis level but aerobic oxidation still the main way for obtaining ATP and promoting T cells proliferation[27]. During T-cell activation, oxidative phosphorylation process would lead to reactive oxygen species that can stimulate some major transcription factors for the immune response[28]. Previous study revealed that Human FCHO1 deficiency reveals role for clathrin-mediated endocytosis in development and function of T cells[10]. we found that FCHO1 was positively associated with the expression of CD4 + T cell, while negatively correlated with the expression levels of CD8 + T cells. TCGA results showed high FCHO1 expression was significantly positively correlated with NK CD56 bright cells, Th17 cells, pDC and NK cells. The enrich score of NK CD56bright cells, Th17 cells, pDC and NK cells

were positively associated with high expression levels of FCHO1. These finding showed that FCHO1 may participate in tumor immune infiltration in COAD.

In conclusion, our findings indicated FCHO1 is overexpressed in COAD and its expression level is associated with clinical characteristics and prognosis of COAD patients. Futhermore, FCHO1 may participate in hypoxia, glycolysis and immune infiltration in COAD. Nevertheless, these results should be further verified by subsequent basic experiments and clinical trials.

Declarations

DATA AVAILABILITY STATEMENT

All available data were analyzed in this study. These can be found here: TCGA (https://portal.gdc.cancer.gov/), GEO (https://www.ncbi.nlm.nih.gov/gds), STRING (https://cn.string-db.org/),GEPIA2(http://gepia2.cancer-pku.cn/#index), R(https://www.r-project.org/), TIMER(https://cistrome.shinyapps.io/timer/) and HPA (https://www.proteinatlas.org/).

ETHICS STATEMENT

Ethical review and approval were not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

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AUTHOR CONTRIBUTIONS

FC, LB wrote the paper. WZ and XD edited the paper. All authors contributed to the article and approved the submitted version.

