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Research article

# The novel m6A writer METTL5 as prognostic biomarker probably associating with the regulation of immune microenvironment in kidney cancer

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ABSTRACT

Nowadays, among all urinary system cancers, the mortality of kidney cancer (KC) has risen to the first, and the incidence has been keeping on the third. Many recent studies have demonstrated that m6A modification regulated by the methyltransferases (writers) is closely related to the tumorigenesis of multiple cancers. In our previous study, we found that the methyltransferase METTL5 had a stronger association with the hazard ratio of KC more than most tumors, indicating its special function in carcinogenesis of KC. Until now, the expression, functions and mechanism of METTL5 in KC are still unclear. In this study, we analyzed the mRNA expression of METTL5 using the data sets from public databases, and revealed that the METTL5 expression was significantly up-regulated in tumor tissues of kidney renal clear cell carcinoma (KIRC) and kidney renal papillary cell carcinoma (KIRP) compared to normal tissues. Also, the METTL5 expression was correlated with the tumor stage and grade, indicating the potential involvement of METTL5 in tumor progression. Additionally, the higher expression of METTL5 predicted poorer prognosis of KIRC and KIRP patients. Subsequently, we revealed that the functions of METTL5 in KIRC might be related to immune modulation, because its co-expressed gene were enriched in immune-relevant pathways including Th17 cell differentiation, Th1 and Th2 cell differentiation, and phosphatidylinositol 3-kinase activity. Next, we disclosed that the METTL5 expression was correlated to the microenvironment score and immune score of KIRC and KIRP, and associated with the infiltration ratios of 25 types of immune cells. Besides, we demonstrated a wide difference of the METTL5's effect on the survival of patients with high and low immune infiltration, further suggesting METTL5 might affect tumor development via modulating the immune microenvironment. The findings of our study provide a novel potential prognostic biomarker and immune drug target for KC.

## 1. Introduction

According to a recent report, kidney cancer (KC) is currently the most lethal of the urinary system malignancies, and its incidence has remained at the third highest level [1]. The most common subtypes of KC are kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), and kidney chromophobe cell carcinoma (KICH) [2]. KIRC is a malignant tumor originating from the renal tubular epithelial

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system [3], accounting for 80–90% of renal malignancy [4]. Annually, there are over 400,000 new KC cases worldwide, and the incidence rate is still on the rise [5, 6, 7]. In general, although the immuno- and targeted-therapy have already been applied in clinical treatment of metastatic KC, most patients still do not achieve remission [8]. Prognostic biomarker is an aid in clinical decision making with respect to treatment options. However, no prognostic indicators or genes have been applied in clinical diagnosis and treatment for KC.

Recently, many studies have demonstrated that m6A modification is one of the most abundant and extensive modifications on mRNAs [9], regulated by methyltransferases (writers), demethylases (erasers), and specific RNA-binding proteins (readers) [10]. As reported, m6A methyltransferases has a wide range of biological functions in human diseases [11]. Several studies have indicated that the abnormal expression of m6A methyltransferases are involved in the modulation of immune microenvironment and the development of malignant tumors, such as KC, colon cancer, esophageal cancer, and breast cancer [12]. m6A modification may change the responses of immune cells to tumor cells, and potentially participate in the regulation of tumor immune escape [13]. Therefore, the m6A methyltransferases seem to be promising drug targets for cancer immunotherapy, which may be used in the clinical treatment and prognostic diagnosis of cancer patients.

Nowadays, methyltransferase 5 (METTL5) has been identified as a novel methyltransferase responsible for the m6A modification on 18S rRNA [14, 15, 16]. Several studies have demonstrated that the deficiency of METTL5 in mouse embryonic stem cells leads to decreased overall translation rate [15] and the delay of cell differentiation [17]. Some researches have also uncovered a role of METTL5 in tumorigenesis of breast cancer, gastric cancer and lung cancer [16, 18, 19]. Meanwhile, two studies have revealed that METTL5 has a regulatory function in immune microenvironment of lung cancer [20, 21]. Huang *et al* demonstrated that METTL5 was an oncogene in pancreatic cancer that promoted cell proliferation, migration, and invasion, and revealed that the oncogenic effect caused by METTL5 overexpression could be abolished by c-Myc knockdown [22]. In addition, Peng *et al* revealed that the depletion of METTL5-mediated 18S rRNA m6A modification resulted in impaired 80S ribosome assembly and decreased translation of mRNAs involved in fatty acid metabolism [23]. These studies indicate that METTL5 may play an important role in tumor initiation and progression. However, the roles and mechanism of METTL5 in kidney cancer tumorigenesis are still barely known.

In our previous study, we found that METTL5 may have a special function in carcinogenesis of KC (Wei Zhang, *et al*). Therefore, in this study, we explored the expression, prognostic value, functions and mechanisms of METTL5 in KC. We first analyzed the expression, survival correlation and prognostic value of METTL5 in distinct subtypes of KC, including KICH (n = 65), KIRC (n = 531) and KIRP (n = 289) using the data sets from The Cancer Genome Atlas (TCGA). Secondly, we explored the probable pathways involved in the function of METTL5 in KC, and studied the correlation between METTL5 and the immune microenvironment in KC patients. Finally, we investigated the potential mechanisms underlying the high expression of METTL5 in KC.

## 2. Materials and methods

### 2.1. TCGA

TCGA database was used to retrieve level 3 RNA-sequencing files of 537 KIRC and 291 KIRP patients (Supplementary Table S1). Individual files were combined into matrix files, and the Ensembl database was used to change gene names from Ensembl IDs to gene symbols. From the symbol matrix, the datasets for KC peoples were taken out.

### 2.2. SangerBox

SangerBox website is an online tool for the analysis of data sets from TCGA and Genotype-Tissue Expression Project (GTEx). In this study, the mRNA expression of METTL5 in multiple cancerous tissues and normal tissues was analyzed using Sangerbox.

### 2.3. Gene expression profiling interactive analysis (GEPIA)

GEPIA [24] is a database for analyzing RNA-sequencing data of tumor and normal samples from TCGA and GTEx database. In this study, the mRNA expression of METTL5 in KICH, KIRC and KIRP tissues and normal tissues (or normal adjacent tissues) was analyzed using the GEPIA database. The |Log2fold change| cutoff was set as 0.267, and the p-value cutoff was set as 0.05.

### 2.4. UALCAN

UALCAN [25] is a comprehensive and interactive network resource for analyzing multiple omics data of cancer. In this study, the protein expression of METTL5 in KIRC tissues and normal adjacent tissues, and the correlation between the METTL5 expression and different clinical signatures were analyzed using the UALCAN database.

### 2.5. LinkedOmics

LinkedOmics [26] is publicly available portal that includes mult i-omics data of 32 cancers from TCGA and Cancer Institute's Clinical Proteomic Tumor Analysis Consortium (CPTAC). Herein, the co-expressed mRNAs of METTL5 were downloaded from LinkedOmics, and the "LinkInterpreter" module was used to perform Gene Set Enrichment Analysis (GSEA) of the METTL5 co-expressed mRNAs based on Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG).

