Comprehensive Analysis of Expression and Prognostic Value for JMJD5 and PKM2 in Stomach Adenocarcinoma

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Abstract

Background: Jumonji C-domain-containing (JMJD) family, a group of genes that regulate epigenetics, is involved in tumor development in several types of cancer. JMJD5 is a member of the JMJD family, and its clinical impact on stomach adenocarcinoma (STAD) remains unclear. Pyruvate kinase M2 (PKM2) promotes metabolism, tumor proliferation, and metastasis in various cancer types. However, the relationship between JMJD5 and PKM2 in STAD is yet to be established. In this study, we investigated the expressions and relationship of JMJD5 and PKM2 in patients with STAD. Furthermore, we evaluated the clinical significance between their expression and prognosis. In addition, we explored the transcriptional and survival effects of other 7 members of the JMJD family including JMJD1B, JMJD1C, JMJD2D, JMJD4, JARID2, HSPBAP1, TYW5 in patients with STAD.

Methods: The expression of JMJD5 and PKM2 in STAD was examined using western blot, quantitative real-time polymerase chain reaction (RT-qPCR), and immunohistochemical staining. Statistical analyses were performed using the SPSS 22.0 statistical software program. The roles of JMJD1B, JMJD1C, JMJD2D, JMJD4, JARID2, HSPBAP1, TYW5 in STAD were examined using UALCAN, GEPIA, Kaplan–Meier Plotter,

the Human Protein Atlas, STRING, the cBiopotal, Metascape databases.

Results: We discovered that the rates of low expression of JMJD5 and high expression of PKM2 in the tumor cells of STAD were 64.52.% and 62.37%, respectively. Moreover, there was a close connection between the expressions of JMJD5 and PKM2. We uncovered that the low expression of JMJD5 was related to poor differentiation (P = 0.002) and large tumor size (P = 0.044). The survival rate was low in patients with low expression of JMJD5 and high expression of PKM2. In addition, we found that JMJD1B, JMJD1C, JMJD2D, JMJD4, JARID2, HSPBAP1, TYW5 were high in the STAD tissues. Besides, gene expression levels were correlated with tumor stage and grade. Survival analysis demonstrated that high expressions of these genes, except JMJD1B, were associated with low survival rates. Moreover, a high mutation rate of these genes (82.22%) was observed in STAD patients.

Conclusions: These findings implied that JMJD1B, JMJD1C, JMJD2D, JMJD4, JMJD5, JARID2, HSPBAP1, TYW5, and PKM2 could serve as potential therapeutic targets in patients with STAD and as novel biomarkers for the disease.

Introduction

Globally, gastric cancer is a major type of cancer, with an incidence rate of 5.6% and a mortality rate of 7.7% in 2020[1]. The carcinogenic effects of gastric adenocarcinoma are multi-factorial, and chronic helicobacter pylori infection, intestinal metaplasia, mucosal atrophy, diet, smoking, and excessive use of preserved foods are important risk factors[2]. With regard to therapeutics, the emergence of drug resistance and severe side effects make the treatment of gastric cancer difficult. Hence, it is extremely important to investigate novel and precise therapeutic targets for the treatment of gastric cancer. Besides, screening and early detection are likely to improve the prognosis of the patients.

Studies have shown that disorders in epigenetic alteration can lead to malignant modifications in the gastric cells[3]. Gastric cancer and adjacent tissues show differences in the epigenetic regulation of oncogenes and tumor suppressor genes [4]. Jumonji C-domain-containing (JMJD) protein family is an epigenetic regulatory complex; in humans, it is composed of 33 members[5]. The JMJD family is a histone lysine demethylase, and inadequate methylation of histone lysine has been observed in many cancers[6]. The activity of the JMJD family is essential for regulating gene expression, cell cycle, and differentiation. On the other hand, its dysregulation and gene damage may be key determinants of cancer[7].

JMJD5 is a member of the JMJD family, which has a major impact in various cancers. JMJD5 is highly expressed in breast, colon, oral squamous, and prostate cancer cells as well as in atypical meningiomas. However, its expression is decreased in liver and lung cancer cells. Thus, depending on the cancer type, JMJD5 might play the role of oncogene or tumor suppressor. JMJD5 induces the occurrence of epithelial–mesenchymal transition (EMT) by enhancing the expressions of Slug and Twist, thereby increasing the metastasis of colon, prostate, and oral cancer cells[8-10]. Furthermore, JMJD5 promotes the development and metabolism of breast cancer cells[11]. However, the roles of JMJD5 in the occurrence and evolution of stomach adenocarcinoma (STAD) remain vague.