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Figures

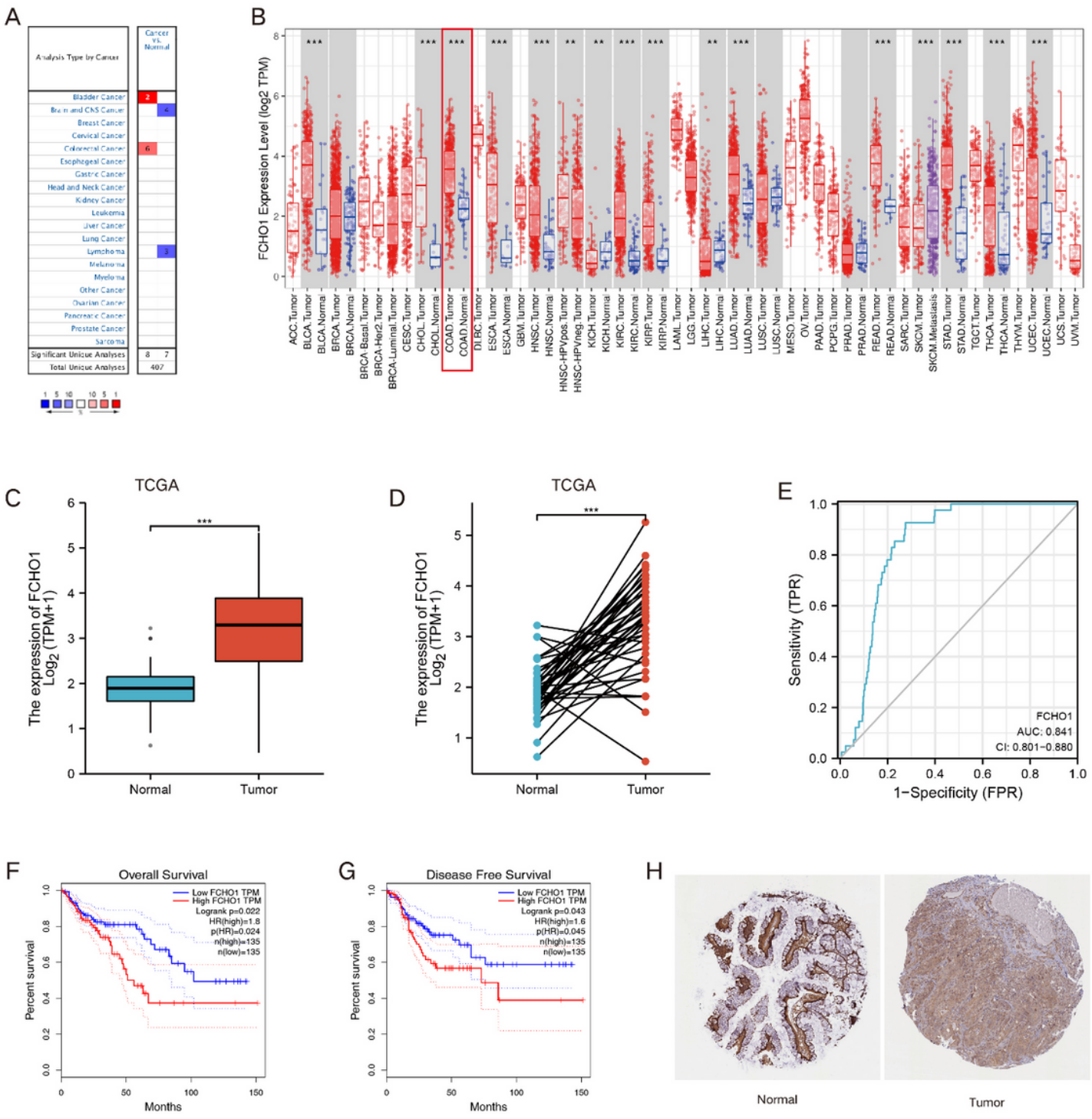

Figure 1

Relationship between FCHO1 overexpression and clinical prognostic characteristics of COAD patients. (A and B) Fcho1 overexpression in Pan-cancer; (C and D) FCHO1 overexpression was expressed in COAD tumor patients and normal tissues; (E) ROC curve of normal specimen combined with adjacent COAD tissue; (F and G) TCGA-COAD database overall survival rate (OS) and Disease Free Survival (DFS)

survival analysis; (H) From the HPA dataset, FCHO1 immunohistochemistry showed shallow staining in normal samples and deep staining in tumor samples.

