TAGLN2 promotes the proliferation, migration, invasion and EMT of renal clear cell carcinoma through PI3K/Akt signaling pathway

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Abstract

The effect of TAGLN2 in clear cell renal cell carcinoma (ccRCC) is till unknown. This paper explored its potential role and mechanism in ccRCC. The expression of TAGLN2 in Pan-cancers was analyzed through the Genotype-Tissue Expression (GTEx) database and The Cancer Genome Atlas (TCGA) database. TCGA-KIRC database were used to analyze the subsequent prognostic survival, pathway enrichment and immune infiltration. Relevant experimental methods could explain the effect of TAGLN2 expression on tumor cell proliferation, migration, invasion, and apoptosis. Apoptosis, proliferation, EMT, and PI3K/AKT signaling pathway related proteins expression can be realized through Western Blot. In TCGA+GTEx database, mRNA-TAGLN2 expression was obviously increased in pan-cancer tissues, and the same result was found in ccRCC patients, based on KIRC analysis results. In addition, TAGLN2 was related to poor clinical stage, pathological grade, and survival prognosis. In vitro, TAGLN2 can be highly expressed in ccRCC tissues and cells. TAGLN2 silencing could inhibit the proliferation, migration, invasion, and EMT in ccRCC cancer cells. Furthermore, TAGLN2 related differential genes enriched in PI3K/AKT signaling pathway were negatively regulated after TAGLN2 silencing. Moreover, TAGLN2 may promote tumor immune escape and increase the risk of distant metastasis in immune infiltration-related analysis. TAGLN2 can be used as a single indicator to explain the survival probability of ccRCC patients. In vitro, TAGLN2 silencing inhibited malignant biological properties of ccRCC by blocking PI3K/AKT signaling pathway. In addition, TAGLN2 contributes to the development of tumor immune escape and may become the potential therapeutic target of ccRCC.

Introduction

The incidence rate of renal cell carcinoma (RCC) continues to rise by 2–4% every year, which is the third most common cancer among the malignant tumors of the urinary system (Capitanio U et al. 2019; Sheng X et al. 2020). Among all RCCs, ccRCC was the main pathological subtype (Hoefflin R et al. 2020). Surgical resection is the most effective treatment strategy of ccRCC. Unfortunately, for some patients, the effect of postoperative chemoradiotherapy on high-grade ccRCC is unsatisfactory. In addition, recurrence or distant metastasis occurs in 20% of patients after surgical treatment (Capitanio, U et al. 2016). Therefore, molecular targeted therapy has become an important complementary treatment measure for ccRCC.

Molecular targeted therapy brings hope to cancer patients. Finding effective biomarkers can provide early clinical diagnosis, precise treatment targets, and prognostic monitoring for cancer patients. Based on the characteristics of drug resistance, relapse, and distant metastasis of ccRCC, much effort has been made to find more molecular therapeutic targets.

In 1998, TAGLN2 was discovered as an actin stress fiber-associated protein with a size of 22 kDa. The main function of TAGLN2 is to bind actin and participate in the reconstruction and regulation of cytoskeleton structure (Suozzi K C et al. 2012; Malarkannan S et al. 2012), which may promote the malignant progress of tumor cells. It is reported that cancer cells express TAGLN2 at a higher level than
normal healthy cells (Dvorakova M et al. 2014; Zhao Z et al. 2021). TAGLN2 knockdown can not only effectively inhibit the proliferation, migration and invasion of bladder cancer (YOSHINO et al. 2011), but also inhibit the proliferation and invasion of colorectal cancer and the invasion of hepatocellular cancer (Shi J et al. 2020). High expression of TAGLN2 leads to poor prognosis of glioma patients (Wei YZ et al. 2017). The current problem is that the specific mechanism of TAGLN2 in the occurrence and development of ccRCC is still unclear. Thus, more exploration is needed to better understand the potential values of TAGLN2 in ccRCC.

In this paper, the effects of TAGLN2 expression on clinicopathological parameters and prognosis of patients with ccRCC were studied by bioinformatics analysis. In addition, the relationship between TAGLN2 and immune cell infiltration was analyzed. The related potential carcinogenic mechanism was further predicted. At the same time, the role and mechanism of TAGLN2 in tumor cells were verified by in vitro cell experiments. To explore the value of TAGLN2 in molecular targeted therapy and prognostic detection in ccRCC.

