Original Article

Low *OGDHL* expression affects the prognosis and immune infiltration of kidney renal clear cell carcinoma

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Background: Oxoglutarate dehydrogenase-like (*OGDHL*) modulates glutamine metabolism to influence tumor progression. Therefore, we aimed to explore the potential role of *OGDHL* in the prognosis of kidney renal clear cell carcinoma (KIRC) and its effect on immune infiltration.

Methods: The Cancer Genome Atlas, Tumor Immune Estimation Resource, Gene Expression Profiling Interactive Analysis, Human Protein Atlas, and The University of Alabama at Birmingham Cancer databases and the GSE53757 dataset were utilized to analyze expression difference and prognosis of *OGDHL* in tumor and normal tissue; diagnostic value was assessed using receiver operating characteristic curves. Correlations with clinical features and survival prognosis were analyzed. Independent prognostic factors were identified using univariate and multifactorial Cox regression analysis. We used the CIBERSORT analysis tool to discover the proportion of tumor-infiltrating immune cells (TIICs) in KIRC patients. Next, the differences in the proportion of TIICs under different *OGDHL* expression were analyzed. Finally, we explored the potential mechanisms by which *OGDHL* expression affects patient survival using Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and Gene Set Enrichment Analysis (GSEA).

Results: *OGDHL* expression was markedly downregulated in KIRC tissues compared to in normal tissues, and the downregulation of *OGDHL* expression was significantly associated with tumor progression (including tumor stage and grade) and poor prognosis. Cox regression analyses revealed *OGDHL* to be an independent prognostic factor for KIRC. CIBERSORT analysis showed that *OGDHL* expression is associated with differences in the proportion of several TIICs, particularly resting mast cells. Finally, GO and KEGG analysis showed that *OGDHL* was associated with extracellular matrix and epithelial cell differentiation involved in kidney development. GSEA indicated that low *OGDHL* was closely related to the activation of carcinogenic signaling pathways, including epithelial mesenchymal transition, tumor necrosis factor alpha and nuclear factor kappa B signaling pathway, negative regulation of apoptotic signaling, collagen formation, etc.

Conclusions: *OGDHL* level can be monitored for diagnosing KIRC. Reduced expression is associated with poor prognosis and immune infiltration of KIRC. *OGDHL* is expected to become a new target for the treatment of KIRC.

Keywords: Oxoglutarate dehydrogenase-like (*OGDHL*); kidney renal clear cell carcinoma (KIRC); prognosis; immune infiltration; cancer mechanism

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Introduction

Renal cell carcinoma (RCC) is a prevalent form of cancer worldwide, with approximately 430,000 new cases annually (1,2). The predominant histological subtype of RCC, kidney renal clear cell carcinoma (KIRC), accounts for approximately 70% of all cases (3). Unfortunately, KIRC lacks reliable early diagnostic indicators, and imaging techniques such as contrast-enhanced tomography or magnetic resonance imaging are required for detection (4). Furthermore, numerous newly diagnosed patients with KIRC are already in advanced stages of the disease, often presenting with distant metastases and displaying limited response to conventional chemotherapy and radiotherapy. Despite significant advances in targeted therapy such as tyrosine kinase inhibitors and mammalian target of rapamycin (mTOR) inhibitors, as well as immunotherapy with programmed cell death protein 1/programmed cell death ligand 1 and cytotoxic T-lymphocyte-associated antigen 4 inhibitors, patients with advanced and metastatic KIRC continue to face an unsatisfactory prognosis (5,6). Therefore, it is important to identify meaningful prognostic biomarkers and elucidate the molecular mechanisms underlying KIRC to identify new therapeutic targets.

The development of cancer cells is intricately linked to energy metabolism, with glutamine serving as the main carbon source after glucose during energy production and anabolism (7). Glutamine promotes mitochondrial metabolism to sustain the proliferation of cancer cells and inhibiting this metabolic process has been shown to effectively induce anticancer effects (8,9). Oxoglutarate dehydrogenase-like (*OGDHL*), a subunit of the oxoglutarate dehydrogenase complex (*OGDHC*), is a crucial component involved in glucose and glutamic acid degradation in the tricarboxylic acid cycle, and is associated with energy metabolism in cancer (10,11). Reduction in *OGDHL* expression or inhibition of *OGDHC* activity would lead to a decrease in glutamine metabolism and an increase in glutamine dependence in cancer cells, which is related to poor liver cancer prognosis (12). Liu *et al.* showed that *OGDHL* regulated the microRNA-214/TWIST1 pathway and inhibited the growth and metastasis of pancreatic cancer (13). Sen *et al.* found that *OGDHL* knockdown would activate the protein kinase B (AKT) signaling pathway to promote cervical carcinogenesis (14). Guo *et al.* reported that breast cancer morbidity was associated with rare coding variants of *OGDHL* (15). Mao *et al.* also showed that low *OGDHL* expression was associated with poor prognosis in thyroid cancer (16).

However, an in-depth exploration of the biological significance and prognostic implication of *OGDHL* in KIRC is yet to be conducted. In the present study, we compared the differential expression of *OGDHL* in normal and tumor tissues by analyzing multiple databases, evaluating its diagnostic value, analyzing the correlation between clinical features and survival prognosis, determining independent prognostic factors, and studying the role and biological function of *OGDHL* in tumor microenvironment (TME). Our ultimate objective was to elucidate the correlation between *OGDHL* expression prognosis, and immune infiltration in KIRC, with the aim of identifying novel biomarkers that can serve as targets for future therapeutic interventions. We present this article in accordance with the TRIPOD reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-961/rc).

Highlight box

Key findings

- Oxoglutarate dehydrogenase-like (*OGDHL*) can be used as an independent risk factor to predict kidney renal clear cell carcinoma (KIRC). Low *OGDHL* is associated with poor prognosis and immunity in KIRC.

What is known and what is new?

- Low *OGDHL* expression is associated with poor prognosis in cancers, including liver, pancreatic and cervical cancer, etc.
- A prognostic model of *OGDHL* in KIRC was constructed to explore the mechanisms associated with immune infiltration and carcinogenesis.

What is the implication, and what should change now?

- *OGDHL* shows promise as a new target for KIRC immunotherapy.

Methods

Data collection

The Cancer Genome Atlas (TCGA) gene expression profiles and clinical characteristics of 535 KIRC tumor and 72 normal samples were obtained from the University of California Santa Cruz Xena database (http://xena.ucsc.edu/). The information of 522 patients with KIRC was selected for further analysis by matching the gene expression data and clinical information. In addition, we incorporated data from the Gene Expression Omnibus database, specifically the GSE53757 dataset (17). This dataset encompasses tumor specimens and their matched normal tissues from a cohort of 72 patients diagnosed with KIRC. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Gene Expression Profiling Interactive Analysis (GEPIA2) and Tumor Immune Estimation Resource (TIMER)

GEPIA2 is an online gene expression analysis tool of considerable versatility, enabling insights into diverse gene expression levels across both tumor and normal tissue contexts (18). TIMER, is an extensively acknowledged biological information analysis tool renowned for its substantial functionality, garnered substantial recognition within scholarly circles (19). GEPIA2 was used exclusively to analyze the differential expression patterns of *OGDHL* in both normal and KIRC contexts, whereas TIMER primarily focused on comprehending the broader implications across various cancer types. Both tools were used to corroborate their impact on the survival prognosis of patients with KIRC. Within the foundational framework of these methodologies, adjustments were made solely pertaining to gene nomenclature and the specific cancer types of interest, while retaining the default system parameters for both tools.