Pyruvate kinase M2 (PKM2) is a protein kinase that phosphorylates the histones for gene translation and is hence crucial in the formation of cyclin D1 and c-Myc[12]. In addition, PKM2 is indispensable for the Warburg effect, transferring a phosphate group from phosphoenolpyruvate for ATP generation[13]. PKM2 can promote tumor growth, metastasis, and chemo-resistance or regulate different signaling pathways in cancer cells[14]. Interestingly, in breast and prostate cancer, JMJD5 expression correlates with a high level of nuclear PKM2, which increases glycolysis and anabolic process[10, 11]. However, to date, the relationship between JMJD5 and PKM2 in STAD remains elusive.

Therefore, in the current research, we examined the expressions of JMJD5 and PKM2 using immunohistochemical staining, western blot, and quantitative real-time polymerase chain reaction (RT-qPCR). We further delineated the interconnection between JMJD5 and PKM2 expression and compared the relationship of these two genes with clinicopathological parameters and survival time. Besides, we applied a range of tools and databases to explore the members of the JMJD family and PKM2 in STAD. We examined their expressions and mutations and their relationship with the clinical features to confirm the expression patterns, potential mechanics, and prognostic values in STAD.

Materials And Methods

2.1 Patients and tissue specimens

Two types of tissue samples were collected. The first type of STAD tissue specimens, 93 paraffin-embedded STAD tissues, and 25 corresponding normal gastric tissues were obtained from the Department of Pathology, the Tumor Hospital of Harbin Medical University from October 2011 to August 2017. The second set included samples from STAD patients who underwent surgical resection at the Tumor Hospital of Harbin Medical University. Fresh STAD tissue samples and normal gastric tissues were collected immediately after surgical resection. Patients who received neoadjuvant therapy or radiotherapy before the surgery were excluded from this work.

A pathologist provided diagnosis and histological typing according to the World Health Organization Consensus Classification and Staging System for gastric cancer. Clinicopathological data were retrieved from the patients' medical records, and the patients were followed up (n = 93) until December 17, 2020, or the date of death. The study was approved by the Ethics Committee of the Harbin Medical University, and written informed consent was obtained from each patient.

2.2 Immunohistochemical staining

The paraffin-embedded tissues were dried at 70°C for at least 3 hours. After deparaffinization and hydration, the tissues were treated in the dark with 3% H_2O_2 for 10 min. Citrate buffer (pH 6.0) or ethylenediaminetetraacetic acid (pH 9.0) (BL617A, Biosharp, Guangzhou, China) was used for antigen retrieval. Each section was then treated with anti-JMJD5 antibody (1:200 dilution, GTX85251, GeneTex, USA) and anti-PKM2 antibody (1:150 dilution, PB9379, BOSTER, California, America) at 4°C overnight. Subsequently, each section was covered with the secondary antibody at room temperature. Later, each tissue was treated with diaminobenzidine (DAB) (ZLI-9017, ZSGB-BIO, China) working solution.

The tumor portion was evaluated by a pathologist who was completely unaware of the tissue information. Immunohistochemical scoring was performed using a semi-quantitative method based on the staining intensity and the proportion of the stained cells. The staining intensity was scored into four categories: no color: 0 points; light brown: 1 point; brown: 2 points; and dark brown: 3 points. The proportion of positively stained cells was scored into five categories: ≤5%: 0 points; 6%–25%: 1 point; 26%–50%: 2 points; 51%–75%: 3 points; and 76%–100%: 4 points. The results were obtained by multiplying the expression level intensity with the proportional score of JMJD5 and PKM2. In the final score, the results were interpreted as follows: 0–2 points: negative for JMJD5 and PKM2 expression (−); 3–4 points: weak positive expression (+); 6–8 points: moderate positive expression (++); and 9–12 points: strong positive expression (+++). Negative and weakly stained samples were regarded as low expressions, while moderate and strongly stained samples were regarded as high expressions of JMJD5 and PKM2.

2.3 Western blot

For this procedure, 20 mg of freshly frozen tissue was taken, cut into fine pieces, and ground well under low temperature. The tissue was treated using RIPA Lysis Buffer (R0010, Solarbio, China) for 20 min. The protein was loaded and separated on sodium dodecyl sulfate polyacrylamide gels (P0012AC, Beyotime Biotechnology, China). The membranes were incubated at 4°C with anti-JMJD5 antibody (1:1000 dilution) and anti-PKM2 antibody (1:1000 dilution) for the entire evening. The membranes were covered with rabbit secondary antibody for 1 h. Subsequently, the membranes were visualized using a chemiluminescence detection kit (AR1171, BOSTER, California, America). Anti-GAPDH was used to ensure equal loading.