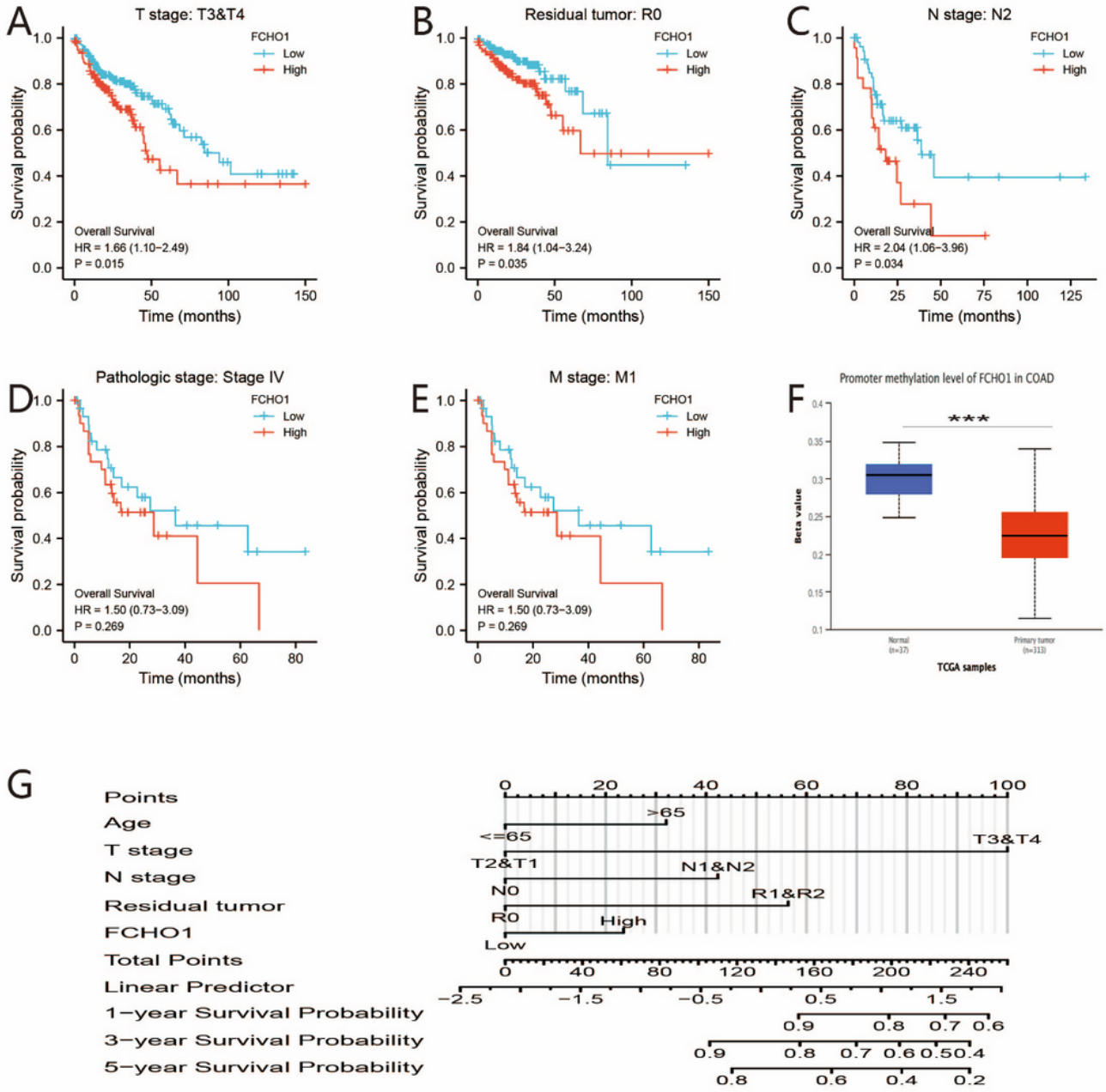

Figure 2

(A-E) The survival characteristics of FCHO1 in patients with COAD were evaluated according to different clinical characteristics; (F) The relationship between FCHO1 and DNA methylation; (G) A nomogram for

predicting probability of patients with 1-, 3- and 5-year overall survival (OS).