### 2.6. Kaplan Meier Plotter

The Kaplan-Meier Plotter [27] is a database that assesses the correlation between gene expression and patient survival rates in different tumors to discover and validate the probable survival biomarkers. In this study, to evaluate the prognostic value of METTL5 in KC patients, the KC patients was classified into METTL5-high-expression group and low-expression group based on the best cut-off value, and the overall survival rate (OS) and the relapse free survival rate (RFS) were then analyzed.

### 2.7. xCell

xCell [28] is an algorithm that calculates the potential proportion of immune cells based on the gene expression matrix. In this study, the correlation between the METTL5 expression and the levels of infiltrating immune cells in KICH, KIRC and KIRP tumors were investigated using the xCell.

### 2.8. Statistical analysis

The HR of METTL5 in KIRC was analyzed using the univariate cox regression, and the p value of METTL5 as an independent prognostic gene in KIRC was investigated using the multivariate cox regression. A p-value less than 0.05 was regarded as statistically significant.

### 2.9. Cell culture

The HK2 cells were cultured in DMEM/F12 medium (Gibco, USA), the A498 cells were cultured in MEM medium (Gibco, USA), and the ACHN and OS-RC-2 cells were cultured in RPMI-1640 medium (Gibco, USA). All culture media were supplemented with 10% foetal bovine serum (FBS) (ExCell Bio, China) and 1% penicillin/streptomycin (Solarbio, China). The cells were cultured in a cell culture incubator at 37 °C with 5% CO2.

### 2.10. RNA extraction and qRT-PCR

The total RNA was isolated from four cell lines according to the manufacturer's instructions using Trizol (Invitrogen). The transScript all-in-one first-strand cDNA synthesis superMix for qPCR (One-step gDNA removal) kit (TransGen Biotech, AT341-01) was used for the reverse transcription of mRNA. After that, the qPCR assays were conducted out with the PerfectStart Green qPCR SuperMix kit (TransGen Biotech, AQ601-02). The primers of METTL5 gene were designed in Primer Premier 5.0, and the primers sequences were METTL5-F: 5-GATCCTGTGCCTTCAAACCCTACG-3.

METTL5-R: 5-CCACTTGTTGCAGGCGACTCTC-3. The primers were synthesized by the Sangon Biotech Company. Among them, GAPDH was an internal reference gene. The relative gene expression was calculated by the $2^{-\Delta\Delta}$CT method. And p value $< 0.05$ was considered statistically significant.

## 3. Results

### 3.1. METTL5 expression was significantly higher in human KIRC and KIRP tissues than normal adjacent tissues

In our previous study, we found that METTL5 had a stronger association with the hazard ratio (HR) of KC more than most tumors, indicating its special function in carcinogenesis of KC (Supplementary Figure 1). In this study, to investigate the role of METTL5 in tumorigenesis, we first analyzed the mRNA expression of METTL5 in tumorous tissues of 33 types of cancers and normal tissues (or normal adjacent tissues) from TCGA and GTEx. The results showed that METTL5 was increasingly expressed in 10 tumors, including cholangiocarcinoma (CHOL), glioblastoma multiform (GBM), head and neck squamous cell carcinoma (HNSC), KIRC, KIRP, liver hepatocellular carcinoma (LIHC), lung squamous cell carcinoma (LUSC), rectum adenocarcinoma (READ), stomach adenocarcinoma (STAD), and uterine corpus endometrial carcinoma (UCEC), as shown in Figure 1A-B. Furthermore, we evaluated the METTL5 expression in distinct independent studies in Oncomine database, and the results revealed that the METTL5 expression was more likely to be elevated in KC, colorectal cancer, head and neck cancer, liver cancer, lymphoma, cervical cancer, and myeloma (Figure 1C).

Because we focused on the role of METTL5 in KC, we then verified the mRNA expression of METTL5 in KICH, KIRC and KIRP tissues versus normal adjacent tissues using the data sets from the GEPIA databases. The results showed that the METTL5 expression was significantly up-regulated in tumor tissues of KIRC and KIRP patients versus normal tissues (Figure 1D). Furthermore, to verify the results of our bioinformatics analyses, we have examined METTL5 expression in one immortalized normal renal epithelial cell line (HK2) and three renal cancer cell lines (A498, ACHN and OS-RC-2) by qRT-PCR assay. Consequently, we found that METTL5 expression in three renal cancer cell lines were statistically significant higher than in immortalized normal renal epithelial cell line (Figure 1E).

### 3.2. The protein expression of METTL5 in distinct KIRC subgroups

As KIRC accounted for the largest proportion of KC [29], we chose KIRC as the representative model for the following study. Firstly, we found that the METTL5 protein was increasingly expressed in KIRC tissues compared with normal adjacent tissues (Figure 2A). To further explore the functions of METTL5 in tumorigenesis, we analyzed the METTL5 protein expression in distinct KIRC patient groups. Shown in Figure 2B, the METTL5 expression was significantly increased in tumor tissues of KIRC patients aged 21–40, 41–60, 61–80 and 81–100 compared with normal tissues. Besides, in different genders, the METTL5 expression was significantly higher in tumor tissues of male and female KC patients than in normal tissues (Figure 2C). In different stages, compared with the normal group, METTL5 exhibited significantly up-regulated expression in KIRC tissues from patients at stage 1, stage 2, stage 3 and stage 4 (Figure 2D). Interestingly, the METTL5 expression was gradually increased with tumor stage, and statistically different between stage 1 and stage 2, stage 1 and stage 4, stage 2 and stage 3, and stage 3 and stage 4. In terms of races, the METTL5 expression was significantly increased in Caucasian compared with the normal group (Figure 2E). As for weight, compared with the control group, the METTL5 expression was significantly higher in KIRC tissues from patients with normal weight, extreme weight, obesity and extreme obesity (Figure 2F). Notably, the METTL5 expression was also gradually elevated with tumor grade, and significantly different between grade 1 and grade 2, and grade 2 and grade 3 (Figure 2G). Our observations in tumor stage and grade indicated that METTL5 might be relevant to the progression of KC. Ultimately, the METTL5 expression was significantly up-regulated in tumorous tissues of KIRC patients with MYC/MYCN mutations than other mutations, which suggested that the functions of METTL5 in KIRC might be related to the MYC/MYCN mutations (Figure 2H).

### 3.3. The higher expression of METTL5 in tumor tissues versus normal adjacent tissues indicated poorer prognosis of KIRC and KIRP patients

As mentioned above, METTL5 was probably a functional gene in KC tumorigenesis. Thus, we assessed whether the METTL5 expression was related to the survival rates of KC patients using the data sets from the Kaplan Meier Plotter database. As a result, the KIRC patients with higher METTL5 expression frequently had poorer OS ($n = 530$, $p = 0.00068$), and RFS ($n = 117$, $p = 0.13$) (Figure 3A). Besides, the KIRP patients with higher expression of METTL5 also had lower OS ($n = 287$, $p = 0.00079$) and RFS ($n = 183$, $p = 0.0052$) (Figure 3B). These results further confirmed that METTL5 was potentially functional in the initiation and progression of KIRC and KIRP. To further investigate the prognostic value of METTL5 in patients with KC, we performed the cox regression analyses based on OS and progression free survival rate (PFS). As a result, the univariate cox regression result disclosed that the METTL5 expression was a risk factor for KC development ($p < 0.0001$) (Figure 3C), and the multivariate cox regression analysis revealed that METTL5 was an independent prognostic gene for KC ($p = 0.0701$) (Figure 3D). Furthermore, the nomogram analysis demonstrated that METTL5 could be used an indicator in combination with other clinical diagnostic indicators to predict the KC prognosis (C-index $= 0.871$, $p < 0.001$) (Figure 3E-F).