Materials And Methods

TCGA data acquisition and processing

The RNA-seq and clinical information of ccRCC patients were collected from the FPKM format of KIRC in TCGA database. The mRNA-TAGLN2 expression was compared between tumor and peritumoral tissue using the Mann-Whitney U test. The median TAGLN2 expression level was divided into high and low groups, survival tool in R Studio was used to examine the link between TAGLN2 expression and clinical subgroup data, and Kaplan-Meier survival curve was drawn. After univariate and multivariate analysis of TAGLN2 expression and clinical pathological parameters, the results were shown as forest maps. Prognostic model-nomogram was established by rms package in R Studio, and using survival tool to estimate the postoperative survival probability of patients. Finally, the accuracy of the model was verified by prognostic Calibration analysis.

TAGLN2 as the target molecule, and the DESeq2 package in R Studio was used for single gene differential analysis, log2 FoldChange>|1|, P < 0.0001 were used as screening conditions. GO, KEGG and GSEA enrichment analysis of differential genes were assessed using the cluster analysis package in R studio. The correlation of TAGLN2 expression with 24 immune cells was investigated using the Gsva package in R Studio.

Clinical samples and cell lines

8 pairs of cancer and paracancer tissues from patients with ccRCC were collected with the ethical consent of Hospital (2022A-206). These tissues were used to extract mRNA and protein. Human renal clear cell carcinoma cell line 786-O and CAKI-1 and normal renal tubular epithelial cell line HK-2 were collected from the Key Laboratory of Urological Diseases in Gansu Province.
HK-2 was cultured in DMEM medium (Basal Media) with 10% serum (FBS), whereas 786-0 and Caki-1 in RPMI 1640 medium (Basal Media) with 10% serum (FBS).

**Cell transfection**

Three silencing targets (Gene Pharma) mediated by lentiviral vectors were used to silence TAGLN2, Silencing control by scramble-sequence sh-nc.

**Quantitive real-time PCR (qRT-PCR)**

RNA was isolated using Trizol (Thermo Fisher). Reverse Transcription Kit (Evo M-MLV II, Accurate Biology) uses 1µg of RNA to synthesize cDNA, The SYBR Green (Accurate Biology) was then used for fluorescent real-time quantitative PCR analysis using cDNA as a template. All data were normalized to GAPDH expression. Relative RNA expression can be calculated by using the $\Delta \Delta Ct$ method. The forward primer of TAGLN2 is TTCCCTAAGAAATCCAAGGAG, reverse primer is CTCCTTAATGTCACGCACGT.

**Western blot (WB)**

Protein isolated from ccRCC tissue samples and cultured cells. Using anti-TAGLN2, GAPDH, Bcl-2, Bax, Caspase-3, Pcna, E-cadherin, N-cadherin, Vimentin, Pi3k, Akt, P-akt antibody (ProteinTech, China). A secondary antibody (ABclonal, China) was then applied to detect the signal by enhanced chemiluminescence. The density of the bands was quantified and normalized to GAPDH using Image-J software.

**Cell viability assay**

Cells were implanted into 96-well plates ($3 \times 10^3$ cells/well). The detection times were 0, 24, 48, 72 and 96 h. Subsequently 10 µL CCK-8 reagent was added to each well and then incubated for 45 min. The absorbance at 450nm was recorded with a microplate reader.

**Colony forming assay**

500 cells were inoculated into 6-well plates. Cells were fixed in methanol and stained with crystal violet after 2 weeks.

**EdU proliferation assay**

EDU-488 (BeyoClick) proliferation kit was used, cells were spread in 24-well plates and when the density reached 40%, 100ul Click Additive solution was added to each well according to the manufacturer's instructions. After 30 minutes at room temperature, the cells were washed 3 times. Nuclei were stained using Hoechst 33342, after 10 minutes at room temperature, the cells were washed 3 times. Finally, cells were observed and photographed under a fluorescence microscope, and the results were analyzed by Image J software.

**Wound healing assay**
Cells were seeded in 6-well plates. When the plates were completely covered, using the tip of 1 ml sterile pipette scratched wounds in each well. The scratch area was observed and photographed at 0 and 12h, respectively.