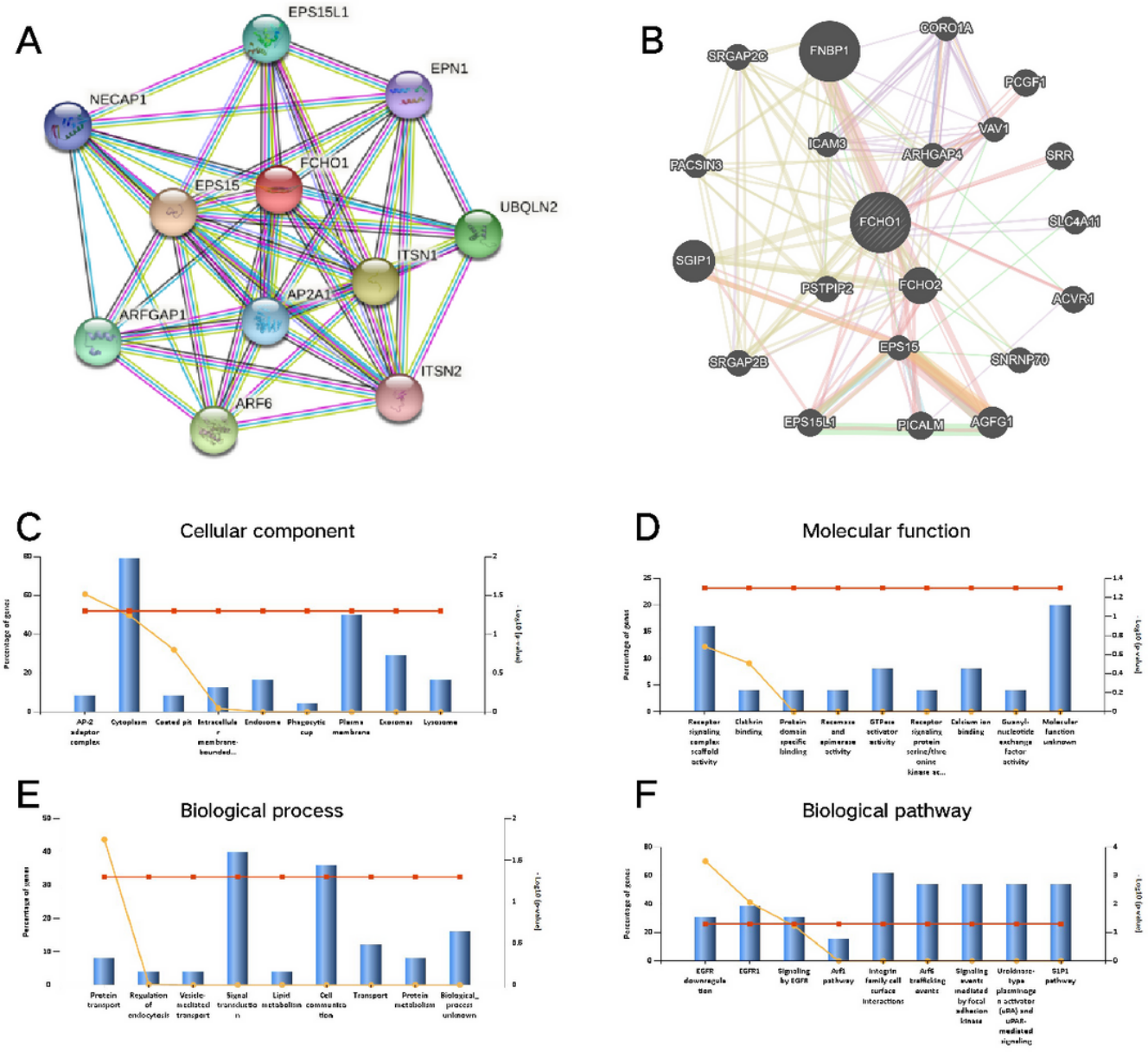

Figure 3

(A and B) The relationship between fcho1 and similar genes is in different databases; (C-F) Enrichment analysis of FCHO1 related functions includes CC, BP, MF and KEGG.

