

WITHDRAWN: Expression and Prognosis Analysis of *JMJD5* in Human Cancers

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The full text of this preprint has been withdrawn by the authors while they make corrections to the work. Therefore, the authors do not wish this work to be cited as a reference. Questions should be directed to the corresponding author.

Abstract

JumonjiC (JmjC) domain-containing protein 5 (*JMJD5*) plays an important role in cancer metabolism. However, the prognostic value of *JMJD5* in most human cancers is still unknown. In this study, we aim to investigate the expression and prognostic value of *JMJD5*, immune cells infiltration, and the correlations among them. We found: The expression of *JMJD5* was significantly lower in human breast carcinoma (BRCA), cholangiocarcinoma (CHOL), liver hepatocellular carcinoma (LIHC) and lung cancer (LUC) but higher in prostate adenocarcinoma (PRAD) and stomach adenocarcinoma (STAD) comparing to their respective normal tissues by online Tumor Immune Estimation Resource (TIMER) and immunohistochemistry (IHC) of tissue microarray sections (TMAs) respectively. And high expression of *JMJD5* has better prognosis only in BRCA, LIHC, LUC but the opposite effect in STAD by the Kaplan-Meier Plotter databases analyses. Further analysis revealed *JMJD5* expression is significant correlation with the abundance of six immune cells infiltration in above four cancers by TIMER, and evaluated the prognostic value by the combination of *JMJD5* expression with either B cells or macrophages tumor infiltration by the COX proportional hazards model. Both the BRCA or lung adenocarcinoma (LUAD) patients with abundance of B cell and the STAD patients with low level of macrophage have a better cumulative survival by TIMER. In conclusion, we provided novel evidence of *JMJD5* as an essential prognostic biomarker in cancers through analyses the correction of the *JMJD5* expression, tumor-infiltrating B cells and macrophages and prognostic value. This study offers new perspectives therapeutic target in BRCA, LUAD and STAD.

Introduction

Proteins containing JmjC domains have been identified as a novel demethylase signature motif contributing to various human cancers through epigenetic remodeling[1-3]. It was predicted that JmjC domain is a metalloproteinase folded with copper protein and a candidate enzyme for regulating chromatin remodeling [4]. In addition to histone demethylase activity, some members of JmjC family also have protein hydroxylase and RNA hydrogenase activities, such as *JMJD5*, *JMJD6*, etc[5]. Besides histone modifications, the substrate of JmjC protein family also included many other function proteins, such as transcription factors, signal molecules, shear related proteins, etc, all of which are involved in physiological and pathological processes such as oxidative stress and cell development[6, 7]. Further studies showed that dysregulation of JmjC family members, such as *JMJD5*, *JMJD6*, *JARID1B* and *JMJD2A* caused abnormal embryonic development or promoted tumor cells proliferation and migration[3, 8].

JmjC domain-containing protein family contains more than thirty members, all of them have the same JmjC domain which owns the activity that catalyzes the demethylation of mono-, di- or trimethylated lysines[9]. *JMJD5* (also known as *KDM8*) is a member of JmjC domain-containing protein family. Recent studies reported that *JMJD5* is a cathepsin L-type protease, which mediates the hydrolysis and cleavage of histone H3 N-tail protein under stress conditions, resulting in a DNA damage response[10]. *JMJD5* only cleaves Kme1 H3 peptides, while little or no cleavage effect of *JMJD5* on dimethyl-lysine (Kme2),

trimethyl-lysine (Kme3), indicating that H3 N-tail cleavage plays a role in gene expression regulation [10]. Another research showed that *JMJD5* might play an important role in cell cycle regulation, *JMJD5* promoted CyclinA1 expression by affecting dimethylation of histon (H3K36) on CDKN1A gene locus and further accelerated cell cycle G2 /M [11]. Knock-out of *JMJD5* in mice caused embryonic lethality, suggesting that *JMJD5* played an important role in mammalian embryogenesis[12].

Studies of a similar protein of *JMJD5* in mouse indicated a potential role for this protein as a tumor suppressor. It was reported that *JMJD5* interacted with *p53* and negatively regulated *p53* function in control of cell cycle and proliferation in human lung cancer[13]. *JMJD5* is up-regulated by hypoxia and is crucial for hypoxia-induced cell proliferation in human breast cancer[14]. Research in liver cancer showed that *JMJD5* is a tumor suppressor gene in human LIHC pathogenesis, and the epigenetic silencing of *JMJD5* promotes LIHC cell proliferation by directly downregulating *CDKN1A* transcription[15]. On the contrary, another research revealed that *JMJD5* is a potential oncogene for colon carcinogenesis[16].

Based on above mentioned, the expression of *JMJD5* is different in human distinct cancers. However, the detail expression level, the immune cells infiltration and prognostic value of *JMJD5* in most human cancers are still unknown. In this study, we sought to examine the detail expression, prognostic value of *JMJD5* and the correlations between expression of *JMJD5* and the infiltration of immune cells in human tumors.

Materials And Methods

Data mining of *JMJD5* mRNA expression by TIMER database

The mRNA expression of *JMJD5* in different types of tumor tissues was analyzed by TIMER[17, 18] database (<http://timer.cistrome.org/>). TIMER is a comprehensive resource for systematical analysis of diverse cancer types. The “Gene-DE” module in TIMER was used to detect the differential expression between tumor tissues and adjacent normal tissues for *JMJD5* across TCGA tumor resources. The mRNA expression levels of *JMJD5* was displayed using box plots showing with the median, spread and outliers by RNA-Seq normalized by transcript per million (TPM) across normal and cancerous tissues. The statistical significance computed by differential analysis (edgeR) is annotated by the number of stars (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ∴: $p < 0.1$).

JMJD5 protein expression analyze byIHC in human tumor tissues and adjacent normal tissues

Materials and ethics statement

All samples were obtained from patients with cancers who had surgery in Huaihe Hospital of Henan University. The tissues fixed in 4% buffered formaldehyde and embedded in paraffin for constructing TMAs. This study was approved by ethics committee of Huaihe Hospital of Henan University, and written informed consent was obtained from each patient. All cases were diagnosed histologically according to the World Health Organization classification.