**Transwell migration and invasion assay**

Migration and invasion experiments were conducted in a 24-well Milli cell chamber (Corning). 2x10^4 cells were respectively seeded in the coated chambers without Matrigel (Corning) for the migration assay and in the coated chambers with 30ul of Matrigel for the invasion assay. Next, serum-free medium was added to the upper chamber while serum medium was added to the lower chamber. After 24 and 48 hours of cell culture, the results of migration and invasion were observed.

**Flow cytometry assay**

Cells were laid on 6-well plates. After 72hours, cells were collected, and suspended in 500µl binding buffer containing 5ul Propidium Iodide (PI) reagent and Annexin V-FITC (Yeasen), respectively. Finally, flow cytometry was used to detect the role of TGALN2 in cell apoptosis.

**Statistical analysis**

Three independent experiment datas were expressed as mean ± SEM. Statistical analysis between two groups was carried out by T test. P < 0.05 was considered statistically significant.

**Results**

1. **TAGLN2 is highly expressed in ccRCC from TCGA database**

Upon the scaled matrix of expression, TAGLN2 is strongly expressed in a variety of malignancies, including ccRCC (Figure.1A). Both paired and unpaired samples of ccRCC showed a substantial increase in TAGLN2 expression (Figure.1B-C). In addition, the examination of the relationship between TAGLN2 and clinicopathological variables in ccRCC patiennts showed that TAGLN2 resulted worse clinical T, N, M and pathological stage and histopathological grade (Fig. 1D-H).

2. **TAGLN2 has a significant prognostic value in ccRCC from TCGA database**

According to the survival analysis, patients with ccRCC had lower overall survival (OS) and higher level of TAGLN2 expression (Figure.2A). In the subsequent subgroup analysis of clinical parameters, TAGLN2 high expression group played a significant role in reducing OS in ccRCC patients (Figure.2B-C). Meanwhile, the ROC curve revealed that TAGLN2 had a compelling sensitivity and specificity to predict ccRCC (Figure.2D). In Figure. 2E-F, univariate COX regression models indicated that TAGLN2 was a single predictor. Finally, TAGLN2 expression data were used to build a prognostic model (Figure.2G), and
calibration analysis was used to confirm the accuracy of the prognostic model (Figure.2H-J). These results indicate that TAGLN2 is an effective prognostic indicator.

3. TAGLN2 is highly expressed in ccRCC tissues and cells

TAGLN2 mRNA and protein expression were found in ccRCC and its surrounding tissues. As shown by qRT-PCR and WB in Figure.3A-C, the expression level of TAGLN2 in cancer tissues was much higher than that in paracancerous tissues. To further explore the role of TAGLN2 in ccRCC, the expression of TAGLN2 in HK-2, 786-O and Caki-1 cell lines could be detected by qPCR and WB. Consistent with the clinical samples, ccRCC cell lines had higher levels of TAGLN2 mRNA and protein expression than normal cells did (Figure.3D-F). We next elucidated the role of TAGLN2 in 786-O, Caki-1 cell lines. First, three TAGLN2-targeting sh-RNAs named sh-1, sh-2 and sh-3 were transfected into Caki-1 and 786-O cells respectively. To confirm the inhibitory effect, the protein expression level of TAGLN2 was determined by WB method. Results showed that sh-3 had the best inhibition efficiency (Figure. 3G-J). So sh-3 was selected to silence the expression of TAGLN2 in subsequent experiments.

4. TAGLN2 promotes the malignant phenotype of ccRCC cells

4.1 TAGLN2 promotes proliferation of ccRCC cells

CCK-8 assay demonstrated reduced viability of ccRCC cells after TAGLN2 silencing (Figure.4A-B). Colony formation assay indicated that the colony forming ability of ccRCC cells was diminished after silencing TAGLN2 (Figure.4C-D). In addition, EDU proliferation experiment showed that TAGLN2 silencing significantly decreased the proliferation of ccRCC cells (Figure.4E-H). According to the aforementioned findings, TAGLN2 knockdown dramatically reduced the proliferation of ccRCC cells.