2.4 RT-qPCR

Total RNA from the tissue was extracted using the Total RNA Isolation Kit (Omega, Norcross, GA). Transcriptor First Strand cDNA Synthesis Kit (Roche, Basel, Switzerland) was used for the reverse transcription of the RNA samples to cDNA. Quantitative analysis of JMJD5 and PKM2 was performed with the help of SYBR® Green Real-time PCR Master Mix (TOYOBO, Osaka, Japan). The primer sequences used were as follows: JMJD5 forward: 5'GCAGAAGTGGAGTTTGGAGTAT-3', reverse: 5'-TCTGGGACCATTCCTCATCT-3'; PKM2 forward: 5'CTACGTGGATGATGGGCTTATT-3', reverse:

5'-GGAAGGTTCACACCCTTCTT-3'; GAPDH forward: 5'-GGACGACAGCCATTTCCTAATA-3', reverse:

5'-GCAGCTTAGCTTGCTGATAAAC-3'. Each sample was examined three times. The results were calculated on the basis of the expression of the threshold cycle (Ct), JMJD5, PKM2, and GAPDH according to the manufacturer's instructions.

2.5 UALCAN

UALCAN (http://ualcan.path.uab.edu/) provides information on The Cancer Genome Atlas (TCGA) level RNA-seq and clinical data for 31 kinds of cancer. The expressions of tumor genes can be analyzed according to the stage, grade, race, or weight of each cancer by means of UALCAN [15]. In this paper, UALCAN was mainly applied to query the expression levels of the members of the JMJD family and PKM2 in STAD and normal tissues. The relationships between the mRNA expression levels of these genes and tumor grades and stages in STAD were searched. The expression differences were examined using the student's t-test. When the *P*-value was <0.05, the differences were considered to be statistically significant.

2.6 Gene Expression Profiling Interactive Analysis (GEPIA)

GEPIA (http://gepia.cancer-pku.cn/) provides the RNA expression levels obtained from TCGA and Genotype-Tissue Expression (GTEx) projects[16]. In this paper, the expressions of the JMJD family members and PKM2 in cancers were analyzed using GEPIA. The correlation between JMJD5 and PKM2 was retrieved. In addition, the genes on GEPIA that were similar to members of the JMJD family and PKM2 were identified. The expression differences were examined using the student's t-test, and when the *P*-value was <0.05, the differences were taken to be statistically significant.

2.7 Kaplan–Meier plotter

The Kaplan–Meier plotter (http://kmplot.com/analysis/) aids in calculating the survival rates of several genes in different types of cancer[17]. The overall survival (OS), progression-free survival (FP), and post-progression survival (PPS) of the STAD patients were analyzed using the Kaplan–Meier survival chart. In short, the nine genes were obtained using the Kaplan–Meier survival graph, and the *P*-value was shown on the graph. The results demonstrated that the differences were statistically significant when the *P*-value was <0.05.

2.8 The Human Protein Atlas

The Human Protein Atlas (https://www.proteinatlas.org/) contains immunohistochemistry data and transcriptome data related to 17 types of cancer. Users can retrieve the protein expression information pertaining to different genes in specific tumors. In this study, the immunofluorescence images in the Human Protein Atlas were searched to clarify the protein localization in STAD.

2.9 STRING

STRING is available at https://string-db.org/. The basic interaction in Strings is "functional binding" or the connection between two proteins that together promotes a particular biological function[18]. In this paper, the co-expression genes of 90 JMJD family members and PKM2 were searched. Besides, the PPI network among the genes was analyzed by using STRING to determine their functions in a comprehensive manner. The edges represent the interactions between different proteins, and the nodes in which the network was disconnected were hidden.

2.10 The cBiopotal

The cBiopotal (http://cbioportal.org) is an in-depth web resource used to examine complicated cancer genomics data. It is an open-source that currently contains five published datasets and 15 interim TCGA datasets [19]. In this study, the Stomach Adenocarcinoma (TCGA, Firehose Legacy) dataset was examined to analyze the mutation data of the JMJD family members and PKM2 in STAD. In addition, the 3D structure of mutation of each gene was demonstrated.

2.11 Metascape

Metascape (http://metascape.org) is a tool used for gene enrichment analysis[20]. Enrichment analysis was performed via the "Custom Analysis" module. The functions of the members of the JMJD family and PKM2 and their 450 genes similar genes were analyzed by Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and Protein–Protein Interaction (PPI) enrichment. GO analysis includes three parts: biological processes (BP), cellular components (CC), and molecular functions (MF). These analyses predict the functional roles and pathways of the genes.