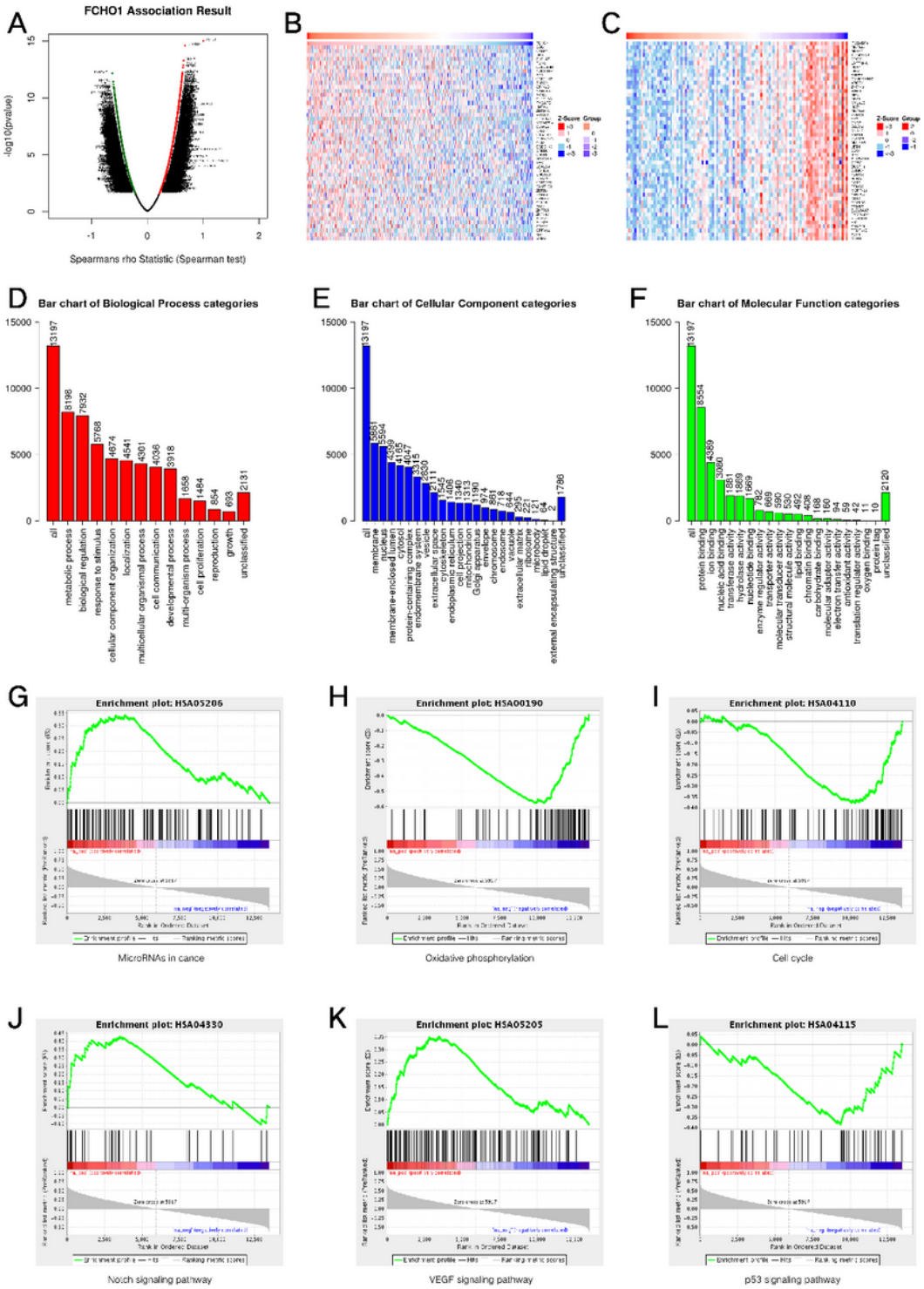

Figure 4

(A-C) FCHO1 interaction gene and heat map; (D-F) Functional enrichment analysis of fCHO1 interacting genes; (G-L) The functional analysis of FCHO1 interacting gene was obtained by GSEA analysis.