Human Protein Atlas (HPA) and The University of Alabama at Birmingham Cancer (UALCAN)

The HPA serves as a discriminating tool to discern the variance in protein expression between normal and pathological tissues using immunohistochemistry (20). UALCAN provided a quantitative approach for corroborating protein expression profiles (21). We validated the expression levels of the *OGDHL* protein using two distinct websites.

Construction of prognostic model

This study used the $\log_2$[fragments per kilobase million (FPKM) +1] data format to investigate the prognostic significance of *OGDHL* expression in tumor samples. Receiver operating characteristic (ROC) curve analysis was performed to assess the diagnostic value of *OGDHL* for KIRC. Additionally, the correlation between *OGDHL* expression and clinical features was analyzed, and both univariate and multivariate Cox regression analyses were performed to determine the independent risk factors associated with the disease. The criteria for all high- and low-expression groups were defined based on the median expression level of *OGDHL* in KIRC samples.

Evaluation of immune infiltration

The CIBERSORT algorithm was used to assess the relative abundance of 22 tumor-infiltrating immune cells (TIICs) across 522 tumor samples. Specifically, the proportion of TIICs in groups with high and low *OGDHL* expression was compared. Furthermore, the proportions of TIICs in normal and tumor tissues were compared. The CIBERSORT algorithm uses the LM22 gene set (https://cibersortx.stanford.edu/) to generate a matrix of annotated gene sets that defines 22 distinct immune cell subtypes.

Gene set enrichment analysis (GSEA)

In our analysis, we included a list of genes that exhibited an expression difference of more than two-fold between normal and KIRC samples ($P<0.05$) (table available at https://cdn.amegroups.cn/static/public/tcr-23-961-1.xlsx). Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis were performed to investigate the potential mechanisms or pathways associated with *OGDHL* and KIRC, including biological processes, cell components, molecular function, and ten *OGDHL*-associated signaling pathways. In addition, the MSigDB database (https://www.gsea-msigdb.org/gsea/msigdb/index.jsp) was used to stratify the samples into two groups based on the median expression of *OGDHL*, and GSEA was used to explore the activation or inhibition of specific mechanisms or pathways.

Statistical analysis

Data processing and statistical analysis were performed

using R software v4.2.2 (http://www.r-project.org/) and the corresponding R package, GraphPad Prism 8.0.2. The present study employed the statistical *t*-test to assess potential discrepancies in *OGDHL* gene expression across distinct groups. The P value of the Kaplan-Meier survival curve was calculated using the log-rank test. Chi-squared test was used to evaluate the correlation between *OGDHL* gene expression and clinical features. Univariate and multivariate Cox regression analyses were used to identify independent prognostic factors. Statistical significance was set as P<0.05.

Results

OGDHL expression was significantly down-regulated in KIRC tissues

We first performed transcript differential expression analysis using DESeq2 and determined that compared with normal tissues, *OGDHL* was down-regulated by 2-fold in KIRC tissues (P_{adj}<0.05; *Figure 1A*). To clarify the specific reason for the difference, we extracted and analyzed the expression levels of the *OGDHL* gene in tumor and normal samples. As shown in the results, *OGDHL* expression was significantly down-regulated in tumor samples (P<0.001; *Figure 1B*). In addition, to verify the reliability of the results, we used two online databases, GEPIA2 and TIMER, from which the same results were obtained, with statistical significance (P<0.05; *Figure 1C,1D*). We used the GSE53757 dataset for investigation (17). The outcomes unequivocally demonstrated the robust validation of *OGDHL* within this dataset (P<0.001; *Figure 1E*). To investigate the disparities in the protein-level expression of *OGDHL*, immunohistochemical observations were conducted using the HPA website. The staining intensity of KIRC specimens was notably diminished compared with that of normal tissues (*Figure 1F,1G*). Similarly, the UALCAN website effectively corroborated the downregulation of *OGDHL* expression in cancerous tissues (P<0.001; *Figure 1H*).

The prognostic significance and diagnostic potential of OGDHL in KIRC

To investigate the effect of *OGDHL* on the survival of patients with KIRC, we conducted a thorough analysis of TCGA-KIRC cohort. We eliminated four duplicate cases and meticulously performed Kaplan-Meier survival analysis on 522 cancer cases that possessed *OGDHL* expression data alongside clinical information. The results indicated that patients with low *OGDHL* expression had poor overall survival (OS) (P<0.001; *Figure 2A*). To ensure that the results were reliable, we again obtained the same results through analysis of the GEPIA2 and TIMER online databases (P<0.001; *Figure 2B,2C*). These results strongly suggest that *OGDHL* is a good predictor of survival in KIRC. We plotted an ROC curve to assess the value of *OGDHL* in the diagnosis of KIRC. The analysis showed that *OGDHL* was highly sensitive in the diagnosis of KIRC and had reliable diagnostic value [area under the curve (AUC) =0.917; 95% confidence interval (CI): 0.885–0.949; *Figure 2D*].

Correlation between OGDHL expression and clinical features

We used the median *OGDHL* expression as the critical point, and 522 patients with KIRC with clinical information matching were divided into two groups (high and low *OGDHL* expression) to determine their relationship with clinicopathological features. Low *OGDHL* expression was significantly associated with the T stage (P=0.018), M stage (P<0.001), pathological stage (P=0.001), histological grade (P=0.018), and sex (P<0.001) (*Table 1*). These factors may play an important role in the prognosis of KIRC; therefore, we conducted an additional stratified analysis. The results showed that there were significant differences in *OGDHL* expression levels between different sexes (P<0.001; *Figure 3A*), which was consistent with the higher incidence of KIRC in males than in females (1). The low expression of *OGDHL* was significantly correlated with the progression of T and M stages (T1 and 2 *vs.* T3 and 4, P<0.001, *Figure 3B*; M0 *vs.* M1, *Figure 3C*), and the expression of *OGDHL* decreased significantly with the progression of tumor pathology, and the expression in stage III and IV was significantly lower than that in stage I and II (P<0.001; *Figure 3D*). Moreover, a similar trend was observed for tumor histological grade (G1 and 2 *vs.* G3 and 4, P<0.01; *Figure 3E*). These results revealed a significant correlation between low *OGDHL* expression and poor prognosis in patients with KIRC.