### 3.4. METTL5 might potentially regulate the immune microenvironment of KC patients

To explore the potential mechanism of METTL5 regulating the occurrence and development of KC, we analyzed the co-expressed genes of METTL5 in KC. As KIRC accounted for the largest proportion of KC [29], we chose KIRC as a representative model for this study. As a result, we obtained 2702 METTL5 co-expressed mRNAs using the data sets from Linkedomics (Supplementary Table S1). The top 50 positively co-expressed genes and negatively co-expressed genes with METTL5 were shown in the heat map (Figure 4A-B). Then, we analyzed these co-expressed mRNAs using the GSEA to explore the probable biological processes and pathways that METTL5 involved in (Figure 4C-F). The GO module named biological process (BP) uncovered that METTL5 was strongly correlated with the immune-related biological processes, such as Th1 and Th2 cell differentiation, and Th17 cell differentiation (Figure 4C). Additionally, other analyses revealed that the functions of METTL5 were related to phosphatidylinositol 3-kinase activity, small GTPase mediated signal transduction, hippo signaling, endoplasmic reticulum, and mitochondrial complex (Figure 4D-F). Based on the results of BP analysis, we speculated that the function of METTL5 in KIRC might be relevant to the regulation of immune microenvironment.

Subsequently, we analyzed the correlation between the METTL5 expression and the ratios of infiltrating immune cells in distinct subtypes of KC using xCell, QUANTISEQ and TIMER algorithms (Figure 5A-C). As a result, the METTL5 expression was significantly correlated with the

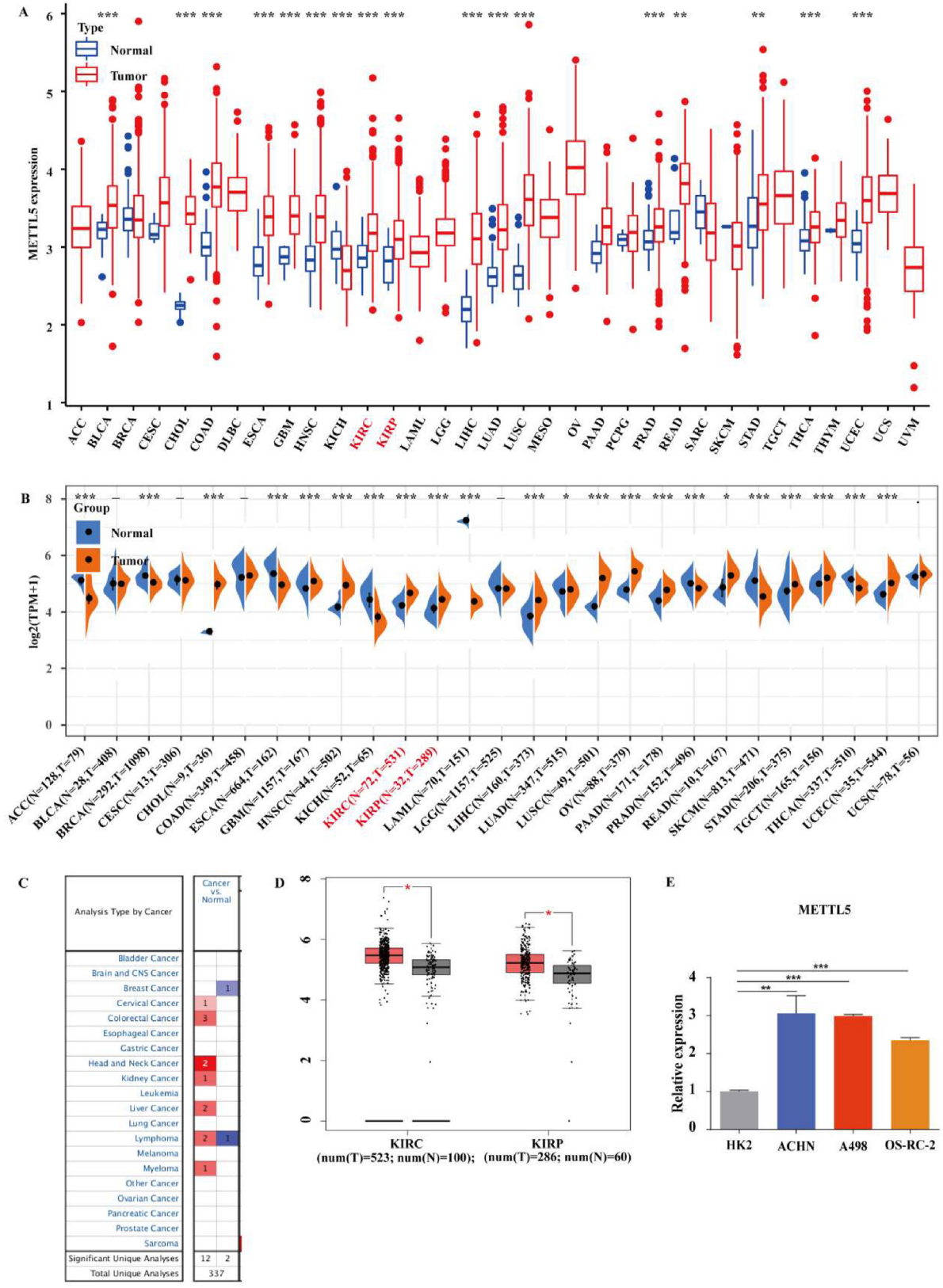


**Figure 1.** The mRNA expression of METTL5 in KC. The mRNA expression of METTL5 in multiple cancers as analyzed using the data sets from (A) TCGA, and (B) TCGA and GTEx. (C) The mRNA expression of METTL5 in KC provided by the Oncomine database and the METTL5 expression was more likely to be elevated in KC than in other cancer. (D) The mRNA expression of METTL5 in KIRC and KIRP tissues and normal tissues as analyzed using the data sets from the GEPIA database, and the METTL5 expression was significantly up-regulated in tumor tissues of KIRC and KIRP patients versus normal tissues. Fold change >1.2, and *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. (E) The validation of METTL5 expression in immortalized normal renal epithelial cell lines (HK2) and renal cancer cells (A498, ACHN and OS-RC-2) by qRT-PCR. Fold change >1.5, and *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and the METTL5 expression in three renal cancer cell lines were statistically significant higher than in immortalized normal renal epithelial cell line.