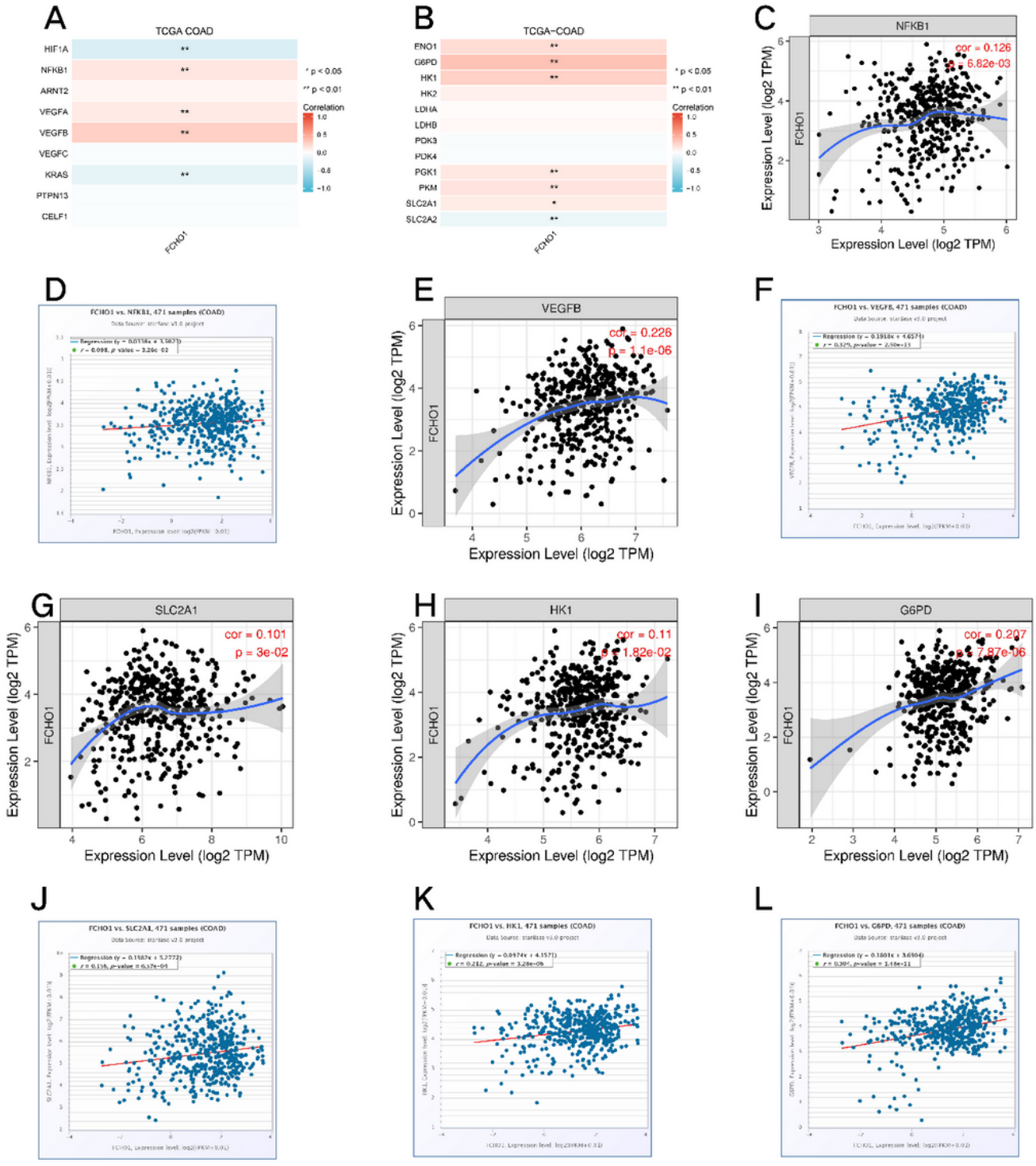

Figure 5

(A and B) Relationship between fcho1 and glycolysis-related genes; (C-L) Validation of FCHO1 and glycolysis-related genes.