2.12 Statistical analysis

Statistical analyses were performed using the SPSS 22.0 statistical software program (New York, USA). Distinctions between two groups were analyzed with Chi-square ($\chi 2$) test. Hazards ratio (HR) and 95% confidence interval (CI) were used to analyze the potential factors affecting

survival. Cox proportional risk model was applied to evaluate the univariate and multivariate regression. The results demonstrated that the differences were statistically significant when the *P*-value was <0.05.

Results

3.1 Expression and correlation of JMJD5 and PKM2 in the STAD tissues

We first determined the expressions of JMJD5 and PKM2 in the STAD tissues by western blot and RT-qPCR. We identified that the number of JMJD5 was remarkably lower in STAD in comparison with the normal gastric tissue (Fig. 1A, C). However, the number of PKM2 was greatly higher in STAD than in normal gastric tissue (Fig. 1B, D).

We further identified the relationship between the expressions of JMJD5 and PKM2. In the group of low JMJD5 expression (n = 60), 33 patients had a high expression of PKM2 (55%) and 27 had a low expression of PKM2 (45%). Contrarily, in the group of JMJD5 high expression (n = 33), 25 patients had a high expression of PKM2 (75.76%) and 8 had a low expression of PKM2 (24.24%). The expression of JMJD5 in the STAD tissue had a significant positive correlation with PKM2 expression ($P = 0.048$) (Table 1). Similarly, the result retrieved from GEPIA showed that JMJD5 had a positive correlation with PKM2 expression in STAD ($P = 0.014$) (Supplementary Fig. 1).

3.2 Correlation between the expressions of JMJD5 and PKM2 and the clinicopathological characteristics of the STAD tissues

The relationship between JMJD5 and the different clinicopathological features is shown in Table 2. Low expression of JMJD5 was related to poor differentiation ($P = 0.002$) and large tumor size ($P = 0.044$). There were no statistical associations of JMJD5 with gender, age, pathological tumor node metastasis (pTNM) stage, primary tumor (pT) classification, lymph node metastasis, CEA, and CA199. Furthermore, PKM2 expression had no significant correlations with gender, age, differentiation, pTNM stage, pT classification, tumor size, lymph node metastasis, CEA, and CA199 (Table 2).

3.3 Correlation of JMJD5 and PKM2 expressions with survival in STAD tissues

Kaplan–Meier survival analysis curves are displayed in Fig. 3. Patients with high expression of JMJD5 had remarkably higher OS (43.46 vs. 29.18 months, $P = 0.008$) and disease-free survival (DFS) (29.07 vs. 19.29 months, $P = 0.065$) than those with low expression of JMJD5 (Fig.3A, C). On the contrary, the results suggested that the prognosis of STAD patients with high expression of PKM2 was poorer than that of patients with low expression of PKM2 with regard to OS (27.69 vs. 42.22 months, $P = 0.018$) and DFS (16.87 vs. 29.83 months, $P = 0.046$) (Fig. 3B, D). Furthermore, STAD patients with both high expression of JMJD5 and low expression of PKM2 had obviously higher OS ($P = 0.001$) and DFS ($P = 0.024$) than the others (Fig. 3E, F).

Moreover, we assessed the relationship among differentiation, stage, pT size, lymph node metastasis, OS, and DFS (Fig.4). Our study found that patients with well-differentiated (Fig.4A), stage I (Fig.4B), T1+T2 pT staging (Fig. 4C), smaller tumor (Fig.4D), and no lymph node metastasis (Fig. 4E) had higher OS and DFS than the others.

3.4 Univariate and multivariate analyses of prognostic factors in STAD tissues

Univariate analysis of OS showed that low expression of JMJD5

($P = 0.010$), high expression of PKM2 ($P = 0.020$), poor differentiation ($P < 0.001$), high pT ($P = 0.001$), positive lymph node metastasis ($P = 0.001$), advanced pTNM stage ($P < 0.001$), and larger tumors ($P = 0.001$) were unfavorable prognostic predictors. However, age, gender, CEA, and CA199 had no prognostic value. Multivariate analysis indicated that differentiation (HR 2.082, 95% CI 1.326–3.269, $P = 0.001$) and pTNM stage (HR 2.811, 95% CI 1.174–6.730, $P = 0.020$) were the independent poor prognostic factors (Fig. 5A, B).

Furthermore, the results of univariate analysis of DFS proved that high expression of PKM2 ($P = 0.048$), poor differentiation ($P = 0.004$), high pT ($P = 0.002$), positive lymph node metastasis ($P < 0.001$), advanced pTNM stage ($P < 0.001$), larger tumor ($P = 0.001$), and CA199 ($P = 0.009$) were the key factors leading to poorer DFS in patients with STAD. Multivariate analysis revealed that differentiation (HR 2.045, 95% CI 1.308–3.199, $P = 0.002$) and pTNM stage (HR 2.714, 95% CI 1.126–6.544, $P = 0.026$) were the independent prognostic factors of DFS, as shown in Fig. 5C, D.