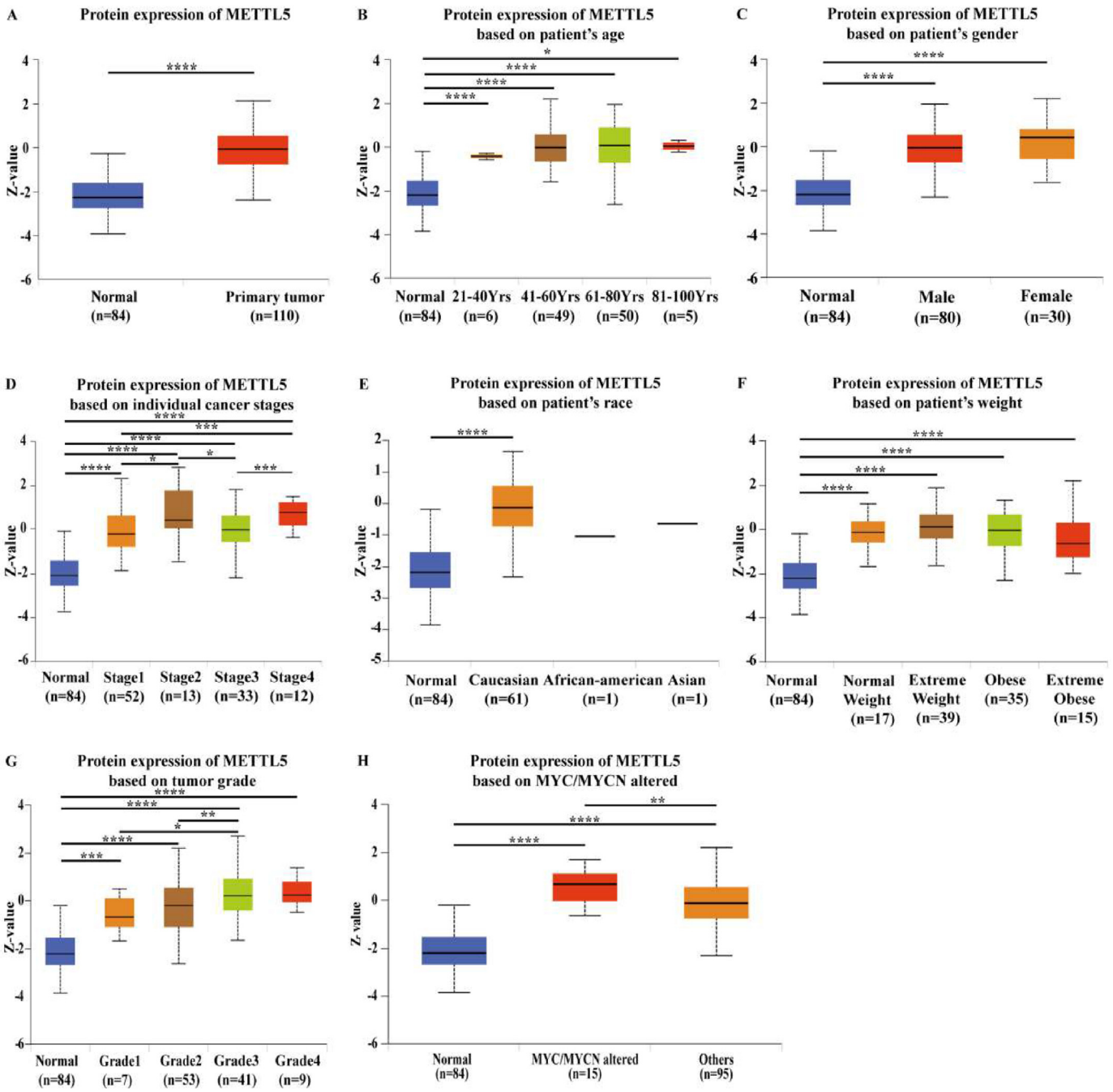


**Figure 2.** The correlation between the protein expression of METTL5 and distinct clinical characters in KIRC patients. (A) The METTL5 expression in KIRC tissues and normal adjacent tissues, and the METTL5 protein was increasingly expressed in KIRC tissues compared with normal adjacent tissues. The correlation between the protein expression of METTL5 and (B) age, (C) gender, (D) stages, (E) race, (F) weight, (G) grade, and (H) MYC/MYCN mutations in KIRC tissues. All the results were analyzed using the data sets from the UALCAN database. Fold change >1.2, and *p < 0.05, **p < 0.01, ***p < 0.001.

microenvironment score and immune score in KIRC, KIRP, and KICH, as analyzed using xCell. Meanwhile, the METTL5 expression exhibited negatively correlated with the infiltrating levels of 18 types of immune cells in KIRC patients, including natural killer (NK) T cell, naive CD8+T cell, effector memory CD8+ T cell, naive CD4+ T cell, central memory CD4+ T cell, T helper 1 (Th1) CD4+ T cell, plasma cytoid dendritic cell, myeloid dendritic cell, mast cell, macrophage M2, macrophage M1, macrophage, eosinophil, class-switched memory B cell, plasma B cell, naive B cell, memory B cell and B cell. Meanwhile, the METTL5 expression was positively correlated with the infiltration ratios of four types of immune cells in KIRC, including memory CD4+ T cell, T helper 2 (Th2) CD4+ T cell, (non-regulatory) CD4+ T cell and common lymphoid progenitor cell. Furthermore, using the algorithm named QUANTISEQ, we found that the METTL5 expression also correlated with the infiltration degrees of B cell and macrophage M2 in KIRC. Besides, the METTL5 expression exhibited negatively correlated with the infiltrating levels of eight types of immune cells in KIRP patients, including effector memory CD4+ T cell, central memory CD4+ T cell, myeloid dendritic cell, monocyte, macrophage M2, macrophage M1, macrophage and eosinophil as analyzed using xCell, and the METTL5 expression was also negatively correlated with the infiltration degrees of macrophage M2 in KIRP when analyzed using QUANTISEQ. In addition, the infiltration degrees of CD8+ T cells, myeloid dendritic cells and B cells were positively correlated with the METTL5 expression, and CD4+ T cell was