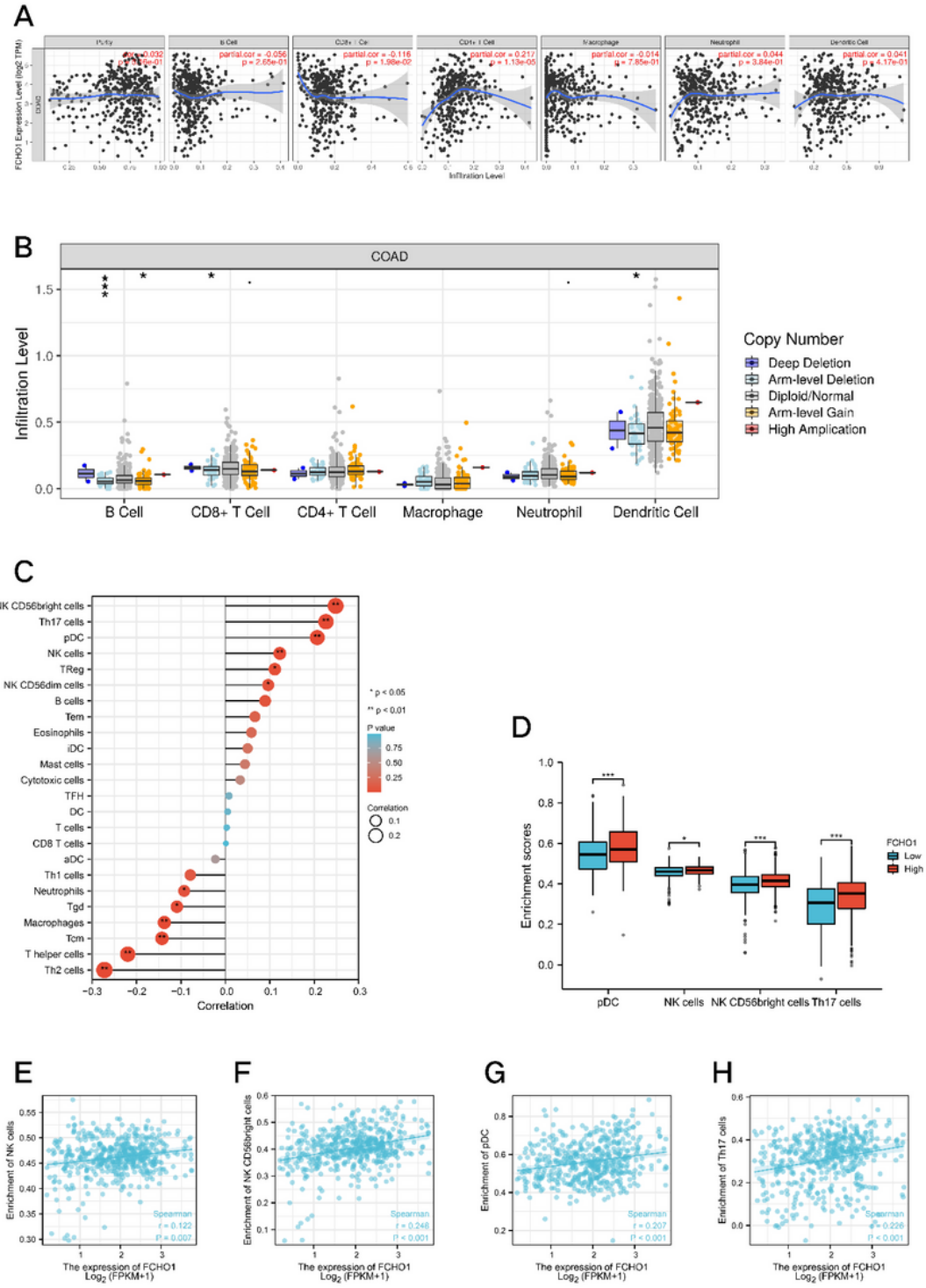

Figure 6

(A) The relationship between fcho1 and related immune cells was analyzed by TIMER; (B) The change of CNV of fcho1 in COAD was analyzed by TIMER; (C-H) The immune factors of FCHO1 in COAD were analyzed in online database.