3.5 Transcriptional levels of members of the JMJD family and PKM2 in STAD tissues

The alternative names, chromosomal locations, and amino acid sequences of members of the JMJD family and PKM2 are depicted in Table 3. We used UALCAN (http://ualcan.path.uab.edu/) to compare the mRNA expressions of JMJD family members and PKM2 between 415 STAD and 34 normal gastric tissues. The results signified that the expressions of JMJD1B, JMJD1C, JMJD2D, JMJD4, JMJD5, JARID2, HSPBAP1, TYW5, and PKM2 were higher in STAD when compared with the normal stomach tissues (Fig. 6). Furthermore, we contrasted the expressions

of JMJD family members and PKM2 using GEPIA (http://gepia.cancer-pku.cn/). The expressions of JMJD1B, JMJD1C, JMJD2D, JMJD4, JARID2, HSPBAP1, TYW5, and PKM2 mRNAs were higher in the tumor tissues than in the normal stomach tissues (Supplementary Fig. 2A). In addition, JMJD1B had the highest expression in STAD, followed by JMJD1C and JMJD4. However, JMJD5 had the lowest expression (Supplementary Fig. 2B). Moreover, we obtained immunofluorescence images in The Human Protein Atlas (https://www.Fproteinatlas.org/) to verify the protein localization. We discovered that JMJD1B/JMJD1C/JMJD2/JMJD5 were all located in the nucleoplasm, JMJD4 in the plasma membrane, JARID2 in the nucleoplasm and mitochondria, and HSPBAP1/TYW5/PKM2 in the mitochondria, nuclear bodies, and cytosol, respectively (Supplementary Fig. 3).

3.6 Relationship of the clinicopathological parameters with JMJD family members and PKM2 in STAD tissues

After determining the expressions of members of the JMJD family and PKM2 in STAD, the relationship of these genes with cancer stage and grade was examined in the UALCAN database (Fig. 7,8). We found an obvious correlation between the mRNA expressions of these genes and the clinicopathological parameters. Importantly, the mRNA expressions of JMJD1B, JMJD1C, JMJD2D, JMJD4, JARID2, HSPBAP1, TYW5, and PKM2 were evidently higher in the advanced stage tumors than in the early stages ($P < 0.05$). The expression level of JMJD5 was also higher in advanced-stage tumors, but there was no significant difference between the normal tissue and advanced-stage tumors (Fig. 7).

In addition, we statistically found that the mRNA expressions of JMJD1B, JMJD1C, JMJD2D, and JMJD5 were the highest in grade III. However, the mRNA expressions of JMJD4, JARID2, TYW5, and PKM2 were the highest in grade II and that of HSPBAP1 was the highest in grade I (Fig. 8).

3.7 Prognostic value of members of the JMJD family and PKM2

We employed the Kaplan–Meier plotter (http://kmplot.com/analysis/) to determine the prognostic value of JMJD family members and PKM2 in STAD patients, including OS, FP, and PPS. The results showed that the members of the JMJD family and PKM2 were remarkably correlated with the prognosis (Fig. 9). In each group, the patients were divided into two sub-groups of low and high expression according to the cut-off value. In the STAD tissues, patients with high expressions of JMJD1C, JMJD2D, JMJD4, JARID2, HSPBAP1, TYW5, and PKM2 had significantly poorer OS/FP/PPS than those with a low expression ($P < 0.05$) (Fig. 9B, C, D, F, G, H, I). On the contrary, patients with high expressions of JMJD5 and JMJD1B had longer OS/FP/PPS ($P < 0.05$) (Fig. 9A, E).

3.8 Genetic mutations in members of the JMJD family and PKM2

We used the cBioPortal online tool (www.cbioportal.org) to explore the alterations in the JMJD family and PKM2 in STAD (TCGA, Firehose Legacy). According to the obtained results, these genes varied in 393 samples out of the 478 patients with STAD (82.22%). Among the STAD tissues, JARID2 had the highest mutation rate of 17%, followed by JMJD1C and JMJD4, which were both 13%. HSPBAP1 had the lowest mutation rate of 0.5% (Fig. 10A, B). We then retrieved the 3D structures of the JMJD family and PKM2, and the common mutations in the STAD sites were color-coded in the latter figures (Supplementary Fig. 4). We then constructed the network for the JMJD family, PKM2, and 90 co-expressed genes. The co-expressed genes comprised the glycolysis-related and cellular growth-related genes, including PGK1, PGAM1, and ANXA2 (Fig. 10C).