TMA construction

One core (1mm diameter) was removed from each paraffin embedded sample and inserted into a blank paraffin block. Each section comprised of up to 80 cores. Two separate TMAs were made, containing 14 kinds of different cancers. (For detail see **Table1**).

IHC of TMAs

The well-made paraffin-embedded TMA blocks were cut at a 4 μ m thickness, which were mounted on microscope slides. The detailed IHC protocol was available in our previous article[19]. Briefly, The sections incubated with the first antibody overnight at 4°C with rabbit anti-human *JMJD5* polyclonal antibody (1:250, Abcam #28883, USA.) were washed with Tris-buffered saline and were then incubated with the MaxVision TM HRP-Polymer anti-Rabbit IHC Kit (Maixin, Fuzhou, China) for 15 min at room temperature. The sections were visualized using the DAB Detection Kit (Maixin, Fuzhou, China), and the reaction was followed by counterstaining with hematoxylin.

Digital imaging of the TMAs

Stained sections from the TMAs were scanned using a ScanScope T2 automated slide-scanner (Aperio Technologies, Vista, CA, USA). These sections were scanned at 200 \times magnification and the digital images were extracted as individual TIFF files.

Evaluating of *JMJD5* protein expression

The evaluation of the IHC staining was performed independently by two authors without knowledge of the clinicpathological information. The quantification of IHC was formalized into a parameter called mean optical density detected by Image-Pro Plus2.0 (Media Cybernetics), which was obtained by dividing the optical density cumulative value of each point on the picture by the area of the target distribution.

Survival analysis in Kaplan-Meier Plotter, PrognoScan and GEPIA database

The prognostic significance of the mRNA expression of *JMJD5* in cancers was evaluated using the Kaplan-Meier plotter[20] (www.kmplot.com), GEPIA (<http://gepia.cancerpku.cn/>) and PrognoScan (<http://dna00.bio.kyutech.ac.jp/PrognoScan/index.html>)[21-23]. All of them are web-based tools to deliver fast and customizable functionalities based on online database including gene expression data and clinical data. We explored the expression of *JMJD5* on RFS in above significant expression cancer types by these three platforms. Survival analyses were carried out to achieve KM plots. P-value < 0.05 was considered to indicate a statistically significant result. Hazard ratios (HRs) with 95% confidence intervals (95%CI) and log-rank P-values were calculated.

Estimation of correlations between *JMJD5* expression level and abundances of six tumor-infiltrating immune cells in TIMER

The relationships between *JMJD5* expression and tumor-infiltrating immune cells were determined using the TIMER which is a web resource for systematical evaluations of the clinical impact of different immune cells in diverse cancer types. In “Gene” module of TIMER, we explored the correlation between expression of *JMJD5* and abundance of tumor-infiltrating immune cells in BRCA, LIHC, LUAD, LUSC and STAD. The abundances of six tumor-infiltrating immune cells (B cells, CD4⁺ T cells, CD8⁺ T cells, macrophages, neutrophils and myeloid dendritic cells) were estimated by TIMER algorithm. Since most immune cell types were negatively correlated with tumor purity, tumor purity is a major confounding factor in this analysis. Therefore, we selected the “Purity” for adjustment. The relationship between the expression of *JMJD5* and tumor purity was also determined in this analysis. The partial spearman’s association analysis was used to determine the correlation coefficient.

Correlations between survival and immune cell infiltrations in TIMER analysis

To clarify more precisely the characteristics of tumor-infiltrating immune cells in the immune microenvironment, we then examined the above six immune-infiltrating cells and performed KM analysis by “Survival” module in TIMER. KM curves were drawn by Cox proportional hazard model for the corresponding above six immune infiltrates and BRCA (1017 patients with 146 dying), LIHC (362 patients with 127 dying), LUAD (482 patients with 174 dying), LUSC (482 patients with 205 dying) and STAD (387 patients with 148 dying). The infiltration level was divided into low and high levels. Hazard ratios (95% CIs) and log-rank P-values were calculated for Cox model and showed on the KM curve plot.

Prognostic impact of combinations of *JMJD5* expression and tumor-infiltrating immune cells

We sought to identify whether the expression of *JMJD5* and the abundance of six tumor-infiltrating immune cells have an effect on the survival of patients in BRCA, LIHC, LUAD, LUSC and STAD. Patients were divided into four groups, 1: low *JMJD5* expression + low tumor-infiltrating immune cells, 2: low *JMJD5* expression + high tumor-infiltrating immune cells, 3: high *JMJD5* expression + low tumor-infiltrating immune cells, and 4: high *JMJD5* expression + high tumor-infiltrating immune cells. COX proportional hazards model was used to draw KM plots for *JMJD5* expression level and immune infiltrates to visualize the survival differences. Expression level of *JMJD5* and six immune infiltrates were divided into low and high levels by 50%. P-value of log-rank test for comparing survival curves of four groups were showed in each plot.

Statistical analysis

Differential *JMJD5* expression was analyzed by digital gene expression data in R (edgeR) on RNA-Seq. Raw counts is annotated by the number of stars (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; · : $p < 0.1$). The *JMJD5* protein expression levels were analyzed with GraphPad Prism5 and were presented as the means \pm SD. Statistical significances were calculated with T-test. Differences were considered statistically significant at $p < 0.05$. The results also annotated by number of stars (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, not significant). The DFS was calculated by the KM method. The log rank test was used to calculate the hazard ratios and logrank P -value in KM Plotter and GEPIA. A univariate Cox regression model was

used to calculate the HR and Cox P value in TIMER. The correlation of gene expression was evaluated using partial Spearman's correlation analysis, also $P < 0.05$ was considered statistically significant, if not specially noted.

Results

Expression of *JMJD5* was different in distinct human cancers

We investigated mRNA expression of *JMJD5* in different cancers using TIMER obtained from The Cancer Genome Atlas tumor (TCGA) database containing cancerous and normal human tissues. The results revealed that mRNA expression of *JMJD5* was significant higher in PRAD (**) and STAD (***), but lower in BRCA (*), GHOL (***), colon adenocarcinoma (COAD) (***), Kidney chromophobe cell carcinoma (***), LIHC (***), LUAD (·), LUSC (***), and Uterine corpus endometrial carcinoma (***), each compared to respective normal tissues. However, there was no difference between cancerous and normal tissues of mRNA expression of *JMJD5* in bladder urothelial carcinoma (BLCA). Unfortunately, it was impossible to obtain the comparison results between cancerous and normal tissues in cervix squamous cell carcinoma (CESC), ovarian cancer (OV) and pancreas invasive ductal carcinoma (PAAD) due to the lack of normal tissues (**Figure 1**).