Prognostic value of OGDHL in KIRC

Previously, we found that patients with different *OGDHL* expression levels have significantly different OS according to the Kaplan-Meier survival analysis. Further investigations

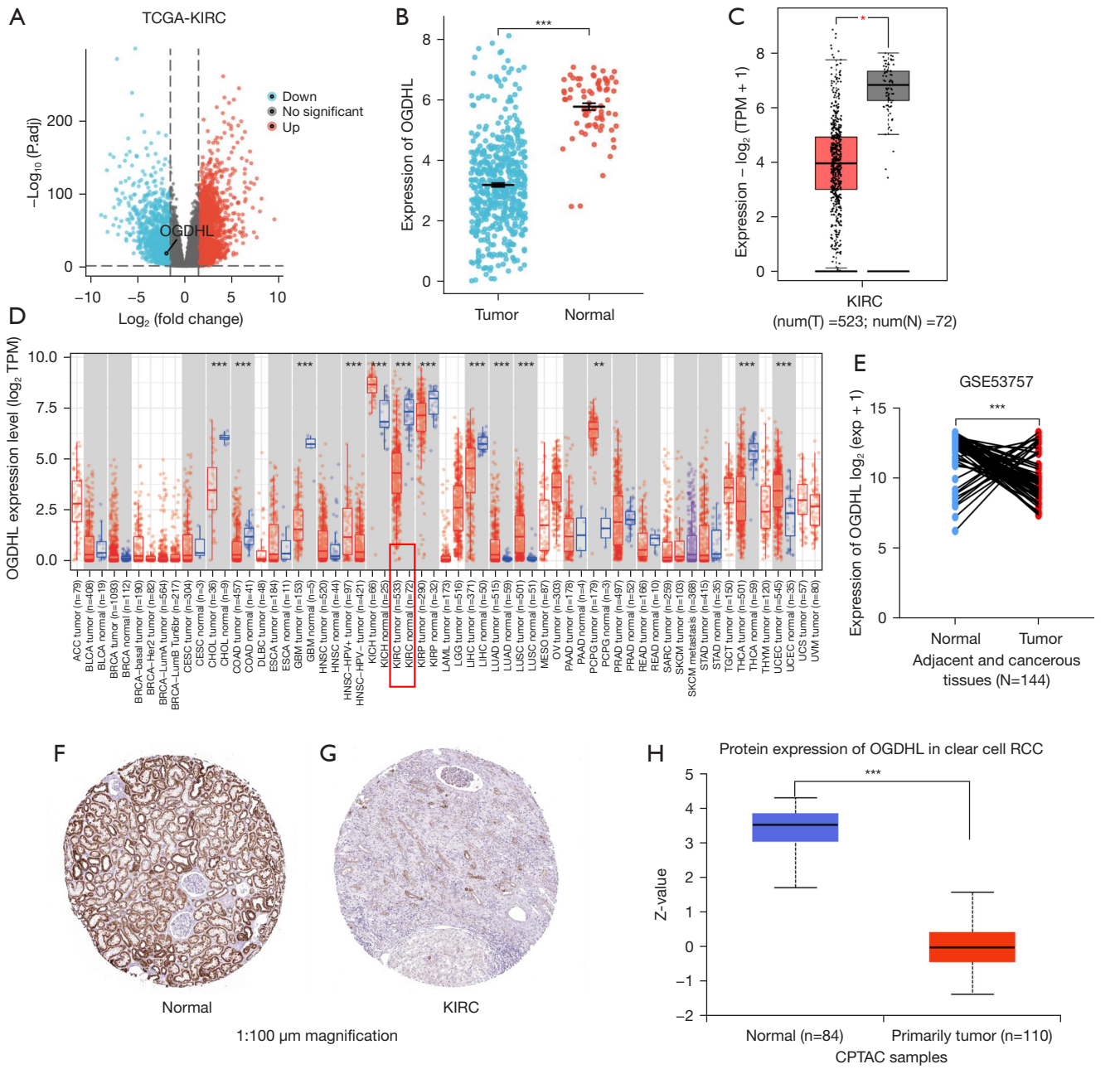

Figure 1 *OGDHL* expression was significantly lower in renal clear cell carcinoma tissues than in normal tissues. (A) *OGDHL* expression was downregulated more than 2-fold in the TCGA-KIRC cohort. (B) Data from 526 tumor and 72 normal samples from the TCGA-KIRC cohort. (C) Data from the GEPIA2 database. (D) *OGDHL* expression data from the TIMER database for pan-cancer analysis. (E) *OGDHL* expression data from the GSE53757 dataset. (F,G) Protein expression of *OGDHL* in immunohistochemistry from HPA. (H) Protein expression of *OGDHL* data from UALCAN. *, P<0.05; **, P<0.01; ***, P<0.001. URL of (F): https://www.proteinatlas.org/ENSG00000197444-OGDHL/tissue/kidney; (G): https://www.proteinatlas.org/ENSG00000197444-OGDHL/pathology/renal+cancer. TCGA-KIRC, The Cancer Genome Atlas-kidney renal clear cell carcinoma; *OGDHL*, oxoglutarate dehydrogenase-like; TPM, transcripts per kilobase million; RCC, renal cell carcinoma; CPTAC, Clinical Proteomic Tumor Analysis Consortium; GEPIA2, Gene Expression Profiling Interactive Analysis; TIMER, Tumor Immune Estimation Resource; HPA, Human Protein Atlas; UALCAN, The University of Alabama at Birmingham Cancer.

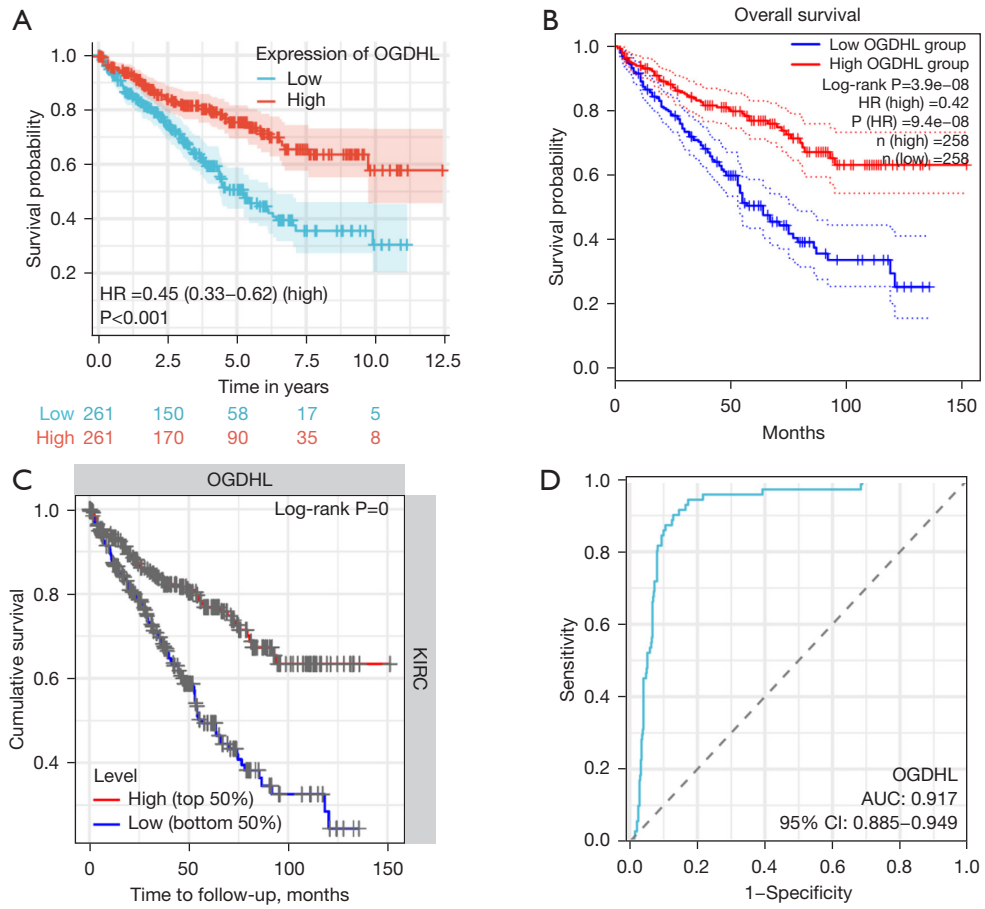

Figure 2 Relationship between *OGDHL* expression and clinical characteristics. (A) *OGDHL* downregulation was significantly associated with OS in the TCGA-KIRC cohort. (B) Validation of the relationship between *OGDHL* expression and survival information from the GEPIA2 database. (C) Validation of the relationship between *OGDHL* expression and survival information from the TIMER database. (D) Value of *OGDHL* for diagnosing KIRC with AUC =0.917. *OGDHL*, oxoglutarate dehydrogenase-like; OS, overall survival; TCGA, The Cancer Genome Atlas; KIRC, kidney renal clear cell carcinoma; HR, hazard ratio; AUC, area under the curve; CI, confidence interval; GEPIA2, Gene Expression Profiling Interactive Analysis; TIMER, Tumor Immune Estimation Resource.