3.9 Predicting the functions and pathways of members of the JMJD family and PKM2 and similar genes in the STAD patients

Next, we applied Metascape (https://metascape.org) for GO, KEGG, and PPI enrichment analyses. The potential functions and pathways of the members of the JMJD family, PKM2, and similar genes are shown in Fig. 11 A–F. The participation of these genes was in the range of BP (20 terms), CC (20 terms), and MF (20 terms). The genes were enriched in several BP terms: covalent chromatin modification, DNA repair, regulation of DNA metabolic process, mRNA processing, NADH processing, protein acylation, positive regulation of GTPase activity, and DNA replication (Fig. 11A). Moreover, cellular components, including transferase complex, chromosomal region, nuclear periphery, nuclear speck, heterochromatin, and nuclear chromosome were significantly associated with members of the JMJD family, PKM2, and similar genes (Fig. 11B). We discovered that molecular functions such as transcription coregulator activity, chromatin binding, helicase activity, nucleoside-triphosphatase regulator activity, histone binding, transcription factor binding, N-acetyltransferase activity, and histone demethylase activity were remarkably regulated by members of the JMJD family, PKM2, and similar genes (Fig. 11C). The genes were enriched in the 12 KEGG pathways, including biosynthesis of amino acids, hypoxia-inducible factor-1 (HIF-1) signaling pathway, central carbon metabolism in cancer, cell cycle, and cysteine and methionine metabolism in cancer (Fig. 11D). Fig. 11E and F demonstrate that the genes were enriched in the PPI network. The genes were mainly associated with DNA repair, transcription elongation from RNA polymerase II promoter, DNA-templated transcription, elongation, and metabolism.

Discussion

In this research, we analyzed the clinical significance of JMJD5 and PKM2 expression in patients with STAD. Our findings demonstrated that the low expression of JMJD5 and the high expression of PKM2 were unfavorable prognostic factors for patients with STAD. This investigation firstly examined the correlation between the expression of JMJD5 and PKM2 in STAD. To find additional factors that affect the prognosis of STAD, we explored the roles of JMJD family members. At present, there are no reports on the roles of JMJD1B, JMJD1C, JMJD2D, JMJD4, JARID2, HSPBAP1, and TYW5 in STAD. Our study uncovered that JMJD1B, JMJD1C, JMJD2D, JMJD4, JARID2, HSPBAP1, and TYW5 might be promising molecular markers of STAD. Hence, their specific functions and precise mechanisms in STAD need further research.

Our results indicated that JMJD5 expression in the STAD tissues was downregulated when compared with the normal tissues. In agreement with our findings, JMJD5 expression was totally lost or compromised in most liver and lung cancer cells, strongly supporting its tumor-suppressive role[21, 22]. Another interesting finding was that low JMJD5 expression levels were related to poor differentiation and large tumor size. However, high expression of JMJD5 was associated with poor differentiation in UALCAN. The reason for this discrepancy might be the sample size. A previous study reported that JMJD5 knockdown stimulated cell proliferation and tumorigenicity in hepatocellular carcinoma by accelerating the G1/S transition in the cell cycle. Besides, JMJD5 inhibited cell proliferation in hepatocellular carcinoma chiefly by activating CDKN1A expression[22]. Our results showed the correlation between low JMJD5 expression and the advanced pTNM stage. However, there was no statistical significance, which might be due to the variations in the detection techniques, scoring methods of JMJD5 expression, sample size, etc.

In this study, we further determined the correlation between JMJD5 and PKM2 in STAD tissues. A potential correlation was found to exist between JMJD5 and PKM2. In the group of high JMJD5 expression, 75.76% of the patients had high expression of PKM2. Interestingly, the patients had worse OS and DFS when the expressions of both JMJD5 and PKM2 were low in comparison with the high expressions of both proteins. This result suggested that JMJD5 played a dominant tumor-suppressive role when JMJD5 and PKM2 were expressed simultaneously. Besides, these findings alluded that JMJD5 played a tumor-suppressive role mainly through other pathways in the STAD tissues. Moreover, studies have testified the connection between JMJD5 and PKM2. In breast and prostate cancers, JMJD5 was associated with PKM2 and translocated PKM2 into the nucleus. JMJD5/PKM2 complex served as a coactivator of hypoxia-inducible factor (HIF-1α), which promoted glycolysis and anabolic process[10, 11]. In the future, the relationship between JMJD5 and PKM2 in STAD needs to be further verified. We intend to use a larger sample size to validate our conclusions.