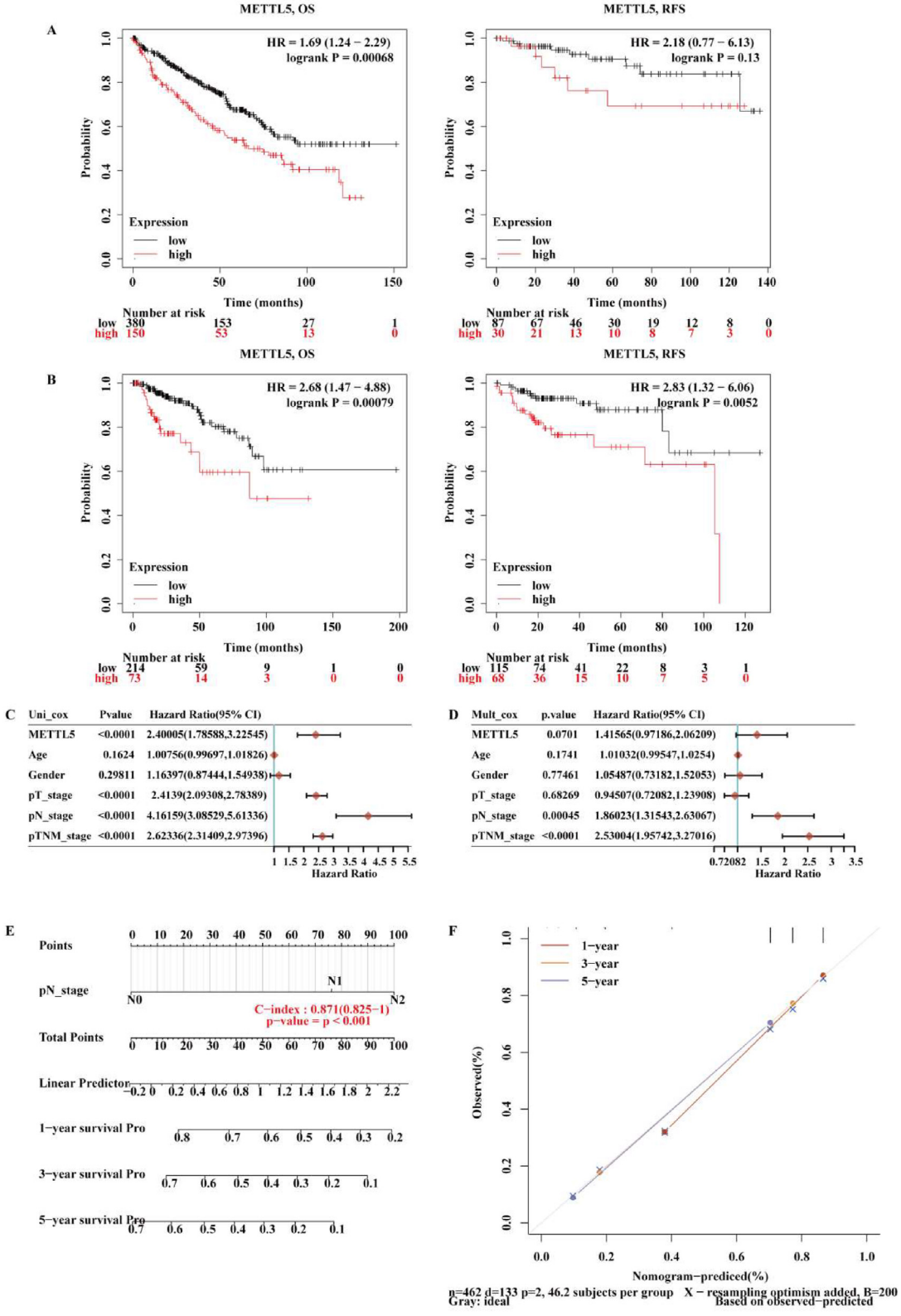


**Figure 3.** The prognostic value of METTL5 in KC. The effects of METTL5 expression on the OS and RFS of (A) KIRC (B) and KIRP patients, the KIRC patients with higher METTL5 expression frequently had poorer OS and RFS. And, the KIRP patients with higher expression of METTL5 also had lower OS and RFS. (C) The HR of METTL5 towards KC as analyzed using the univariate cox regression and disclosed that the METTL5 expression was a risk factor for KC development ($p < 0.0001$). (D) the prognostic value of METTL5 for KC as analyzed using the multivariate cox regression and revealed that METTL5 was an independent prognostic gene for KC ($p = 0.0701$). (E–F) METTL5 could be used in combination with other clinical diagnostic indicators to predict the progression and prognosis of KC as investigated by nomogram analysis.

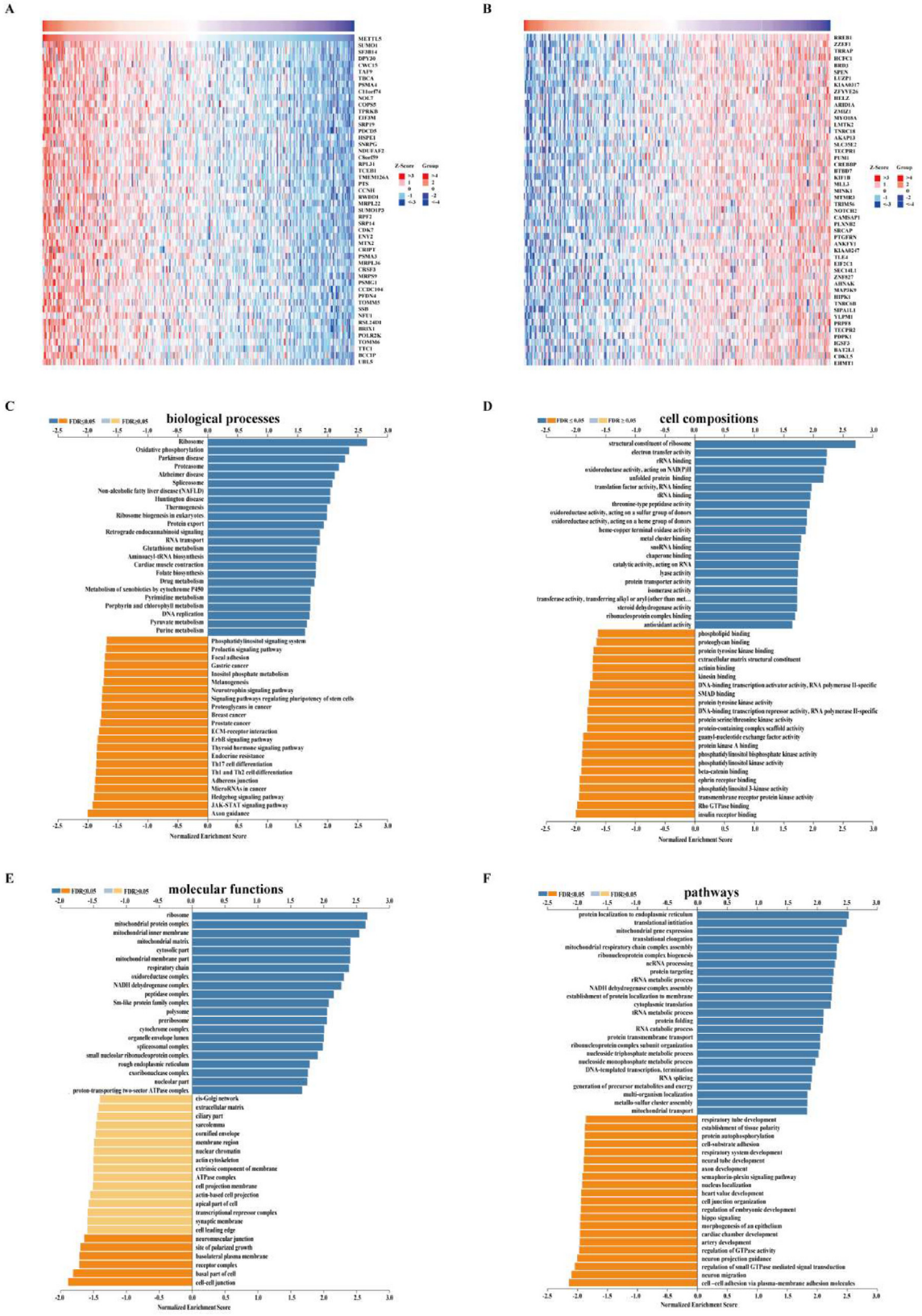


**Figure 4.** The biological processes and pathways that METTL5 potentially involved in the carcinogenesis of KC. (A) The top 50 positively co-expressed and (B) top 50 negatively co-expressed mRNAs of METTL5 in KC tissues. We analyzed these co-expressed mRNAs using the GSEA to explore the probable biological processes and pathways that METTL5 involved in. (C) The biological processes, (D) cell compositions, (E) molecular functions, (F) and pathways that the METTL5 co-expressed genes enriched in.