To confirm the *JMJD5* protein expression and estimate its clinical significance in cancers, we explored the protein expression of *JMJD5* in TMAs from 14 kinds of different cancers obtained from patients who had surgery in Huaihe Hospital of Henan University by using IHC analysis. The quantification of IHC was formalized into mean optical density detected by Image-Pro Plus software. As shown in (**Figure 2A**), the protein expression of *JMJD5* was significant higher in BLCA (*), CESC (*), OV (*), PAAD (*), PRAD (**) and STAD (***) than in their respective normal tissues, but was significantly lower in BRCA (*), CHOL (*), KIRC (**), LIHC (*), LUAD (**) and LUSC (*) than in their each normal tissues (**Figure 2B**). *JMJD5* protein level was higher in COAD than respective normal tissue, but there is no statistical significant difference (**Figure 2A**). Additionally, we found *JMJD5* exists in different parts of tumor cells by IHC staining, either in nuclei or in cytoplasm, or in both. The *JMJD5* protein expression was observed only in nuclei in BRCA and LUAD (**Figure 2B**), only in cytoplasm in COAD, OV, PAAD, PRAD, STAD and CHOL, kidney renal clear cell carcinoma (KIRC), LIHC, LUSC and Uterine corpus endometrial carcinoma (**Figure 2A and B**), and in both nuclei and cytoplasm in BLCA and CESC (**Figure 2A**).

Expression of *JMJD5* can serve as prognostic marker in BRCA, LIHC, LUC and STAD

We proceeded to determine whether the expression of *JMJD5* is associated with the prognosis of cancer patients via online resource of Kaplan-Meier Plotter, GEPIA and PrognoScan. Among the cancers (BLCA, BRCA, CESC, OV, PAAD, PRAD, STAD, CHOL, KIRC, LIHC, LUAD and LUSC) with significantly different expression of *JMJD5*, we identified *JMJD5* as prognostic marker by analyzing relapse free survival (RFS) in BRCA (n=3955, RFS: HR=0.75, 95% CI from 0.67 to 0.83, log-rank P = 1.4e-07) (**Figure 3A**), LIHC (n=364, RFS: HR=0.6, 95% CI from 0.42 to 0.85, log-rank P =0.0033) (**Figure3B**), LUC (n=1927, RFS: HR=0.81, 95% CI from 0.71 to 0.92, log-rank P =0.001) (**Figure3C**) and STAD (n=881, RFS: HR=1.25, 95% CI from 1.05 to

1.49, log-rank $P = 0.011$) (**Figure 3D**). These results showed that the patients with significant higher expression of *JMJD5* have an improved survival rate in BRCA, LIHC and LUC. On the contrary, the patients with low expression of *JMJD5* were correlated with a better survival rate in STAD. In brief, the expression level of *JMJD5* impacted RFS and could be served as prognostic marker in BRCA, LIHC, LUC and STAD.

Expression of *JMJD5* was strongly correlated with tumor-infiltrating immune cells in BRCA, LIHC, LUAD, LUSC and STAD

To further explore the potential relationships between the expression of *JMJD5* and infiltration of immune cells, we analyzed the correlation between *JMJD5* expression and six tumor-infiltrating immune cells (B cells, CD4⁺ T cells, CD8⁺ T cells, macrophages, neutrophils, and myeloid dendritic cells) focusing on BRCA, LIHC, LUAD, LUSC and STAD. The results demonstrated that the expression level of *JMJD5* has significant negative correlation with tumor purity, positive correlation with infiltration of B cell, CD4⁺ T cell, CD8⁺ T cell and macrophage and no relation with infiltrating of neutrophil, and myeloid dendritic cell in BRCA (**Figure 4A**). The expression level of *JMJD5* has no relation with tumor purity and infiltrating of macrophage, neutrophil, and myeloid dendritic cell, but significant positive correlation with infiltration of B cell, CD4⁺ T cell and CD8⁺ T cell in LIHC (**Figure 4B**). The expression level of *JMJD5* has significant negative correlation with tumor purity, positive correlation with infiltration of B cell, CD4⁺ T cell, macrophage, neutrophil and myeloid dendritic cell and no relation with CD8⁺ T cell in LUAD (**Figure 4C**). The expression level of *JMJD5* has significant negative correlation with tumor purity, positive correlation with infiltration of B cell, CD4⁺ T cell, CD8⁺ T cell, macrophage, neutrophil and myeloid dendritic cell in LUSC (**Figure 4D**). The expression level of *JMJD5* has no relation with tumor purity, but significant positive correlation with infiltration of B cell, CD4⁺ T cell, CD8⁺ T cell, macrophage and myeloid dendritic cell in STAD (**Figure 4E**). Taken together, the expression level of *JMJD5* was strongly correlated with immune cells infiltration in BRCA, LIHC, LUAD, LUSC and STAD.

Correlation analysis between survival rate and immune cells infiltration in BRCA, LIHC, LUAD, LUSC and STAD

We analyzed the association between clinical outcome and abundance of six immune cells infiltration in TIMER database. Kaplan–Meier (KM) survival analyses displayed that the more amount of tumor-infiltrating B cells the longer survival rate in BRCA (log-rank $P = 0.046$) and LUAD (log-rank $P = 0$) (**Figure 5A and C**, labeled with blue box). The lower amount of tumor-infiltrating macrophages the longer survival rate in STAD (log-rank $P = 0.004$) (**Figure 5E**, labeled with blue box). In addition to the tumors described above, the other immune cells tumor-infiltrating levels have no correlation with survival rate in the other types of tumors (**Figure 5A-E**). To sum up, B cells could be used as a prognostic marker for BRCA or LUAD and macrophages could be served as a prognostic marker for STAD.