4.2 TAGLN2 promotes ccRCC cells migration and invasion

To investigate the effect of TAGLN2 on ccRCC cell migration and invasion, wound healing, migration and invasion assays were carried out. After TAGLN2 silencing, the lateral migration ability of cells was downregulated (Figure.5A-D). The longitudinal migration ability of ccRCC cells was found to be downregulated in the trans well migration assay (Figure.5E-H). In addition, the invasion ability of ccRCC cells was found to be obviously inhibited in the trans well invasion assay (Figure.5I-L).

Figure.4 (A) The CCK8 assay results of sh-TAGLN2 at 0-, 1-, 2-, 3-, 4 day intervals in 786-O. (B) The CCK8 assay results of sh-TAGLN2 at 0-, 1-, 2-, 3-, 4 day intervals in CAKI-1. (C) The monoclonal cell assay results of sh-TAGLN2 in 786-O. (D) The monoclonal cell assay results of sh-TAGLN2 in CAKI-1. (E-F) The EDU proliferation experiment results of sh-TAGLN2 in 786-O. (G-H) The EDU proliferation experiment results of sh-TAGLN2 in CAKI-1. (*P<0.05, **P<0.01, ***P<0.001.)

Figure.5 (A-B) Wound healing assay results in 786-O. Images of all groups at 0- and 12-h time intervals post injury. (C-D) Wound healing assay results in CAKI-1. Images of all groups at 0- and 12-h time
intervals post injury. (E-F) Migration assay results showed that the sh-TAGLN2 group in 786-O has a weaker migration ability. (G-H) Migration assay results showed that the sh-TAGLN2 group in CAKI-1 has a weaker migration ability. (I-J) Invasion assay results showed that the sh-TAGLN2 group in 786-O has a weaker invasion ability. (K-L) Invasion assay results showed that the sh-TAGLN2 group in CAKI-1 has a weaker invasion ability. (*P < 0.05, **P < 0.01, ***P < 0.001.)

4.3 TAGLN2 influences ccRCC cells apoptosis.

Flow cytometry showed that TAGLN2 silencing influenced apoptosis of 786-O and Caki-1 cells (Figure.6A-D). WB data showed the presence of early apoptosis related protein expression, in which pro-apoptotic protein Bax improved and anti-apoptotic protein Bcl-2 declined whereas late late apoptosis-related protein Caspase-3 did not show significant changes. Moreover, after silencing TAGLN2, the expression of PCNA was significantly decreased (Figure.6E-H).

4.4 knocking-down TAGLN2 inhibits EMT

Epithelial-mesenchymal transition (EMT) significantly affects the distant metastasis of ccRCC. In order to study the role of TAGLN2 in the development of ccRCC, the protein levels of E-cadherin, Vimentin and N-cadherin were measured. We found that expression of the E-cadherin was elevated after TAGLN2 knockdown in 786-O cell line, while there was no significant increase in Caki-1 cell line. Expression of Vimentin and N-cadherin were decreased significantly after TAGLN2 knockdown compared with control samples using sh-nc, both in 786-O and Caki-1 cell lines (Figure.6I-L). These data suggested that TAGLN2 inhibited the EMT of ccRCC cell lines and thus reduce the distant metastasis ability of ccRCC.

5. TAGLN2 regulates PI3K/AKT signaling pathway

To elucidate the related signaling pathways of TAGLN2, GO enrichment and KEGG pathway analysis were performed on TAGLN2-related differential genes. According to GO enrichment analysis in Figure.7A, differential genes are closely related to cellular immunity. Through enrichment analysis of KEGG pathway, the differential gene is rich in PI3K/AKT signaling pathway (Figure.7B). In addition, differential could also be enriched in the PI3K/AKT signaling pathway (Figure.7C), which is confirmed by GSEA enrichment analysis results. According to the results of bioinformatics research, the effects of TAGLN2 on PI3K/Akt signaling pathway were observed. It was found that the expression levels of PI3K, AKT and P-Akt in 786-O cells and CAKI-1 cells decreased significantly after TAGLN2 silencing (figure.7D-I). These partial results were consistent with the enrichment analysis of KEGG pathway. Because PI3K/AKT pathway is regulated by TAGLN2, TAGLN2 silencing can negatively regulate the activity of PI3K/AKT pathway and further inhibit the progress of ccRCC.