Table 1 Correlation of OGDHL expression with clinical features of KIRC

Characteristic	Total	High (n=261), n (%)	Low (n=261), n (%)	P
Age	522			0.661
<60 years		124 (47.5)	118 (45.2)	
≥60 years		137 (52.5)	143 (54.8)	
Gender	522			<0.001
Female		122 (46.7)	59 (22.6)	
Male		139 (53.3)	202 (77.4)	
T stage	522			0.018
T1 & 2		180 (69.0)	153 (58.6)	
T3 & 4		81 (31.0)	108 (41.4)	

Table 1 (*continued*)

Table 1 (*continued*)

Characteristic	Total	High (n=261), n (%)	Low (n=261), n (%)	P
N stage	254			0.256
N0		132 (95.7)	106 (91.4)	
N1		6 (4.3)	10 (8.6)	
M stage	492			<0.001
M0		218 (90.1)	196 (78.4)	
M1		24 (9.9)	54 (21.6)	
Pathological stage	519			0.001
Stage I & II		176 (68.0)	139 (53.5)	
Stage III & IV		83 (32.0)	121 (46.5)	
Histological grade	514			0.018
G1 & 2		131 (51.4)	105 (40.5)	
G3 & 4		124 (48.6)	154 (59.5)	

OGDHL, oxoglutarate dehydrogenase-like; KIRC, kidney renal clear cell carcinoma.

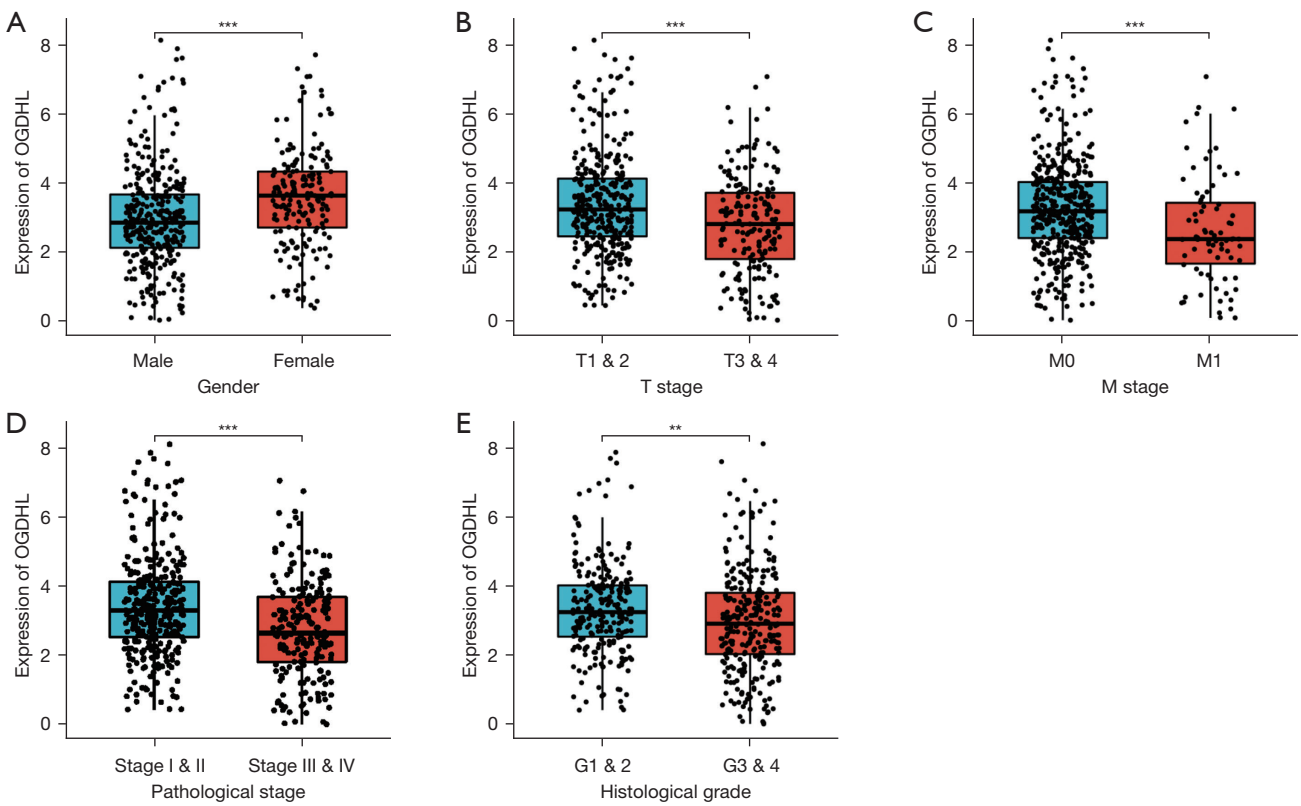

Figure 3 Relationship between *OGDHL* expression and clinical characteristics. (A) *OGDHL* expression levels were significantly lower in men than in women with KIRC. (B-E) Increased T-stage, distant metastasis, pathological stage, and histological grade resulted in a significant decrease in *OGDHL* expression levels. *OGDHL*, oxoglutarate dehydrogenase-like. **, P<0.01; ***, P<0.001. OGDHL, oxoglutarate dehydrogenase-like; KIRC, kidney renal clear cell carcinoma.

Table 2 Univariate and multivariate Cox regression analysis of KIRC prognostic factors

Characteristics	Total (N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Age	522				
≥60 years	280	Reference			
<60 years	242	0.563 (0.411–0.772)	<0.001	0.696 (0.504–0.961)	0.027
Gender	522				
Male	341	Reference			
Female	181	1.054 (0.772–1.440)	0.740	1.375 (0.976–1.938)	0.069
Pathological stage	519				
Stage I & II	315	Reference			
Stage III & IV	204	3.927 (2.847–5.417)	<0.001	2.791 (1.987–3.922)	<0.001
Histological stage	514				
G1 & 2	236	Reference			
G3 & 4	278	2.679 (1.895–3.787)	<0.001	1.948 (1.351–2.809)	<0.001
OGDHL	522				
High	261	Reference			
Low	261	2.198 (1.605–3.009)	<0.001	2.127 (1.511–2.994)	<0.001

KIRC, kidney renal clear cell carcinoma; CI, confidence interval; *OGDHL*, oxoglutarate dehydrogenase-like.

are warranted to gain a deeper understanding of the factors associated with the prognosis of KIRC. Univariate and multivariate Cox regression analyses were performed on important clinical features (sex, age, pathological stage, and histological grade) and *OGDHL* expression levels (*Table 2*). Patients <60 years of age had a significantly lower risk of death than patients ≥60 years of age [hazard ratio (HR) =0.696, 95% CI: 0.504–0.961, P=0.027]. The progression of pathological stage, histological grade and low OGDHL expression were independently associated with OS, and the risk of death was significantly increased (HR =2.791, 95% CI: 1.987–3.922, P<0.001; HR =1.948, 95% CI: 1.351–2.809, P<0.001; HR =2.127, 95% CI: 1.511–2.994, P<0.001). Sex did not significantly affect the prognosis, but the difference was no statistical significance. These results indicate that age, pathological stage, histological grade and *OGDHL* expression are independent prognostic factors for KIRC.