Prognostic value of combinations of *JMJD5* expression and tumor-infiltrating immune cells

To further investigate the prognostic value of combinations of *JMJD5* expression and tumor-infiltrating immune cells. Here, we made a COX proportional hazards model analysis of clinical outcome, the immune cell infiltration and expression of *JMJD5* in BRCA, LUAD and STAD. The KM plots curve showed that patients with low expression of *JMJD5* have an improved survival rate comparing their tumor with higher B cell infiltration to lower B cell infiltration in BRCA (n=1100, OS: HR=0.658, P =0.0487) (**Figure 6A**) and LUAD (n=515, OS: HR=0.553, P =0.00415) (**Figure 6B**). The STAD patients with low expression of *JMJD5* have a better survival rate comparing their tumor with lower macrophage infiltration to higher macrophage infiltration (n=415, OS: HR=1.7, P=0.02) (**Figure 6C**). The combination of *JMJD5* expression with either B cells tumor infiltration or macrophages tumor infiltration could be served as a new tumor prognostic value.

Discussion

It is known that JmjC domain-containing protein family contains more than thirty members, and many members are aberrant expression or dysregulated in many kinds of human cancers and can regulate the proliferation and invasion of tumor cells[24, 25]. For example, the aberrant expression of some family members, such as *PHF8*, *KDM3B* and *JMJD2A*, can promote the proliferation and metastasis of tumor cells in PRAD and BRCA[26, 27]. *JMJD5* is a member of JmjC domain-containing protein family, but the expression, prognosis value and especially tumor immune infiltration in most human cancers are still unclear. In this study, we presented the protein expression of *JMJD5* in almost all human cancers for the first time and found that *JMJD5* was overexpression in STAD and high expression of *JMJD5* indicated poor survival. Conversely, *JMJD5* was underexpression in BRCA, LIHC and LUC and low expression of *JMJD5* associated with a poor outcome. Therefore, *JMJD5* is not only potential biomarkers of prognosis but may also be therapeutic target for BRCA, LIHC, LUC and STAD.

Recent research reported that tumor-infiltrating immune cells present in the tumor microenvironment either inhibiting or supporting the growth and development of tumors[28]. Tumor-infiltrating immune cells in the tumor microenvironment include those mediating adaptive immunity, T lymphocytes, dendritic cells and occasional B cells, as well as effectors of innate immunity, macrophages, polymorphonuclear leukocytes and rare natural killer cells[29]. Recently, these studies displayed that the existence of B cells in human tumors is associated with a favourable response to immunotherapy in *Nature*[30-32].

Furthermore, macrophages present in tumors are known as tumor-associated macrophages. They are re-programmed to inhibit lymphocyte functions through release of inhibitory cytokines[33, 34]. Up till now, JmjC domain-containing protein family has not been well-studied in immunooncology. There is no report on the relationship between *JMJD5* and immune cell infiltration. To address this deficiency, our results proved that mRNA expression of *JMJD5* could reflect immune cells infiltration in BRCA, LIHC, LUAD, LUSC and STAD and the infiltration by high B cells was a key discriminative feature of patients with low *JMJD5* in BRCA and LUAD with improved survival. Also the amount of immune-infiltrating macrophages can serve as a prognostic marker for STAD. This finding may have broad applications in tumor targeting therapy and immunotherapy.

In summary, our results displayed that the lower expression of *JMJD5* was in human BRCA, CHOL, LIHC and LUC but higher in PRAD and STAD comparing to their respective normal tissues. And high expression of *JMJD5* has better prognosis only in BRCA, LIHC, LUC but the opposite effect in STAD. Tumor-infiltrating B cells and microphages were independent prognosticators useful for evaluating the immune microenvironment of BRCA, LUAD and STAD. *JMJD5* might be an essential prognostic biomarker in cancers. This study offers new perspectives therapeutic target in BRCA, LUAD and STAD. Our further study aimed at the strategies how to regulate the expression of *JMJD5* and Tumor-infiltrating B cells and microphages in BRCA, LUAD and STAD, which may be a promising therapeutic approach in tumor treatment.

Abbreviations

Bladder urothelial carcinoma (BLCA); Breast invasive carcinoma (BRCA); Cervix squamous cell carcinoma (CESC); Cholangiocarcinoma (CHOL); Colon adenocarcinoma (COAD); Hazard ratio (HR); Immunohistochemistry (IHC); JumonjiC (JmjC); JumonjiC domain-containing protein 5 (JMJD5); Kidney renal clear cell carcinoma (KIRC); Kaplan–Meier (KM); Liver hepatocellular carcinoma (LIHC); Lung cancer (LUC); Lung adenocarcinoma (LUAD); Lung squamous cell carcinoma (LUSC); overall survival (OS); Ovarian cancer (OV); Pancreas invasive ductal carcinoma (PAAD); Prostate adenocarcinoma (PRAD); Relapse free survival (RFS); Stomach adenocarcinoma (STAD); Tissue microarray (TMA); 95% confidence intervals (95% CIs).

Declarations

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Z.S. and W.S. designed and supervised the entire study. H.L. and Q.L. conducted the experiments and analyzed data. H.L. and H.J. collected all samples for TMAs and analyzed the IHC results. W.S. wrote the manuscript. J.Z, H.Z, X.M, L.W and R.D. contributed to the discussion. Z.S. and Q.L. reviewed and edited the manuscript. All authors reviewed and approved the final manuscript.

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Tables

Table 1. All samples used in TMAs.