Figure.7(A)GO enrichment analysis of TAGLN2 related up-regulated differential genes from TCGA. (B) KEGG pathway enrichment analysis of TAGLN2 related up-regulated differential genes from TCGA. (C) Gene set enrichment analysis (GSEA) results based on the TAGLN2 mRNA expression in ccRCC from TCGA. (D-E) 786-O cells were transfected with sh-TAGLN2, proteins related to PI3K/AKT were detected by
WB. (F-I) CAKI-1 cells were transfected with sh-TAGLN2, proteins related to PI3K/AKT were detected by WB. (*P < 0.05, **P < 0.01, ***P < 0.001.)

6. TAGLN2 is involved in immune cell responses

Based on the correlation between differential genes and cellular immune response in GO enrichment analysis, we looked into how TAGLN2 and immunological infiltration are related. The level of infiltration of different immune cells (e.g., Treg, Th2 cells, Macrophages, and NK CD56 bright cells) was positively correlated with the expression of TAGLN2 (Figure.8A). Treg cells were significantly positively correlated with TAGLN2 expression, and Treg cell infiltration levels were significantly different between TAGLN2 high expression group and TAGLN2 low expression group (Figure.8B-C). In addition, the results between Th2 cells and TAGLN2 cells were the same as those of Treg cells (Figure.8D-E). These findings imply that TAGLN2 is associated with the prognosis and immune infiltration of ccRCC.

Discussion

ccRCC is a common subtype of RCC, with high incidence, poor prognosis, strong invasiveness and frequent metastasis rate (Xie Y et al. 2017), most patients do not achieve a good prognosis (Xia Y et al. 2017). Therefore, it is very important to study the molecular mechanisms of the development of ccRCC and find effective biomarkers to assist diagnosis, as well as provide corresponding molecular therapeutic targets. TAGLN2 is involved in actin cytoskeleton remodeling (Liu J et al. 2020). Numerous cancer-related mechanisms of migration, proliferation, differentiation, and apoptosis involve this protein either directly or indirectly. The carcinogenic effect of TAGLN2 has been widely reported in recent years (Zhang Y et al. 2010; Haijian et al. 2018; Jin H et al. 2021).

In this work, we discovered that TAGLN2 was highly overexpressed in ccRCC and other Pan-Cancer analyses based on TCGA database analysis. TAGLN2 can lead to worse clinical stage, pathological grade and survival prognosis of ccRCC patients. The prognostic analysis model constructed with TAGLN2 as the analysis variable has good accuracy for the long-term survival rate of ccRCC patients. Therefore, we believe that TAGLN2 has a significant value in assessing the prognosis of ccRCC patients. Subsequently, the effect of TAGLN2 on tumor immune infiltration deserves further study, Treg cells and Th2 cells were significantly positively correlated with TAGLN2 expression. In the tumor microenvironment, Treg cells are considered to be the main factor that helps tumor cells evade immune surveillance (Watson M et al. 2021), Th2 immune skewing can promote distant metastasis of tumors (Jma B et al. 2022). Therefore, TAGLN2 may be implicated in regulating the expression level of immune cells in the tumor microenvironment and inducing immune escape.

In vitro studies showed that TAGLN2 silencing decreased the proliferation, colony formation, migration, invasion, and EMT of ccRCC cell lines. A statistical difference was found in Bcl-2 and Bax but no significant change in Caspase-3 by examining the expression of apoptosis related proteins. Anti-apoptotic protein Bcl-2 and pro-apoptotic protein Bax are upstream signals of apoptosis, which could be blocked in the process of downward propagation. Therefore, the apoptotic executive protein caspase-3
does not change. So, TAGLN2 silencing may affect apoptosis, but does not trigger apoptosis, obviously. EMT is closely associated with loss of E-cadherin expression and upregulation of mesenchymal-related proteins like N-cadherin and Vimentin (Jolly M K et al. 2017). we found that, after TAGLN2 silencing, EMT was reversed. So, silencing TAGLN2 can inhibit the development of EMT and reduce the distant metastasis ability of tumor cells.