Additionally, we searched for the characteristics of the JMJD family members in cancer. ULCLAN and GEPIA datasets revealed that the expressions of JMJD1B, JMJD1C, JMJD2D, JMJD4, JARID2, HSPBAP1, and TYW5 were higher in the STAD tissues, which were closely associated with OS, FP, and PPS. Moreover, the expressions of these genes were linked to tumor stage and grade. Similarly, JMJD1C, JMJD2D, JMJD4, and JARID2 were upregulated in many kinds of cancer, such as esophageal and colon cancers. Furthermore, the expressions of these genes were positively correlated with the clinical parameters[23-28]. In addition, researchers have shown that high expressions of JMJD1C, JMJD4, and HSPBAP1 could lead to metastasis and poor prognosis in esophageal cancer, colon cancer, etc.[23, 25, 27, 29, 30]. Interestingly, the results demonstrated that the expression of JMJD1B was low in colorectal cancer and that the low expression was positively correlated with lymph node status, Dukes' classification, and TNM stage[31]. This finding suggested that JMJD1B played different roles in different cancers. Collectively, our results indicate that these genes might be potential biomarkers for STAD. In addition, they might serve as excellent prognostic markers in STAD patients and guide the clinical treatment.

In this study, through GO, KEGG, and PPI enrichment analyses, we established that JMJD family members, PKM2, and similar genes played important roles in metabolic processes, N-acetyltransferase activity, and histone demethylase activity, HIF-1 signaling pathway, cell cycle, p53 binding, etc. We showed that JMJD1B was necessary for p53-mediated cell cycle checkpoint and cell death by maintaining the H4R3me2s demethylation[27]. The knockdown of JMJD2D reduced cell proliferation and metastases in colorectal tumors. The possible mechanisms were JMJD2D demethylated H3K9me3 at promoters of β-catenin target genes and enhanced glycolysis through activation of the HIF1 signaling pathway[26, 32]. Knockdown of JARID2 obviously inhibited the proliferation and metastasis of cancer cells. Mechanistically, JARID2 negatively regulated cyclin D1 (CCND1) expression by increasing H3K27 trimethylation. Moreover, knockdown of JARID2 reduced EMT and the phosphorylation levels of PI3K and Akt[28, 33-38]. In conclusion, our findings have opened up the possibility that these genes play a remarkable role in the tumorigenesis of STAD through the foresaid mechanisms. However, further research is needed to obtain clarity.

There are some limitations to our study. First, we did not collect enough patient samples; thus, our findings should be confirmed by studying larger samples. In addition, some data were retrieved from databases; hence, further *in vitro* and *in vivo* studies are required to support our results.

Abbreviations

STAD：stomach adenocarcinoma; JMJD: Jumonji C-domain-containing;

PKM2: pyruvate kinase M2; JARID2: Jumonji And AT-Rich Interaction Domain Containing 2; HSPBAP1: heat shock 27kDa associated protein 1; TYW5: tRNA-yw Synthesizing enzyme 5; IHC: immunohistochemical; OS: Overall survival; DFS: disease-free survival; FP: progression-free survival; PPS: post-progression survival; GEPIA: Gene Expression Profiling Interactive Analysis; GTEx: Genotype-Tissue Expression projects; TCGA: The Cancer Genome Atlas; EMT: epithelial-mesenchymal transition; DAB: diaminobenzadine; HR: hazard ratio; CI: confidence interval; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; PPI: protein-protein interaction; BP: biological processes; CC: cellular components; MF: molecular functions; SOX9: SRY-type high-mobility group box-9; HCC：Hepatocellular carcinoma; CCND1: cyclin D1; OHyW: hydroxywybutosine; 2-OG：2-oxoglutarate; HIF-1α: hypoxia-inducible factor; PCR: Polymerase chain reaction.

Declarations

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Competing interests

All authors declared that there were no conflicts of interest.

Authors' contributions

Ling Qi and Yan-Jing Li developed the idea, designed the research and performed data analysis work. Ling Qi, Li-Sha Li and Chao Zhan drafted the manuscript. Yanjing Li, Xiao-Xue Du and Cheng-Xin Song reviewed the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable

Consent for publication

All authors have approved the manuscript for submission.

Availability of data and materials

Some or all data, models, or code generated or used during the study are available in a repository or online in accordance with funder data retention policies.