TCGA Abbr.	Organ	Cancer type	T(n.)	N(n.)
BLCA	Bladder	Urothelial carcinoma	5	5
BRCA	Breast	Invasive ductal carcinoma	5	5
CESC	Cervix	Adenocarcinoma	5	5
		Squamous cell carcinoma	5	5
CHOL	Biliary tract	Cholangiocarcinoma	5	5
COAD	Colon	Adenocarcinoma	5	5
KIRC	Kidney	Renal clear cell carcinoma	5	5
LIHC	Liver	Hepatocellular carcinoma	5	5
LUAD	Lung	Adenocarcinoma	5	5
LUSC		Squamous cell carcinoma	5	5
LULCC		Large cell carcinoma	5	5
OV	Ovary	Serous adenocarcinoma	5	5
PAAD	Pancreas	Invasive ductal carcinoma	5	5
PRAD	Prostate	Adenocarcinoma	5	5
STAD	Stomach	Tubular adenocarcinoma	5	5
UCEC	Uterus	Endometrioid adenocarcinoma	5	5

Figures

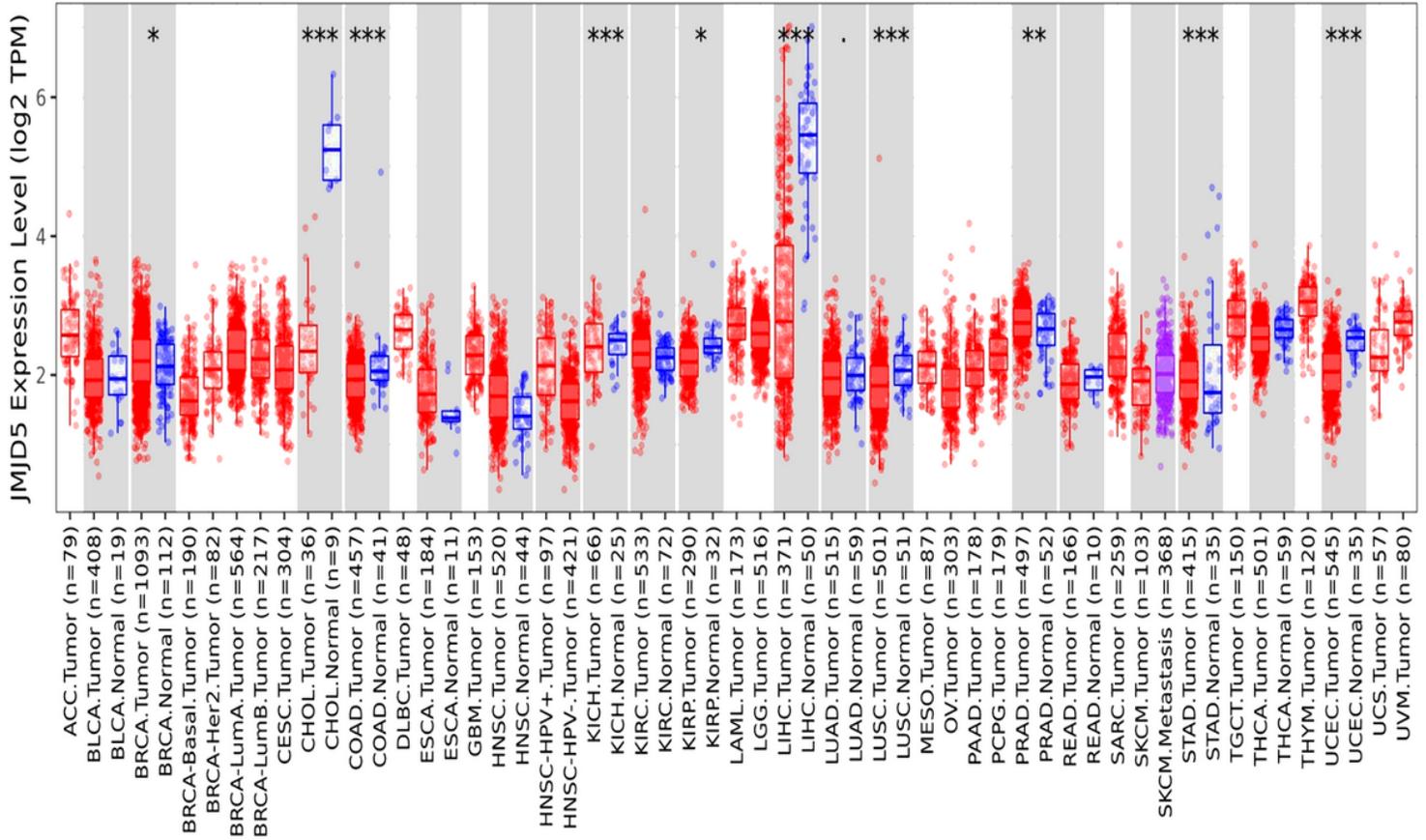


Figure 1

Human mRNA expression of JMJD5 in different cancer tissues compared with normal tissues in cancers from TCGA data in TIMER. Box plots showed the distributions (median, spread and outliers) of the JMJD5 mRNA levels (log2 TPM) by RNA-seq data, as displayed in gray columns when normal data are available. The number of samples was shown at the bottom. P-value significant codes: $0 \leq *** < 0.001 \leq ** < 0.01 \leq * < 0.05 \leq \cdot < 0.1$.