Studies on the role of the PI3K/AKT pathway in tumor development and spread are extensive (Li Y et al. 2016). It is indispensable in regulating cancer cells growth, migration, apoptosis and survival (J Chen et al. 2011; Brian M Slomovitz et al. 2012; Popolo A et al. 2017). In recent renal cancer studies, inhibition of PI3K/AKT signaling pathway was found to reduce cancer cell proliferation (Lin H et al. 2021), migration (Peng X S et al. 2020), invasion (Chen P et al. 2019), EMT (Chen H et al. 2019) and promote cell apoptosis (Zhang S et al. 2017). In this study, the PI3K/AKT signaling pathway was first identified by enrichment analysis of TAGLN2-related differential genes. Subsequent WB analysis conformed that the activity of this signaling pathway was significantly reduced after TAGLN2 silencing. The above experimental results may lead to the speculation that TAGLN2 may promote ccRCC cell proliferation, migration, invasion, and EMT by stimulating the PI3K/AKT signaling pathway, while inhibiting apoptosis. This study suggests that TAGLN2 is promising as a target for ccRCC treatment.

In this paper, there are still some deficiencies. Firstly, we only discussed the effects of TAGLN2 on ccRCC cells in vitro, but the effects of TAGLN2 on tumor size, weight, proliferation and apoptosis in the subcutaneous tumorigenesis model of nude mice need to be confirmed by further experiments. Secondly, whether there are direct acting protein between TAGLN2 and PI3K/AKT signaling pathways that affect the activity this pathway also deserves to be explored in subsequent experiments. Finally, the overexpression of TAGLN2 was insufficient in this study to further verify its influence and mechanism on the malignant biological behavior of 786-O and CAKI-1 cell lines.

Conclusion

Therefore, the evidence of the close correlation between TAGLN2 and poor prognosis of ccRCC patients may serve as a single indicator for predicting the likelihood of survival in this class of patients. In vitro, TAGLN2 silencing could hamper ccRCC proliferation, migration, invasion and EMT by inhibiting the PI3K/AKT signaling pathway. According to our research, TAGLN2 may control immune cell invasion and aid in tumor immune escape, which may be a promising therapeutic target for ccRCC.

Declarations

Statements & Declarations

Funding

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**Ethical approval**

This study involving human participants was conducted in accordance with the ethical standards of the institutional and national research committee and the Helsinki Declaration 2013. This study was conducted by the Ethical Committee of the Lanzhou University Second Hospital (2022A-206).

**Author Contributions**

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Yang He], [Bin Zhang] and [Dali Han]. The first draft of the manuscript was written by [Yuelin Du], [Xingxing Zhang] and [Hongbo Wang]. The project was funded by [Zhongjin Yue] and [Panfeng Shang], and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

**Declaration of competing interest**

The authors declare no conflict of interest.

**Data Availability**

*The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request*

**References**


Figures
Figure 1

(A) The mRNA expression levels of TAGLN2 in pan-cancers from TCGA+GTEx database. (B) The mRNA expression levels of TAGLN2 in unpaired ccRCC samples from TCGA. (C) The mRNA expression levels of TAGLN2 in paired ccRCC samples from TCGA. (D) The mRNA expression levels of TAGLN2 in T stage from TCGA. (E) The mRNA expression levels of TAGLN2 in N stage from TCGA. (F) The mRNA expression levels of TAGLN2 in M stage from TCGA. (G) The mRNA expression levels of TAGLN2 in pathologic stage from TCGA. (H) The mRNA expression levels of TAGLN2 in histologic grade from TCGA. (*P < 0.05, **P < 0.01, ***P < 0.001.)
Figure 2

(A) The OS of ccRCC patients was evaluated according to high and low TAGLN2 expression from TCGA. (B-C) The OS of patients in T stage was evaluated according to the level of TAGLN2 expression from TCGA. (D) ROC curve of TAGLN2 from TCGA. (E) Univariate Cox hazard regression analysis of six clinicopathological variables (T stage, N stage, M stage, Pathologic stage, Histologic stage, TAGLN2) in ccRCC from TCGA. (F) Multivariate Cox hazard regression analysis of six clinicopathological variables (T stage, N stage, M stage, Pathologic stage, Histologic stage, TAGLN2) in ccRCC from TCGA. (G) Nomogram based on six clinicopathological variables (T stage, N stage, M stage, Pathologic stage, Histologic stage, TAGLN2) in ccRCC from TCGA. (H) 1-year calibration curve. (I) 3-year calibration curve. (J) 5-year calibration curve. (*) \( P < 0.05 \), (**) \( P < 0.01 \), (***) \( P < 0.001 \).
Figure 3