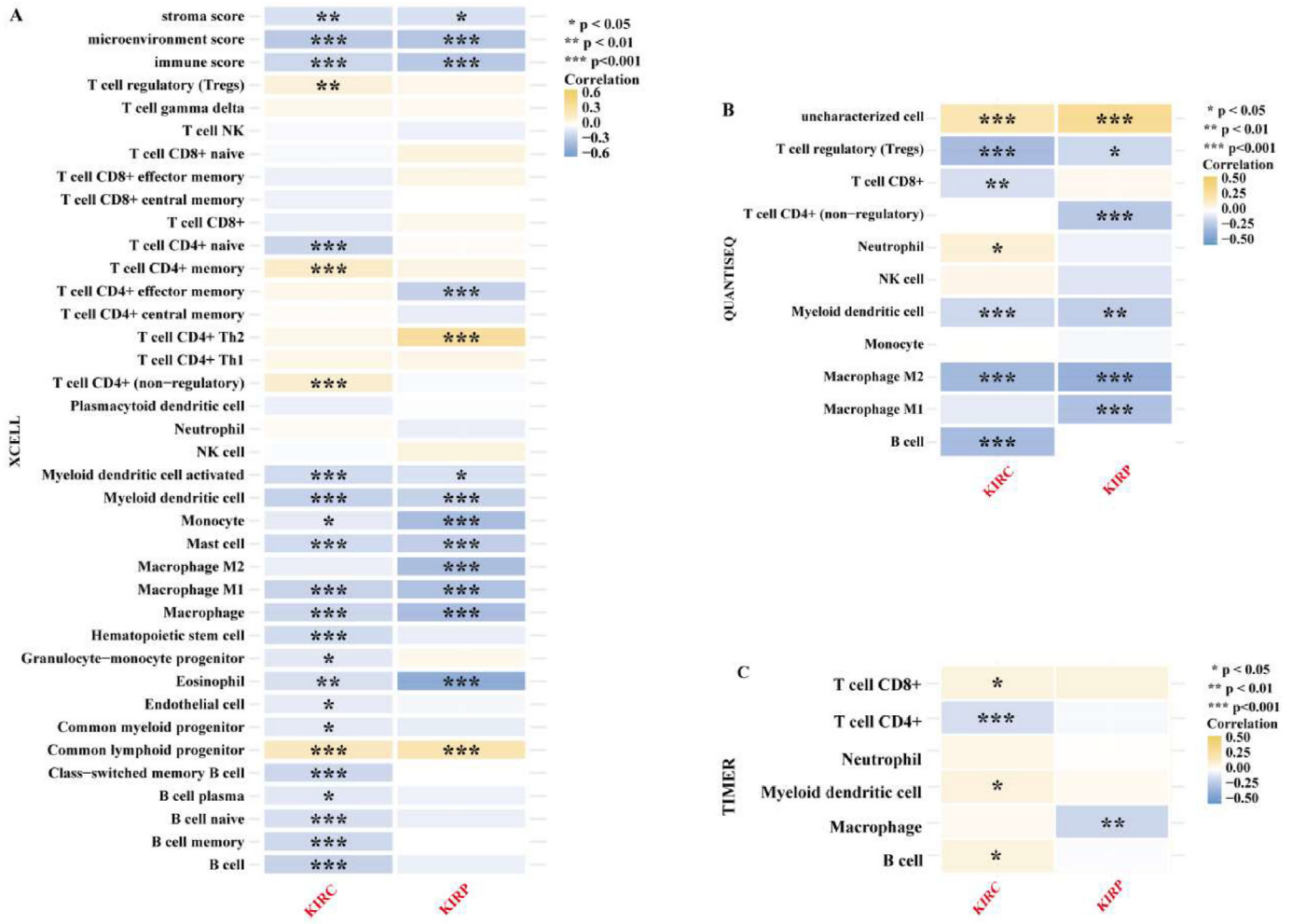


**Figure 5.** The correlation between the METTL5 expression and the ratios of infiltrating immune cells in distinct subtypes of KC. The correlation between the METTL5 expression and the levels of infiltrating immune cells in KICH, KIRC and KIRP as analyzed using the (A) xCell, (B) QUANTISEQ algorithms, and (C) TIMER 2.0 (*p < 0.05, **p < 0.01, ***p < 0.001).

negatively correlated with the METTL5 expression in KIRC as analyzed using TIMER 2.0. And the infiltration level of macrophages was negatively correlated with the METTL5 expression in KIRP.

As we know, the infiltrating immune cells have great effects on the progression of tumors [30]. The above-mentioned results revealed that the METTL5 expression was significantly correlated with the immune-related scores and infiltrating ratios of a variety of immune cells in KIRC and KIRP patients. To explore whether the probable immune regulation by METTL5 affected tumor development, we divided KIRC and KIRP patients into high/low infiltrating groups respectively, and investigated the effects of METTL5 expression on the OS of patients using the data sets from the Kaplan Meier Plotter database. As a result, the expression of METTL5 had different effects on the OS of KIRC and KIRP patients with different degrees of immune infiltration (Figure 6, Supplementary Figure 2). Moreover, this phenomenon was more pronounced in KIRP patients (Figure 6). Regarding to the basophils, CD8+ T cells, mesenchymal stem cells, and, macrophages, the METTL5 expression apparently had a stronger effect on the OS of patients with lower infiltration levels than patients with higher infiltration levels. For B cells, CD4+ memory T cells, nature killer T cells, and type 1 T helper cells, the METTL5 expression harbored a stronger influence on the OS of patients with higher infiltration levels than patients with lower infiltration levels. These results suggested that METTL5 might affect the patient survival by regulating the infiltration of multiple immune cells.

### *3.5. Two potential reasons for the elevated expression of METTL5 in KIRC tissues versus normal tissues*

To investigate the potential reasons involving in the up-regulated METTL5 expression in KIRC tissues versus normal adjacent tissues, we performed a multi-omics analysis to search the upstream transcription factors (TFs) of METTL5. Firstly, we obtained 19 experimentally-confirmed TFs of METTL5 identified in kidney tissues from the hTFtarget database (Supplementary Table S1). Among the 19 TFs, the expression of AR, EP300, NCOR1 and POLR2A were highly correlated with METTL5 as analyzed by LinkedOmics (correlation coefficient >0.45 or < -0.45). As is known to all, the alteration of protein content or modification of TFs both might cause changes in downstream gene transcription, thus we analyzed the alteration of AR, EP300, NCOR1 and POLR2A in protein and phosphorylation levels, and the results showed that the phosphorylation level of EP300 protein at T1698 was significantly increased in KIRC tissues versus normal tissues (Figure 7A), and the phosphorylation level of POLR2A at Y1860 was significantly decreased in KIRC tissues versus normal tissues (Figure 7B). These results demonstrated that the changes in phosphorylation contents of EP300 and POLR2A might be one of the reasons for the up-regulation of METTL5 expression in KIRC tissues.