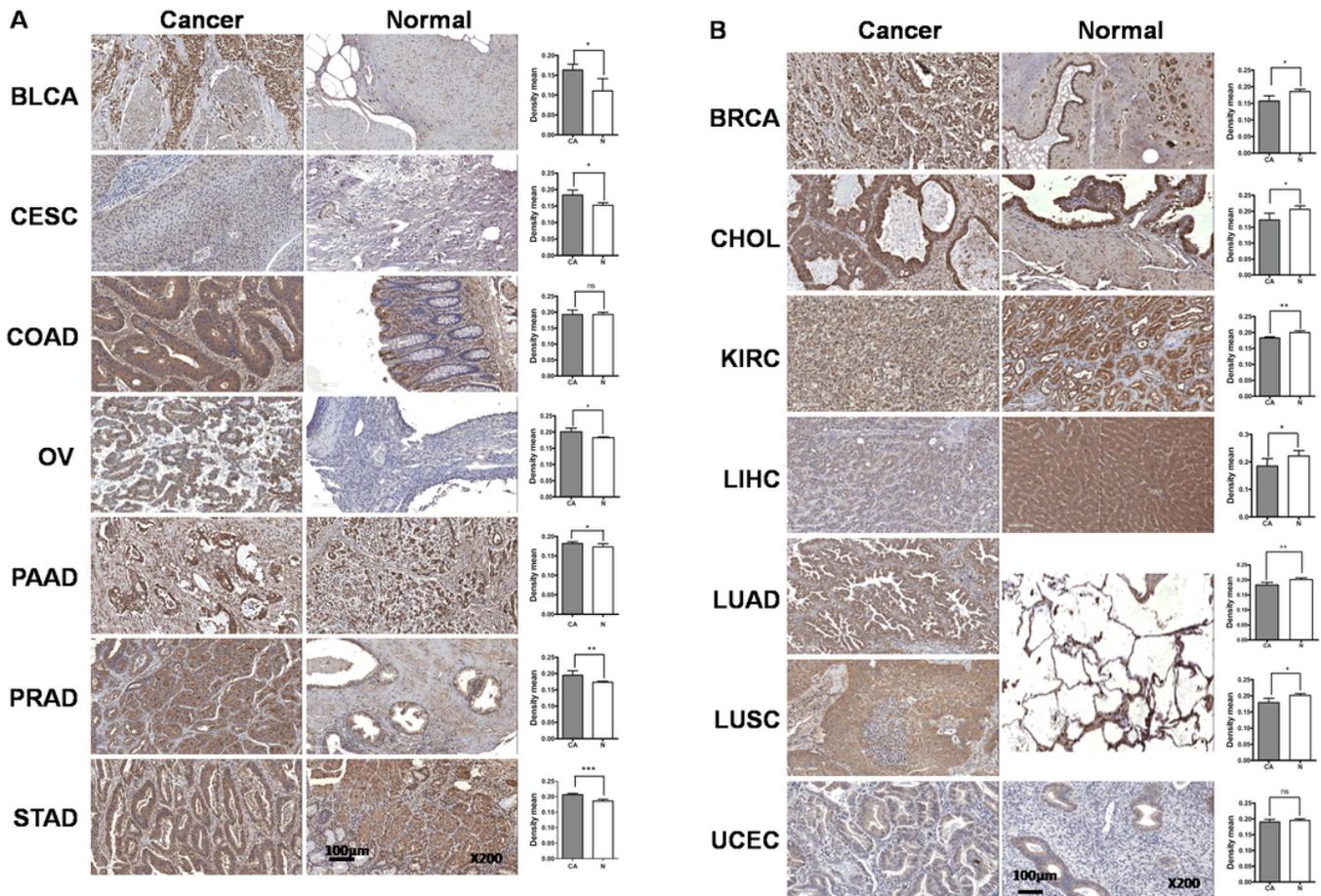


Figure 2

IHC staining of JMJD5 protein in different cancer tissues. A: The protein expression of JMJD5 was significantly higher in BLCA, CESC, OV, PAAD, PRAD and STAD than in their respective normal tissues. B: JMJD5 protein expression was significantly lower in GHOL, KIRC, LIHC, LUAD and LUSC than in their respective adjacent normal tissues. The quantification of IHC was formalized into mean optical density detected by Image-Pro Plus2.0. Bars show the means \pm SD. Difference was statistically significant (* $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$). Original magnification: 200 \times . Scale bar: 100 μ m.

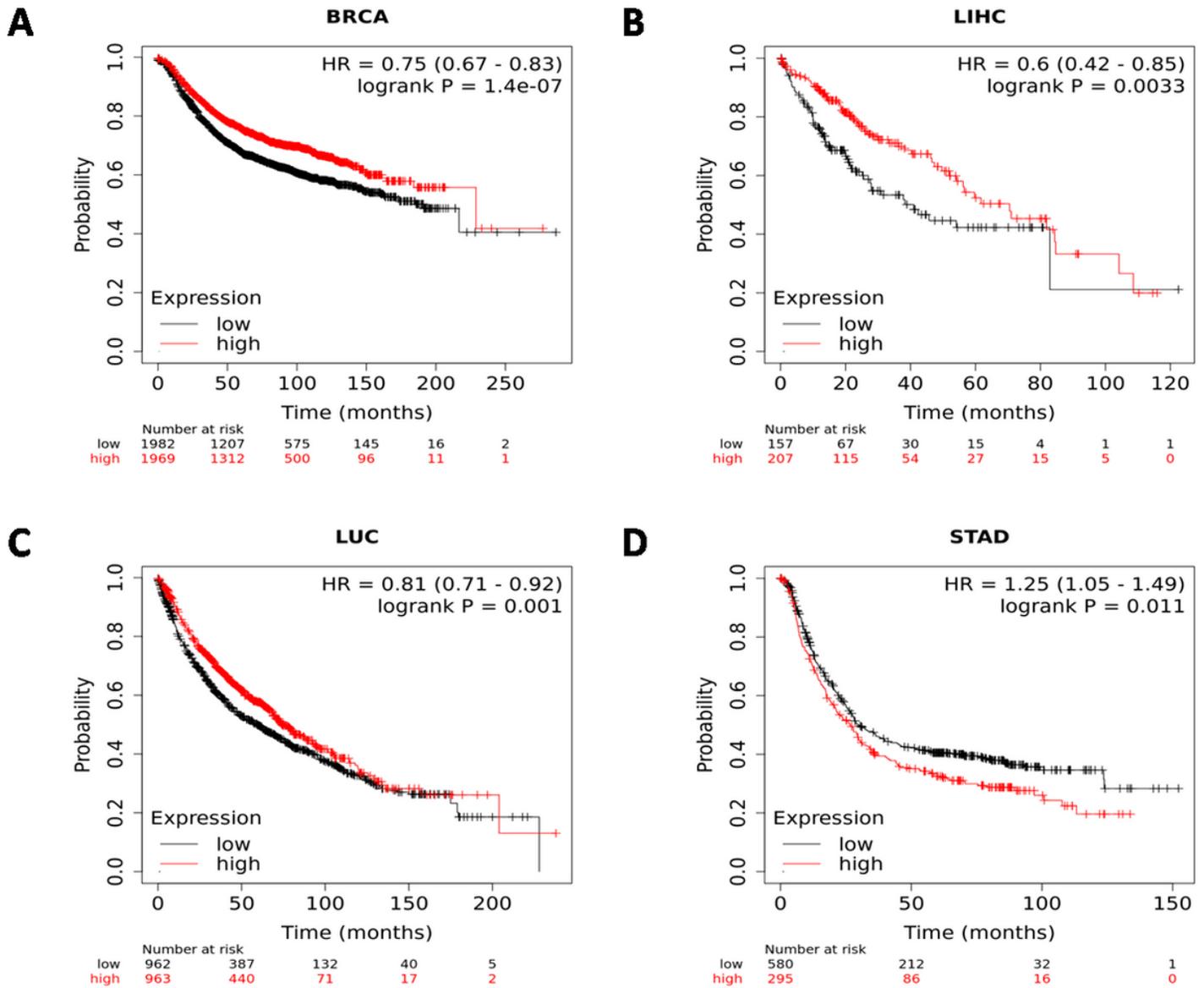


Figure 3

Kaplan-Meier survival curves comparing the high and low expression of JMJD5 in different types of cancers. A: RFS of BRCA, B: RFS of LIHC, C: RFS of LUC and D: RFS of STAD. Red curve represents patients with high expression of JMJD5, while black curve represents patients with low expression of JMJD5. HRs with 95% CIs and log-rank P-values were calculated and shown in each curve. $P < 0.05$ was considered statistically significant.