Subsequently, we stratified these prognostic factors. The results showed that when age ≥60 years (P=0.001), age <60 years (P<0.001), patients were male (P<0.001), G3 and 4 (P<0.001), the OS of patients with low *OGDHL* expression was significantly lower than high *OGDHL* expression, and no further statistical significant differences were observed (*Figure 4A-4D*).

Correlation analysis between OGDHL expression and tumor infiltrating immune cells

The degree of immune cell infiltration in tumor tissues is closely related to patient prognosis. Díaz-Montero *et al.* have shown that KIRC possesses a high degree of immunogenicity, which leads to immune dysfunction by inducing immunosuppressive cell infiltration (22). However, the relationship between *OGDHL* expression and tumor infiltrating immune cells in KIRC is not yet fully understood. We first analyzed the proportion of 22 TIICs from TCGA-KIRC samples using the CIBERSORT algorithm. The results showed a difference in the proportion of TIICs in each sample (*Figure 5A*). Subsequently, differences in the proportion of TIICs types between the two groups (high and low *OGDHL* expression) were further analyzed. The results showed significant differences in the proportions of the eight TIICs types (*Figure 5B*). Plasma

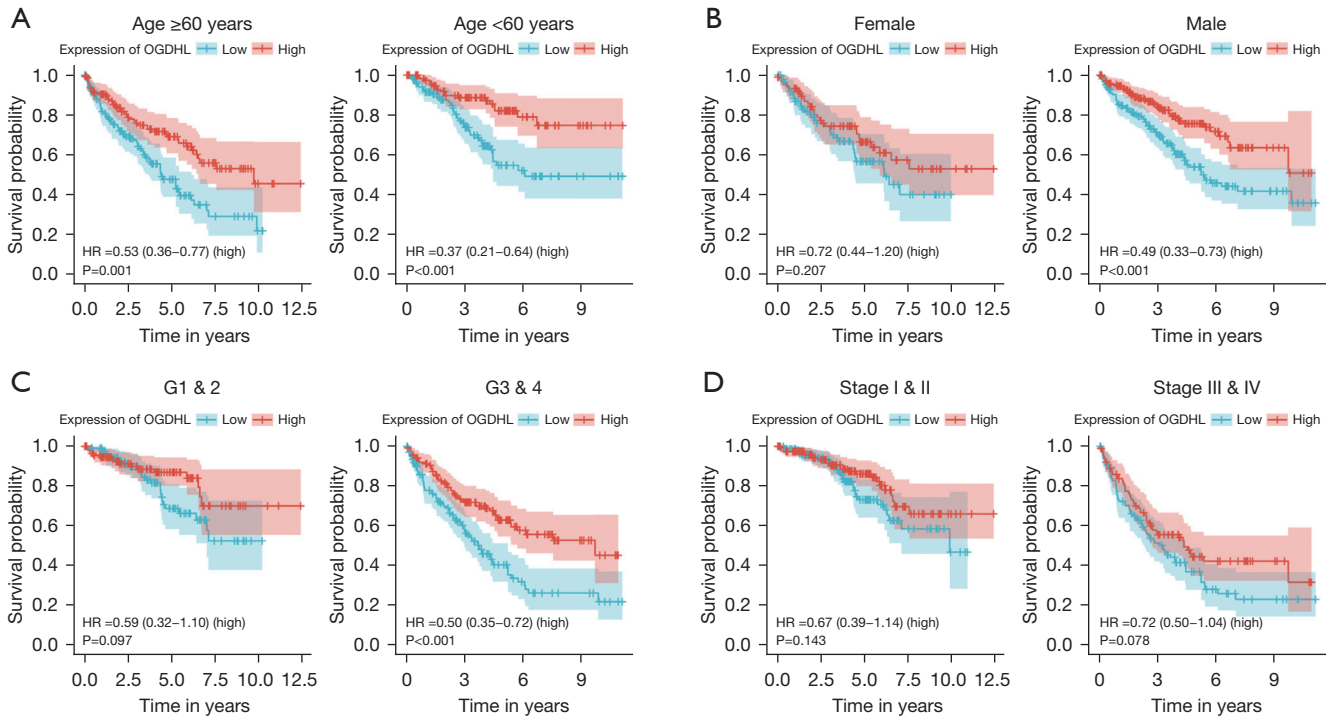

Figure 4 Prognostic value of *OGDHL* expression in patients with KIRC. (A-D) Stratified survival analysis to determine the prognostic value of *OGDHL* for OS based on age, sex, histological grade, and pathological stage. *OGDHL*, oxoglutarate dehydrogenase-like; KIRC, kidney renal clear cell carcinoma; OS, overall survival; HR, hazard ratio.

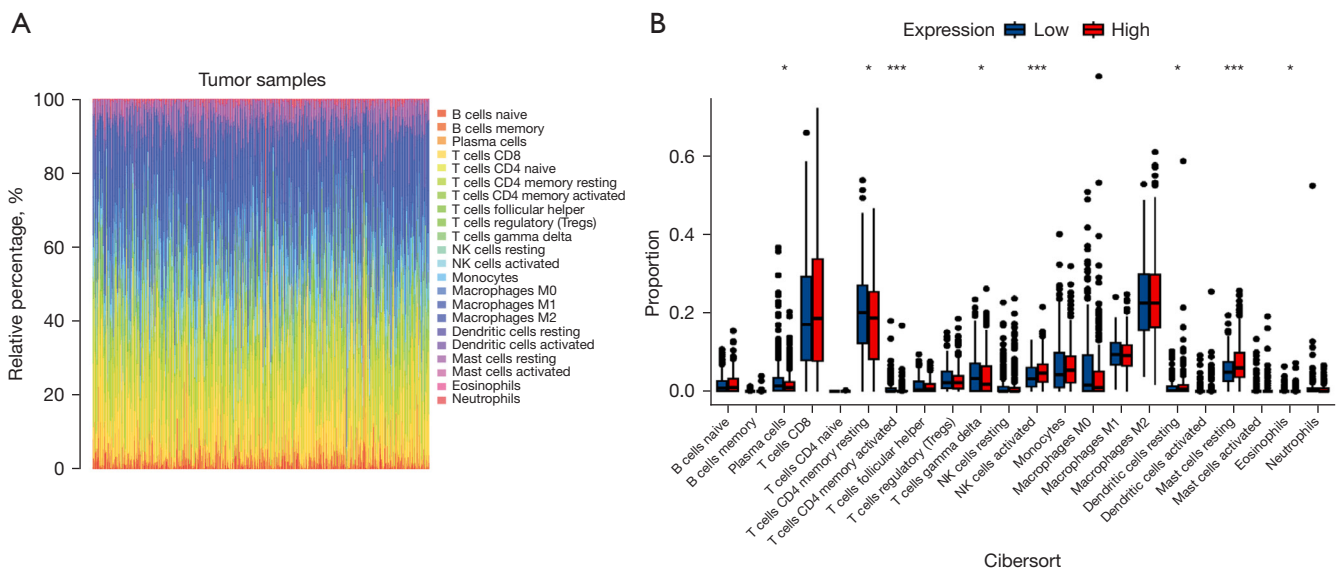

Figure 5 Proportion of TIICs in tumor samples and analysis of differences. (A) The proportion of tumor-infiltrating immune cells from the TCGA-KIRC cohort. (B) Proportion of tumor-infiltrating immune cells at different expression levels of *OGDHL* in KIRC cells. *, P<0.05; ***, P<0.001. TIICs, tumor-infiltrating immune cells; TCGA, The Cancer Genome Atlas; KIRC, kidney renal clear cell carcinoma; *OGDHL*, oxoglutarate dehydrogenase-like.