(A) The relative expression of TAGLN2 mRNA level in 8 pairs of tumor tissues. (B-C) The relative expression of TAGLN2 protein level in 8 pairs of tumor tissues. (D) The relative expression of TAGLN2 mRNA level in ccRCC cells. (E-F) The relative expression of TAGLN2 protein level in ccRCC cells. (G-H) The relative expression of sh-TAGLN2 protein level in 786-O. (I-J) The relative expression of sh-TAGLN2 protein level in CAKI-1. (*P < 0.05, **P < 0.01, ***P < 0.001.)
Figure 4

(A) The CCK8 assay results of sh-TAGLN2 at 0-, 1-, 2-, 3-, 4 day intervals in 786-O. (B) The CCK8 assay results of sh-TAGLN2 at 0-, 1-, 2-, 3-, 4 day intervals in CAKI-1. (C) The monoclonal cell assay results of sh-TAGLN2 in 786-O. (D) The monoclonal cell assay results of sh-TAGLN2 in CAKI-1. (E-F) The EDU proliferation experiment results of sh-TAGLN2 in 786-O. (G-H) The EDU proliferation experiment results of sh-TAGLN2 in CAKI-1. (*P < 0.05, **P < 0.01, ***P < 0.001.)
Figure 5

(A-B) Wound healing assay results in 786-O. Images of all groups at 0- and 12-h time intervals post injury. 
(C-D) Wound healing assay results in CAKI-1. Images of all groups at 0- and 12-h time intervals post injury. 
(E-F) Migration assay results showed that the sh-TAGLN2 group in 786-O has a weaker migration ability. 
(G-H) Migration assay results showed that the sh-TAGLN2 group in CAKI-1 has a weaker migration ability. 
(I-J) Invasion assay results showed that the sh-TAGLN2 group in 786-O has a weaker invasion ability. 
(K-L) Invasion assay results showed that the sh-TAGLN2 group in CAKI-1 has a weaker invasion ability. (*P < 0.05, **P < 0.01, ***P < 0.001.)
Figure 6

(A-B) 786-O cells were transfected with sh-TAGLN2, apoptotic cells was measured by flow cytometric analysis. (C-D) CAKI-1 cells were transfected with sh-TAGLN2, apoptotic cells was measured by flow cytometric analysis. (E-F) 786-O cells were transfected with sh-TAGLN2, proteins related to apoptosis and proliferation were detected by WB. (G-H) CAKI-1 cells were transfected with sh-TAGLN2, proteins related to apoptosis and proliferation were detected by WB. (I-J) 786-O cells were transfected with sh-TAGLN2, proteins related to EMT were detected by WB. (K-L) CAKI-1 cells were transfected with sh-TAGLN2,
proteins related to EMT were detected by WB. (*P < 0.05, **P < 0.01, ***P < 0.001.)
Figure 7

A GO enrichment analysis of TAGLN2 related up-regulated differential genes from TCGA. (B) KEGG pathway enrichment analysis of TAGLN2 related up-regulated differential genes from TCGA. (C) Gene set enrichment analysis (GSEA) results based on the TAGLN2 mRNA expression in ccRCC from TCGA. (D-E) 786-O cells were transfected with sh-TAGLN2, proteins related to PI3K/AKT were detected by WB. (F-I) CAKI-1 cells were transfected with sh-TAGLN2, proteins related to PI3K/AKT were detected by WB. (*P < 0.05, **P < 0.01, ***P < 0.001.)
Figure 8

(A) Correlation analysis between expression levels of TAGLN2 and immune cell infiltration in ccRCC from TCGA. (B) TReg cells were significantly positively correlated with TAGLN2 expression from TCGA. (C) TReg cells infiltration level in different TAGLN2 expression groups from TCGA. (D) Th2 cells were significantly positively correlated with TAGLN2 expression from TCGA. (E) Th2 cells infiltration level in different TAGLN2 expression groups from TCGA. (*P < 0.05, **P < 0.01, ***P < 0.001.)

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