In addition, we also investigated the DNA changes of METTL5 in KIRC patients using the data sets from the cBioPortal database. The results

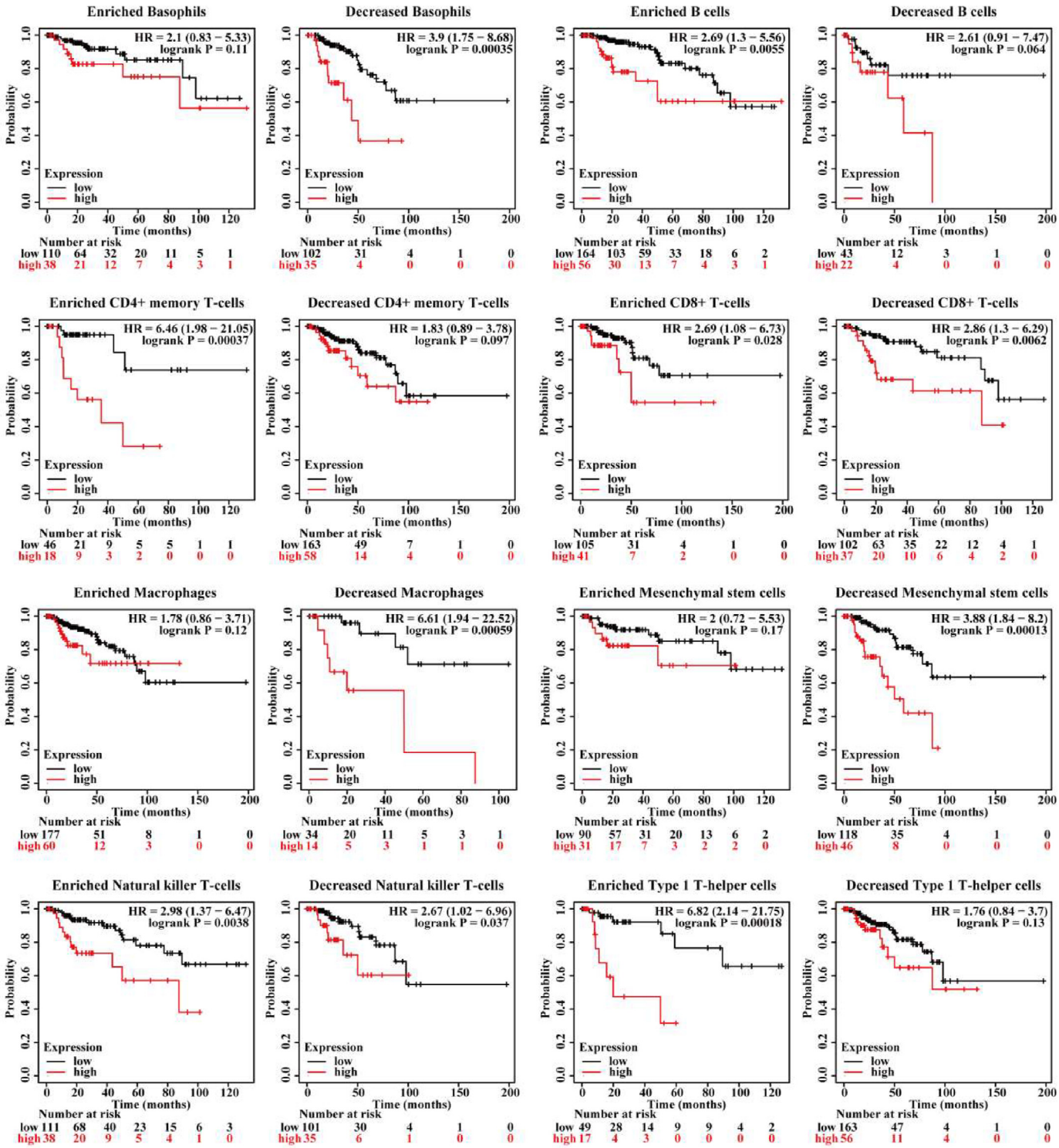


**Figure 6.** METTL5 might affect tumor development through modulating the immune cell infiltration. The Kaplan-Meier survival curves of METTL5 in KIRP patients with high or low infiltration levels of immune cells. Regarding to the basophils, CD8+ T cells, mesenchymal stem cells, and, macrophages, the METTL5 expression apparently had a stronger effect on the OS of patients with lower infiltration levels than patients with higher infiltration levels. For B cells, CD4+ memory T cells, nature killer T cells, and type 1 T helper cells, the METTL5 expression harbored a stronger influence on the OS of patients with higher infiltration levels than patients with lower infiltration levels.

showed that over 70% of METTL5 mutations in KIRC patients were amplification (Figure 7C), and the METTL5 expression was higher in the amplification group than the median values of all mutations (Figure 7D). This indicated that the amplification of METTL5 might also be a reason for the increased expression of METTL5 in KIRC tissues versus normal adjacent tissues.

## 4. Discussion

The prognostic genes can distinguish high-risk patients from low-risk patients at the molecular level, providing an individualised care plan, that will ensure optimum patient outcomes. However, there are no prognostic genes in clinical diagnosis and treatment of KC. In this study, we