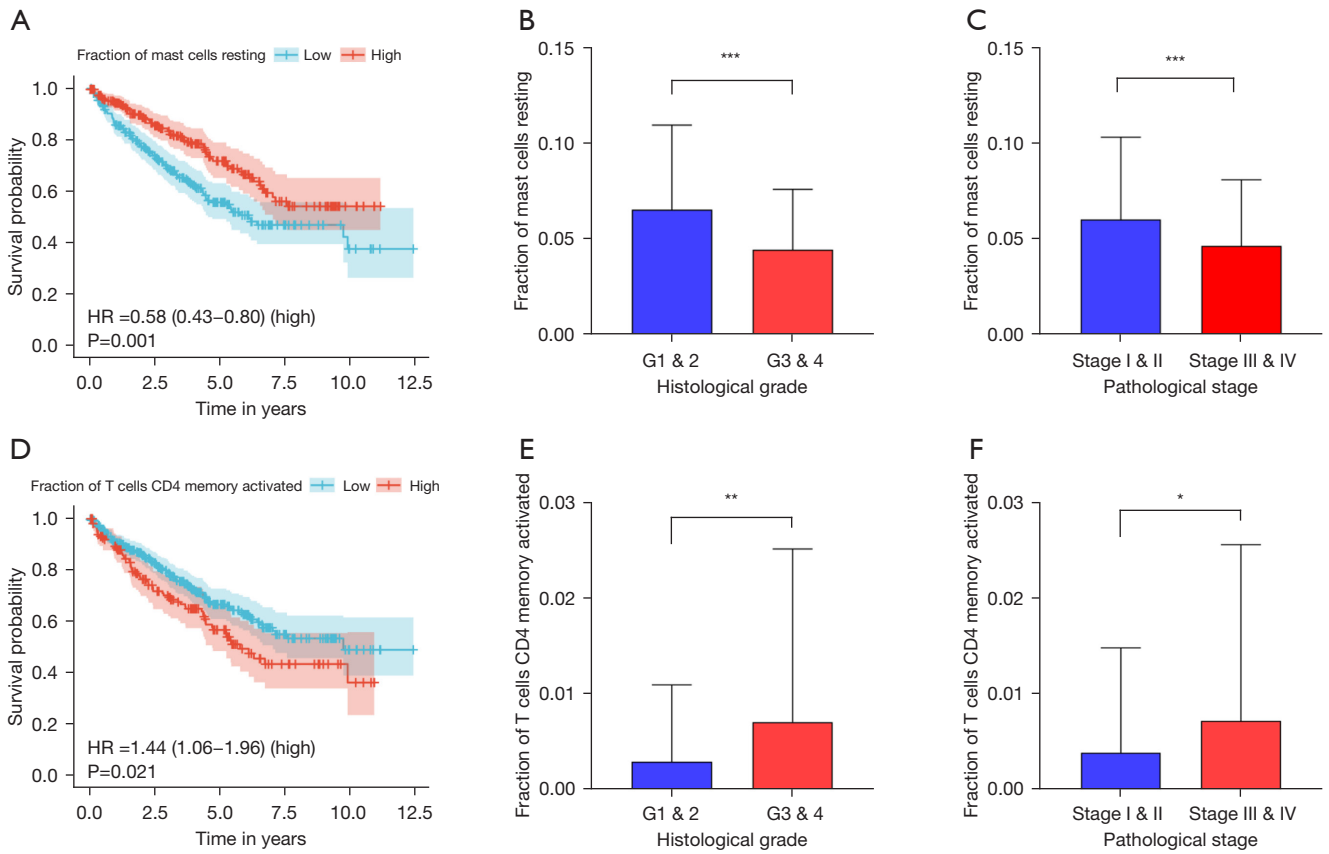

Figure 6 Prognostic value of TIICs in KIRC. (A) The greater the fraction of resting mast cells, the better the OS rate. (B,C) The fraction of resting mast cells correlated significantly with histological grade and pathological stage. (D) The greater the fraction of T cells CD4 memory activated, the lower the OS rate. (E,F) The fraction of T cells CD4 memory activated correlated significantly with histological grade and pathological stage. *, P<0.05; **, P<0.01; ***, P<0.001. TIICs, tumor-infiltrating immune cells; KIRC, kidney renal clear cell carcinoma; OS, overall survival; HR, hazard ratio.

cells (P<0.05), T cells gamma delta (P<0.05), $CD4^+$ memory resting T cells (P<0.05), natural killer (NK) cells activated (P<0.05), $CD4^+$ activated cells (P<0.001), resting mast cells (P<0.001), and eosinophils (P<0.05). Therefore, the identification of dissimilar proportions of TIICs alone does not provide a comprehensive explanation for the prognostic implications in KIRC, and thus necessitates further stratified investigations.

Prognostic value of TIICs in KIRC

In the above results (*Figure 5B*), we identified several TIICs with statistically significant differences in proportion and reviewed the relevant literature using PubMed. Finally, we selected two types of TIICs, resting mast cells and T cells CD4 memory activated, to focus on the prognostic value of KIRC (23,24). The results indicated that lower levels of resting mast cells were significantly associated with worse OS (P=0.001, *Figure 6A*), histological grade (P<0.001, *Figure 6B*) and pathological stage (P<0.001, *Figure 6C*) deterioration, whereas T cells CD4 memory activated showed the opposite (P=0.021, *Figure 6D*; P<0.01, *Figure 6E*; P<0.05, *Figure 6F*).

OGDHL functional enrichment analysis

To further explore the function of *OGDHL*, GO and KEGG functional enrichment analysis were performed using the RStudio. GO functional enrichment analysis showed that *OGDHL* may be significantly related to peptidase activity, transmembrane transport, signal transduction, extracellular matrix, kidney formation, and epithelial cell differentiation

involved in kidney development (*Figure* 7*A*). KEGG analysis indicated that substance metabolism, signal transduction, interleukin (IL)-17 and peroxisome proliferator-activated receptor (PPAR) signaling pathways were significantly enriched (*Figure* 7*B*). Next, we performed GSEA between the high and low *OGDHL* expression groups using multiple gene sets in the MSigDB database to better define the pathways of activation or repression. The results showed that multiple signaling pathways were significantly activated in the low *OGDHL* expression group, including epithelial-mesenchymal transition (EMT), tumor necrosis factor alpha (TNFα)/nuclear factor kappa B (NF-κB) signaling pathway, negative regulation of apoptotic signaling pathway, collagen formation, chemokine response and *HDAC3* targets up. However, immune system development was inhibited (*Figure* 7*C*-7*E*).