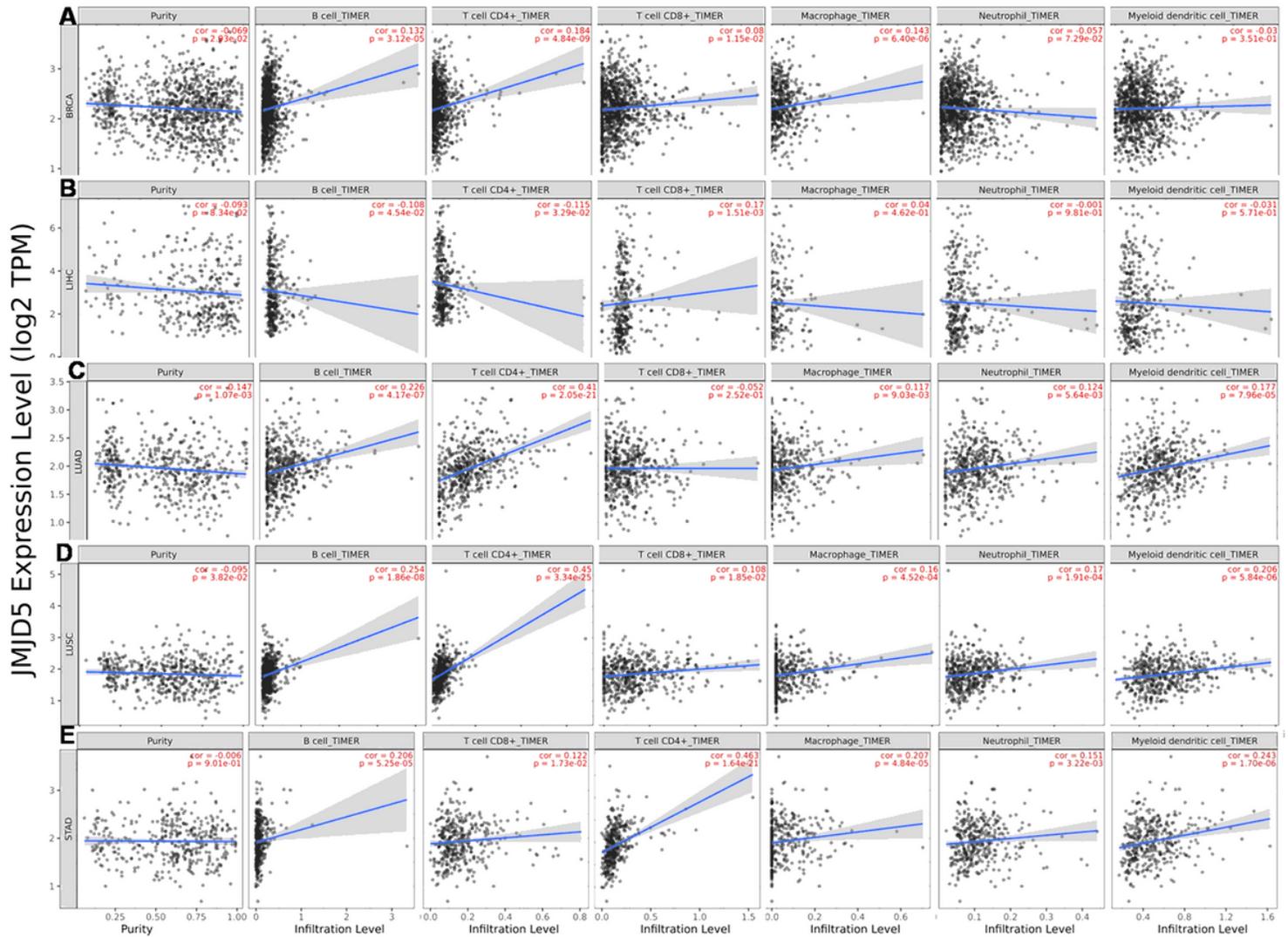


Figure 4

Correlation of JMJD5 expression with six tumor-infiltrating immune cells in BRCA, LIHC, LUAD, LUSC and STAD. Scatter plots were generated using the online tool TIMER to identify different profiles of infiltrating-immune cells associated with JMJD5 expression. Each dot represents a single tumor sample. $P < 0.05$ is considered as significant. (A) JMJD5 expression has significant negative relation with tumor purity, significant positive correlation with tumor-infiltrating levels of B cell, CD4+ T cell, CD8+ T cell, and macrophage, and no relation with in BRCA. (B) JMJD5 expression has no relation with tumor purity and significant positive correlation with tumor-infiltrating levels of B cell, CD4+ T cell in LIHC. (C) JMJD5 expression has significant negative relation with tumor purity and significant positive correlation with infiltrating levels of B cell, CD4+ T cell, macrophage in LUAD. (D) JMJD5 expression has significant negative relation with tumor purity and significant positive correlation with infiltrating levels of B cell, CD4+ T cell, CD8+ T cell, Macrophage and myeloid dendritic cell in LUSC. (E) JMJD5 expression has no relation with tumor purity, significant positive correlation with infiltrating levels of B cell, CD4+ T cell, CD8+ T cell, Macrophage and myeloid dendritic cell in STAD.