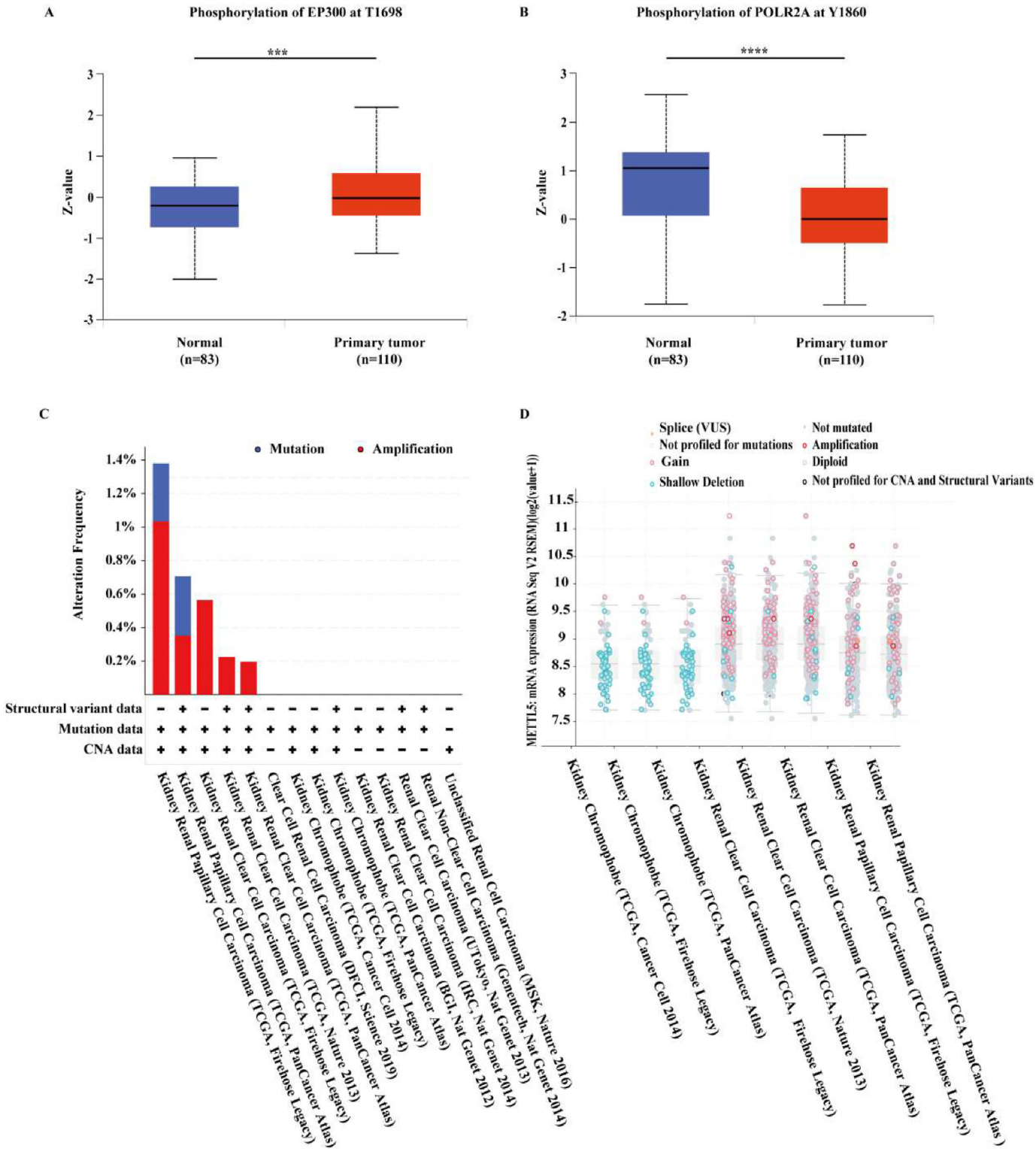


**Figure 7.** Two potential reasons for the higher expression of METTL5 in KIRC tissues versus normal tissues. (A) The alteration of EP300 and (B) POLR2A in phosphorylation level, the phosphorylation level of EP300 protein at T1698 was significantly increased in KIRC tissues versus normal tissues, and the phosphorylation level of POLR2A at Y1860 was significantly decreased in KIRC tissues versus normal tissues. (C) The mutation frequency of METTL5 in KC patients, and over 70% of METTL5 mutations in KIRC patients were amplification. (D) The expression of METTL5 in tumor tissues from KC patients with distinct METTL5 mutations, and the METTL5 expression was higher in the amplification group than the median values of all mutations. Fold change >1.2, and *p < 0.05, **p < 0.01, ***p < 0.001.

revealed that METTL5 was significantly up-regulated in KIRC and KIRP tissues versus normal controls (Figure 1), and the Kaplan Meier analysis and cox regression analysis uncovered METTL5 as a potential independent prognostic gene of KC (Figure 4). Therefore, METTL5 may be applied as one probable prognostic indicator for KC in clinic.

Recently, METTL5 has been identified as a methyltransferase responsible for the m6A modification of 18S rRNA [14, 15]. Several recent studies have shown that METTL5 has an important role in tumorigenesis of some cancers, such as pancreatic cancer, breast cancer, and gastric cancer [16, 18, 19]. For example, METTL5 can promote the

progression of pancreatic cancer by modulating the translation of c-Myc [31]. METTL5 may trigger the translation initiation in breast cancer and then induce the proliferation of cancer cells [16]. Also, METTL5 is one potential prognostic biomarker in gastric cancer [18]. In our previous studies, we discovered that METTL5 had a stronger association with the HR of KC more than most tumors (Wei Zhang, *et al*), suggesting a special role of METTL5 in the initiation and progression of KC. In this study, we disclosed the probable immune functions of METTL5 in KC. To the best of our knowledge, it may be the first time for the discovery of METTL5's functions in regulating the immune microenvironment (Figures 4, 5, and 6).

As is known to all, the infiltrating NK cells, NKT cells and CD8+ T cells within cancers frequently kill tumor cells [32, 33], and regulatory T cells (Tregs) and M2 macrophages always promote tumor progression [34, 35]. In this study, we observed that the METTL5 expression was negatively correlated with the infiltrating ratios of NK and NKT cells, suggesting that the oncogenetic functions of METTL5 in KIRC and KIRP might be relevant to the infiltration inhibition of NK and NKT cells. The previous studies have shown that tumor infiltrating B cells have a strong correlation with the patient response to immune checkpoint inhibitor (ICI) treatment [36, 37, 38]. Our study revealed that the expression of METTL5 was negatively correlated with the ratios of infiltrating B cells in KIRC, suggesting that METTL5 might have an effect on the responses of ICI treatment. The relationship between the METTL5 expression and immune response may be further studied in the future.

Here, we demonstrated the expression, prognostic value, potential mechanism, and probable regulatory upstream factors of METTL5 in KC using the data sets from the public databases. But there are still some questions, which needs to be answered in future. For instance, the METTL5 expression in KICH tissues was not consistent with its effects on patient survival, which indicated a larger cohort might be needed for further confirmation. Secondly, although the correlation between METTL5 expression and the infiltrating degrees of immune cells in KC had been disclosed in this study, the mechanism of METTL5 regulating the immune microenvironment in KC still needed to be further explored. In addition, bacterial infections are a common complication of cancer patients undergoing chemotherapy or radiation therapy [39, 40], which also contribute to immune cell infiltration. This study did not exclude patients with possible bacterial infections. Therefore, METTL5 expression needs to be further examined in patients with confirmed absence of bacterial infection.

In conclusion, we revealed a higher mRNA expression of METTL5 in KIRC and KIRP tissues versus normal adjacent cells, uncovered its prognostic role in KC, and demonstrated its potential immunological functions in tumorigenesis. All in all, we revealed for the first time that METTL5 might be a potential survival-related gene that regulated the immune microenvironment of KC.

## Declarations

### *Author contribution statement*

Wei Zhang: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data.

Yumei Chen: Analyzed and interpreted the data; Wrote the paper.

Zhipeng Zeng: Performed the experiments; Analyzed and interpreted the data.

Yue Peng: Performed the experiments; Wrote the paper.

Lintai Li; Nan Hu: Performed the experiments.

Xucan Gao; Wanxia Cai: Analyzed and interpreted the data.

Lianghong Yin; Yong Xu; Donge Tang: Conceived and designed the experiments.

Xinzhou Zhang; Yong Dai: Contributed reagents, materials, analysis tools or data.

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### *Data availability statement*

Data included in article/supplementary material/referenced in article.

### *Declaration of interest's statement*

The authors declare no conflict of interest.

### *Additional information*

No additional information is available for this paper.

## Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2022.e12078.

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