Discussion

KIRC is a malignant neoplasm that, poses a significant threat to the human health. Despite remarkable progress in the study of KIRC treatment, patient prognosis remains suboptimal (6). Therefore, there is an imperative need to identify novel biomarkers that can serve as therapeutic targets in KIRC. However, the role of *OGDHL* in KIRC remained unclear.

In our study, we used TCGA, GEPIA2 and TIMER databases to verify that *OGDHL* was significantly down-regulated in KIRC. Consistent with this observation, other findings were obtained from the GSE53757 dataset (17). Subsequent validation using HPA and UALCAN revealed a marked reduction in the protein expression of *OGDHL* in neoplastic tissue. Finally, through survival analysis, diminished expression of *OGDHL* was substantiated as a harbinger of adverse OS outcomes. These findings provide a preliminary glimpse into the potential of *OGDHL* to function as a putative tumor suppressor gene, exerting its influence on the pathogenesis of KIRC. Other studies, have confirmed the inhibitory effect of *OGDHL* in other cancers, including liver, pancreatic, cervical, breast, and thyroid cancer (12-16). To assist in the diagnosis, the diagnostic value of *OGDHL* was assessed using ROC curves, and the results were promising, with *OGDHL* performing sensitively for the diagnosis of KIRC (AUC =0.917). Next, we analyzed the correlation between *OGDHL* and the clinical characteristics of patients with KIRC. The results showed that the expression level of *OGDHL* was negatively correlated with pathological features that could reflect the deterioration process (e.g., pathological stage and histological grade). More importantly, we found from univariate and multifactorial Cox analyses that *OGDHL* expression levels were an independent prognostic predictor of OS, which was confirmed in a stratified analysis based on age, sex and histological grade. However, there was no significant difference in survival between patients with stage I and II and stage III and IV, which could possibly be attributed to the relatively lower degree of malignancy of tumors in the stage I and II groups, whereas in the stage III and IV groups, tumors exhibited a high degree of invasiveness. Despite these unsatisfactory results, low expression of *OGDHL* generally indicates, a poor prognosis for patients with KIRC and is a prognostic biomarker worthy of recognition.

Malignant tumor development is intricately intertwined with metabolic reprogramming, wherein glutamine is a primary carbon source for cancer cell energy metabolism (7). Presently, RCC is widely acknowledged as a metabolic disorder, with alterations in energy metabolism pathways implicated in adverse prognostic outcomes, including increased cancer cells uptake of glutamine (25). Furthermore, studies have reported that glutathione is the chief product of the reprogrammed metabolism of glutamine (26,27). Lucarelli *et al.* elucidated that within KIRC tissue, nearly all amino acids, except save for glutamine and glutamic acid, exhibit substantial reduction, potentially linked to an increase in glutathione. Glutathione confers an advantage to cancer cells in the oxidative stress-laden TME, thus underscoring the potential of targeting intermediates in glutamine metabolism, such as α-ketoglutarate, as a latent therapeutic strategy (28). Previous studies corroborated the pivotal role of *OGDHL* in glutamine degradation. Diminished *OGDHL* expression orchestrates a heightened α-ketoglutarate/citrate ratio, thereby mediating the reductive carboxylation of glutamine, reinforcing the cancer cells antioxidative machinery, and in turn, fostering proliferation and survival (12). Collectively, these findings suggest the prospective utility of *OGDHL* as a prognostic biomarker and a potential therapeutic target in KIRC.

Cancer development is closely related to the TME. TIICs play a crucial role in KIRC (22). We used CIBERSORT to analyze the immune infiltration results of KIRC and found that the expression of *OGDHL* was positively correlated with the proportion of resting mast cells, whereas the opposite was true for the activation of T cells CD4$^+$ memory was the opposite. Dudeck *et al.*

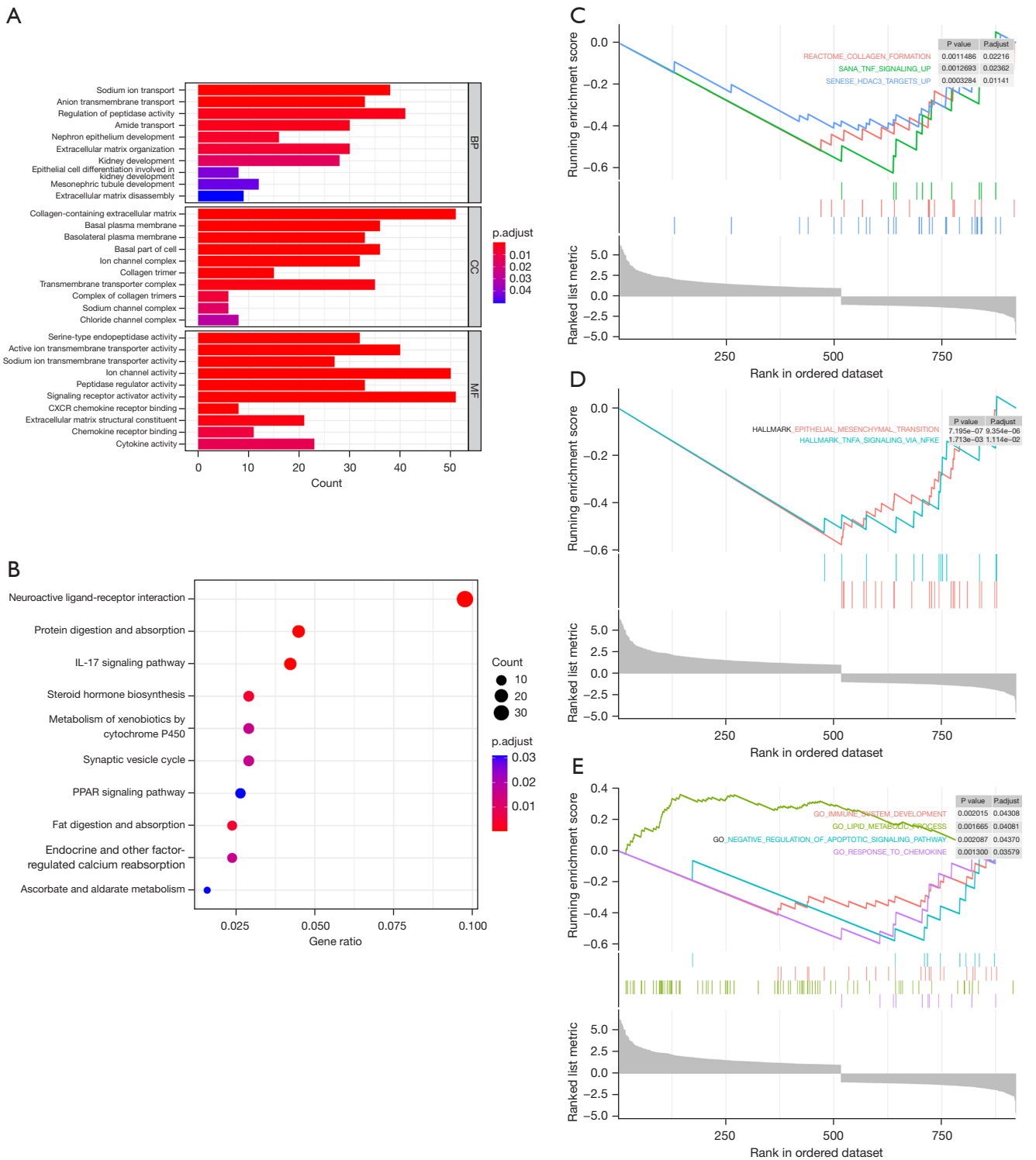

Figure 7 *OGDHL* functional enrichment analysis. (A) GO analysis showing BP, CC, and MF associated with *OGDHL*. (B) KEGG analysis showing ten signaling pathways associated with *OGDHL*. (C) GSEA analysis using curated gene sets gene sets. (D) GSEA analysis using hallmark gene sets. (E) GSEA analysis using ontology gene sets. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; GSEA, Gene set enrichment analysis; *OGDHL*, oxoglutarate dehydrogenase-like; BP, biological processes; CC, cellular component; MF, molecular function.