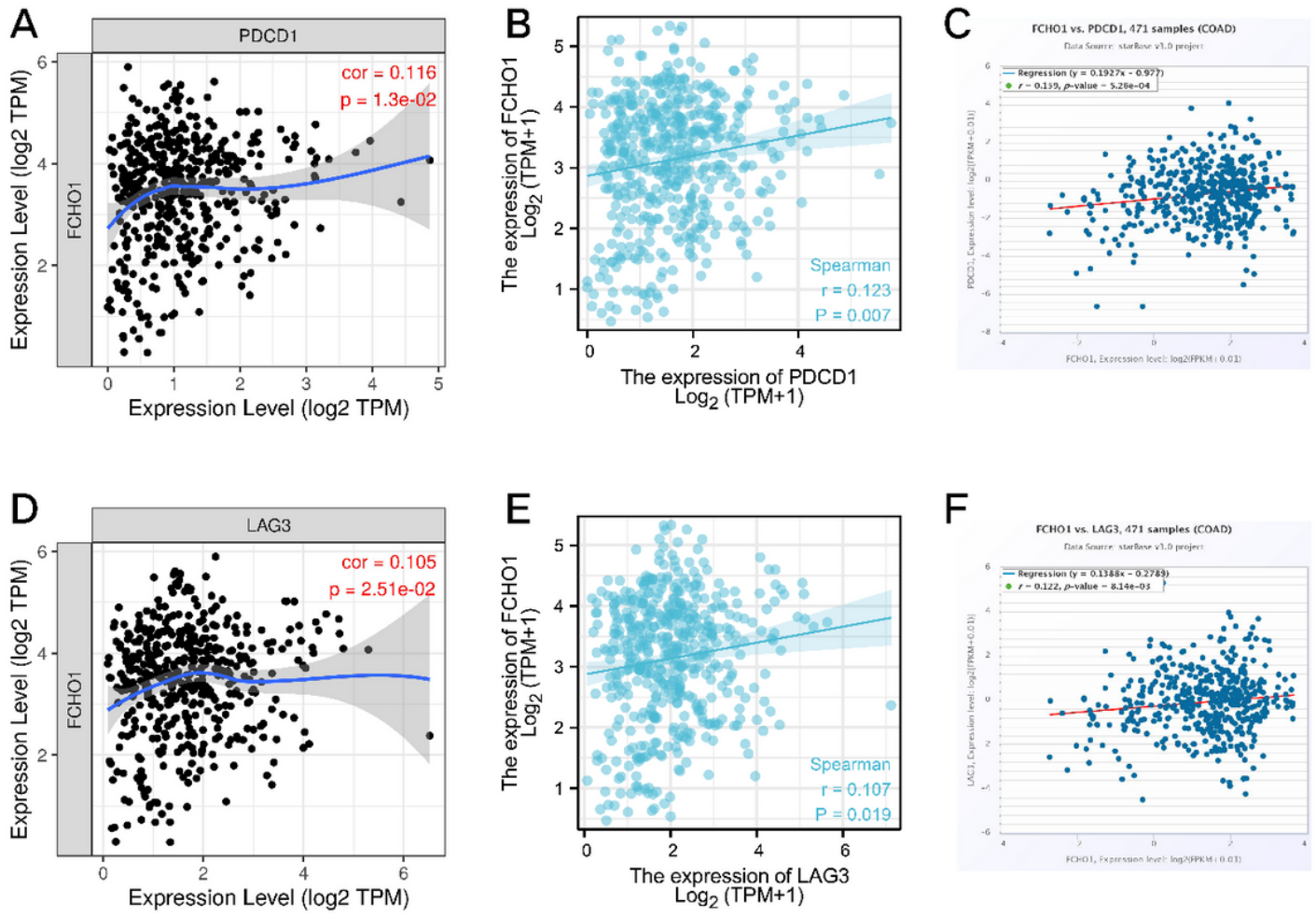

Figure 7

(A-F) Study and verification of FCHO1 and immune checkpoints in COAD by online database.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplementfigure.docx