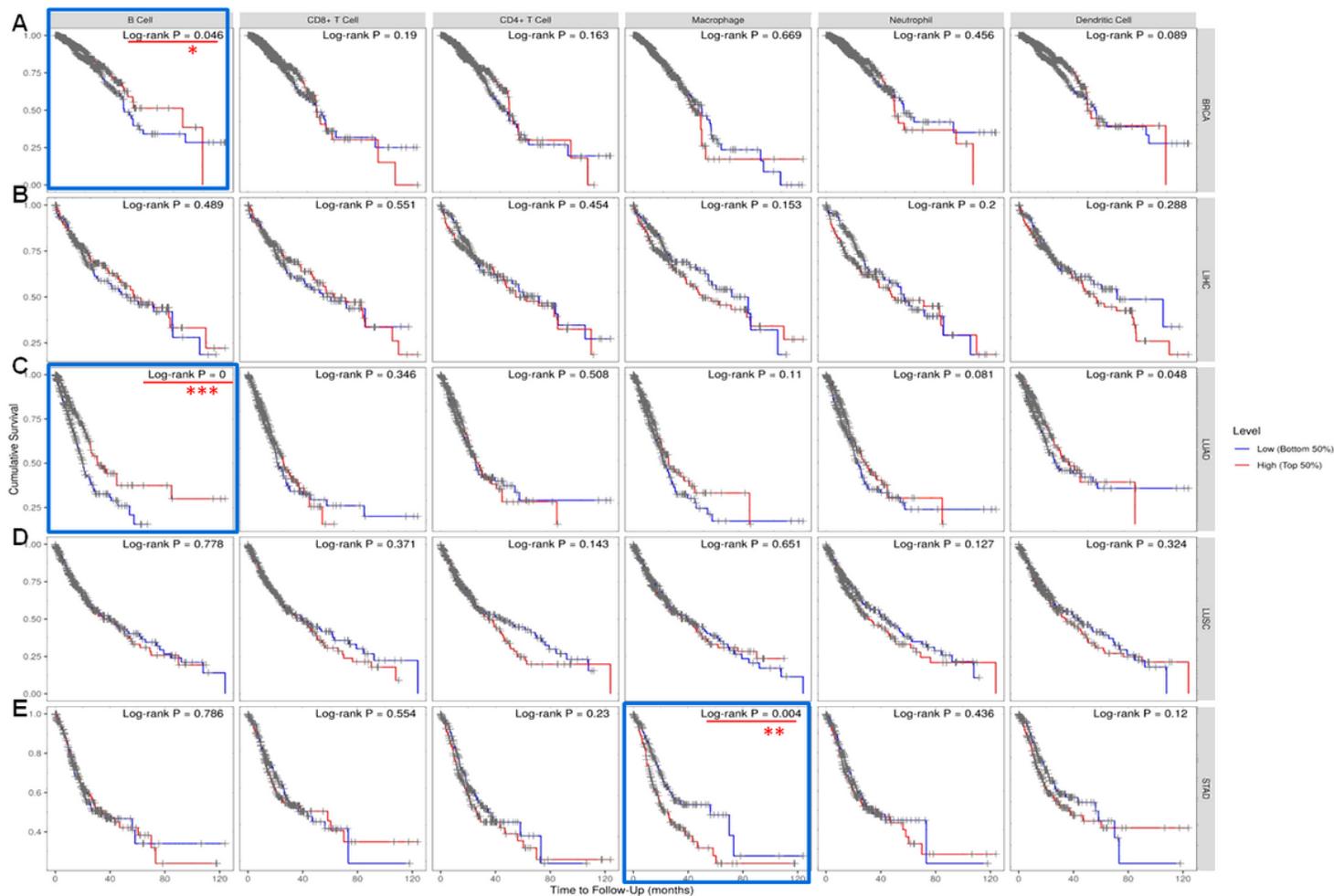


Figure 5

Kaplan-Meier survival curves showing comparison of overall survival between high (red) and low (blue) infiltration of immune six immune cells infiltration in BRCA, LIHC, LUAD, LUSC and STAD. P-values obtained from log-rank test.

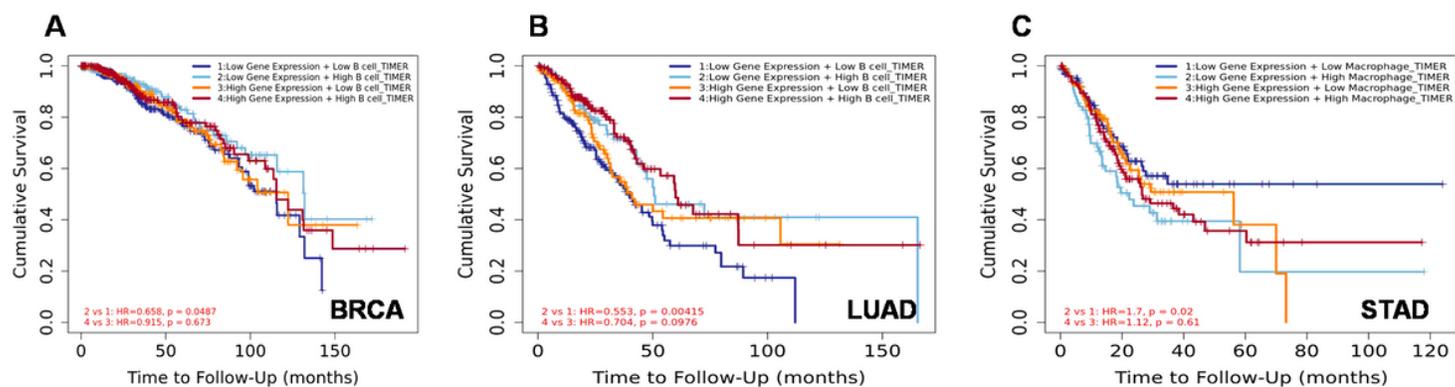


Figure 6

Kaplan-Meier plots for JMJD5 expression level and immune cell infiltrates to visualize the survival differences in BRCA, LUAD and STAD. A and B: Patients with low expression of JMJD5 have an improved

survival rate comparing their tumor with higher B cell infiltration to lower B cell infiltration in BRCA(A) and LUAD(B). The patients with low expression of JMJD5 have a better survival rate comparing their tumor with lower macrophage infiltration to higher macrophage infiltration.