reported that mast cells are an important part of the immune sentinel, that can enhance T cell-mediated immune response and exert anti-tumor effects (29). It has been reported that mast cells can also inhibit cancer by mediating the expression of anti-tumor heparin, histamine, IL-6 and TNFα (30). Our study verified this finding, which provides a direction for future treatment strategies for KIRC (*Figure 6A-6C*). The anti-tumor effect of $CD4^+$ memory T cells have been widely reported (31). However, our results were not consistent with this finding, although, the opposite was true (*Figure 6D-6F*). Feuerer *et al.* also reported that the proportion of $CD4^+$ memory T cells in the bone marrow of patients with breast cancer was notably higher than that in healthy donors, and the increase in memory T cells was related to the size of the primary tumor (32). This may be interpreted as an attempt by the immune system to attack the growing number of cancer cells in patients. However, there is still a lack of sufficient evidence, and it is worth considering the causes and underlying mechanisms. Tumor angiogenesis exhibits a conspicuous nexus with the intricate milieu of the TME (33). Angiogenesis is the principal hallmark of KIRC and plays a central role in various stages of progression. Anti-angiogenic therapy has evolved into an indispensable approach for addressing KIRC (34). Reportedly, approximately 92% of patients with KIRC exhibit somatic mutations in the Von Hippel-Lindau (VHL) gene. This process activates of numerous biological pathways, including pro-angiogenic factors (VEGF and PDGF) and genes involved in metabolism, thereby influencing the TME. Existing research has substantiated a notable elevation in the KIRC utilization of glutamine. Moreover, glutamine deprivation therapy has the potential to emerge as a novel treatment for individuals with VHL-deficient RCC (28,35). The orchestration of metabolic regulatory strategies has also been reported to engender transformative alterations in the TME. Inquisitors have marshaled glutaminase inhibitors that obstruct the synthesis of metabolites and abrogate cellular proliferation (36). As a pivotal rate-limiting enzyme in glutamine metabolism, *OGDHL* may influence the TME patterns by inhibiting angiogenesis or modulating glutamine metabolism, thereby affecting KIRC progression. Therefore, further investigations are necessary.

However, the potential function of *OGDHL* in KIRC has not been reported. We used the TCGA-KIRC database for GO and KEGG analyses of *OGDHL*-co-expressed genes and elucidated several mechanisms related to *OGDHL*, including extracellular matrix formation, signal transduction, kidney formation and epithelial cell differentiation, which are involved in kidney development (*Figure 7A*,*7B*). As a matter of common knowledge, KIRC is a malignant tumor originating from renal tubular epithelium (3). Notably, GO analysis showed that *OGDHL* was associated with epithelial cell differentiation, which is involved in kidney development (*Figure 7A*), indicating that *OGDHL* is closely related to the occurrence of KIRC.

We also performed GSEA to confirm whether signaling pathways were activated or inhibited by *OGDHL* in KIRC. The results showed that several important signaling pathways were significantly activated in the *OGDHL* low expression group, such as EMT, TNFα/NF-κB signaling pathway, negative regulation of apoptotic signaling pathway, collagen formation, *HDAC3* targets up, and chemokine response signaling pathway activation (*Figure 7C-7E*). In the field of pathology, it has been observed that KIRC cells have an origin in renal tubular epithelial cells, and the occurrence, invasion, metastasis, and therapeutic resistance of malignancies are associated with the phenomenon of EMT (3,37). Chemokines significantly influence the TME, play a dual role in regulating the development and progression of cancer by mediating the transportation of immune cells to the TME and affecting the proliferation, metastasis, and invasion of tumor cells by targeting non-immune cells within the TME, including tumor cells and vascular endothelial cells (38). In tumors, the extracellular matrix (ECM) specific to the tumor is known to replace the normal ECM and exhibit greater collagen density and rigidity. Collagen, a major component of the ECM, has been shown to not only promote cancer cells growth and migration but also affect immune cell function within the TME (39). The regulation of apoptosis is an important way for the human body to eliminate cancer cells. However, tumor cells resist apoptotic signals or evolve various mechanisms to evade apoptosis, eventually leading to treatment resistance and recurrence (40). Our results showed that low *OGDHL* expression activated the negative regulation of apoptotic signals, which was associated with a poor prognosis in KIRC. In addition, we found that low *OGDHL* expression up-regulated of HDAC3 expression. Histone deacetylases (HDACs) play a crucial role in regulating cellular functions such as cell survival and proliferation through their ability to deacetylate histones. Aberrant expression of *HDACs* has been observed in various types of cancers. Wei *et al.* reported that *HDAC3* was highly expressed in cancer cells and significantly promoted breast cancer (41). Zhang *et al.* showed that

targeting *HDAC3* could inhibit glutamine uptake by cancer cells, thereby promoting tumor regression (42). Another study demonstrated the feasibility of achieving the same goal by targeting the glutamine transporter protein *SLC1A5* (43). As mentioned earlier, cancer cells growth is highly dependent on glutamine, and the downregulation of *OGDHL* reduces glutamine metabolism by inhibiting *OGDHC* activity while promoting increased dependence on glutamine by cancer cells, possibly through the involvement of *HDAC3* or the glutamine transporter protein *SLC1A5* (7,12,43). However, this hypothesis remains speculative and requires substantiation through experimental investigations. Our results also found that the activation of TNFα/NF-κB signaling pathway was associated with low *OGDHL* expression. TNFα/NF-κB as a classical pathway, its role in cancer has been widely and deeply studied, and it is not discussed here (44,45).

Although the present study highlights the potential impact of *OGDHL* on the prognosis of KIRC, several limitations must be acknowledged. Firstly, the findings are primarily derived from TCGA database; therefore, external validation is required. Second, the mechanism underlying the higher proportion of $CD4^+$ memory T cells in KIRC tumor tissues than in normal tissues remains unclear and requires further investigation. Additionally, the specific effects of *OGDHL* on *HDAC3* and the glutamine transporter *SLC1A5* should be explored. Importantly, the influence of *OGDHL* on tumor angiogenesis or the modulation of glutamine metabolism, thereby inducing alterations in specific mechanisms of the TME, is a research avenue requiring in-depth exploration. Further fundamental research is required to elucidate the precise role of *OGDHL* in renal clear cell carcinoma.

Conclusions

Our study shows that *OGDHL* expression is significant in the diagnosis of KIRC and that reduced *OGDHL* expression is related to poor prognosis and immune infiltration in patients with KIRC. *OGDHL* is a novel biomarker of KIRC and is expected to become a therapeutic target in the future.

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Footnote

Reporting Checklist: The authors have completed the TRIPOD reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-961/rc

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Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-961/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. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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