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Tables

Table 1 Correlation between JMJD5 and PKM2 expression

JMJD5 expression	Number of cases	PKM2 expression		*P*-value
		low expression	high expression	
low expression	60	27	33	**0.048**
high expression	33	8	25	

Table 2 Clinicopathologic characteristics of 93 patients with STAD

Characteristics	All patients	JMJD5		*P*-value	PKM2		*P*-value
		Low	High		Low	High	
	（n=93）	（n=60）	（n=33）		（n=35）	（n=58）	
Gender							
Male	73	45	28	0.269	30	43	0.188
Female	20	15	5		5	15	
Age							
<60	60	38	22	0.748	22	38	0.795
≥60	33	22	11		13	20	
Differentiation							
G3	45	37	8	**0.002***	14	31	0.453
G2	39	20	19		17	22	
G1	9	3	6		4	5	
pTNM stage							
I	8	3	5	0.217	5	3	0.296
II	18	13	5		7	11	
III	67	44	23		23	44	
pT classification							
T1/T2	12	6	6	0.524	6	6	0.072
T3	32	21	11		7	25	
T4	49	33	16		22	27	
Tumor size							
<5	25	12	13	**0.044***	13	12	0.083
≥5	68	48	20		22	46	
Lymph node metastasis							
Absent	18	11	7	0.737	10	8	0.081
Present	75	49	26		25	50	
CEA							
<5	77	50	27	0.853	32	45	0.087
≥5	16	10	6		3	13	
CA199							
<35	71	46	25	0.921	27	44	0.888
≥35	22	14	8		8	14	

Table 3 The alternate name and chromosomal location of genes

Protein	Alternate name(s)	Chromosomal location	Amino acids
JMJD1B	KDM3B,5qNCA, JHDM2B	5q31.2	1761
JMJD1C	KDM3C, TRIP8, JHDM2C	10q21.3	2540
JMJD2D	KDM4D, JHDM3D	11q21	523
JMJD4	-	1q42.13	463
JMJD5	KDM8, FLJ13798	16p12.1	416
JARID2	JMJ	6p22.3	1246
HSPBAP1	PASS1	3q21.1	488
TYW5	C2orf60	2q33.1	315
PKM2	PKM	15q23	531

Table 4 The prognostic values of JMJD family members and PKM2 in STAD (Kaplan-Meier plotter)

JMJD family	OS				FP				PPS			
	cases	HR	95%CI	*p*-value	cases	HR	95%CI	*p*-value	cases	HR	95%CI	*p*-value
JMJD1B(210878_s_at)	881	0.72	0.6-0.85	1.40E-04	645	0.66	0.54-0.81	4.60E-05	503	0.49	0.39-0.62	2.90E-10
JMJD1C(224933_s_at)	881	1.68	1.35-2.08	2.40E-06	645	1.7	1.31-2.19	4.40E-05	503	2.27	1.73-3	1.90E-09
JMJD2D(220278_at)	881	1.43	1.21-1.7	3.40E-05	645	1.38	1.12-1.7	0.002	503	1.48	1.18-1.86	0.00055
JMJD4(222671_s_at)	881	1.32	1.02-1.7	0.033	645	1.54	1.15-2.06	0.0033	503	1.58	1.19-2.1	0.0016
JMJD5(241045_at)	881	0.78	0.62-0.98	0.033	645	0.77	0.6-0.99	0.043	503	0.68	0.51-0.9	0.0064
JARID2(203297_s_at)	881	1.65	1.39-1.95	5.80E-09	645	1.82	1.48-2.23	8.40E-09	503	1.89	1.51-2.37	1.70E-08
HSPBAP1(219284_at)	881	1.56	1.31-1.86	3.70E-07	645	1.59	1.3-1.95	4.90E-06	503	1.58	1.27-1.96	4.30E-05
TYW5(241825_at)	881	1.77	1.35-2.32	3.10E-05	645	1.47	1.12-1.94	0.0059	503	1.98	1.42-2.75	3.90E-05
PKM2(201251_at)	881	2.48	2.02-3.03	<1E-16	645	2.3	1.86-2.83	1.2E-15	503	3.54	2.8-4.49	<1E-16

Figures

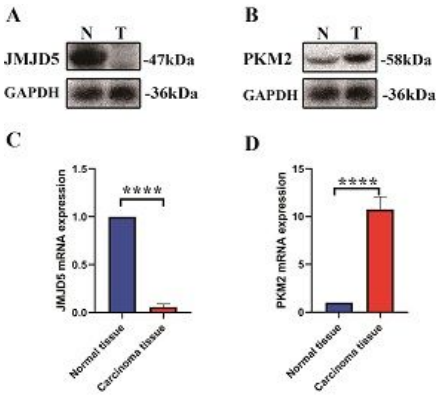

Figure 1

Western blot and RT-qPCR of JMJD5 and PKM2 protein in STAD. (A) The expression of JMJD5 in STAD, and the normal tissue using western blot. (B) The expression of PKM2 in STAD, and the normal tissue using western blot. (C) The expression of JMJD5 in STAD, and the normal tissue using RT-qPCR. (D) The expression of PKM2 in STAD, and the normal tissue using RT-qPCR. N: Normal, T: Tumor

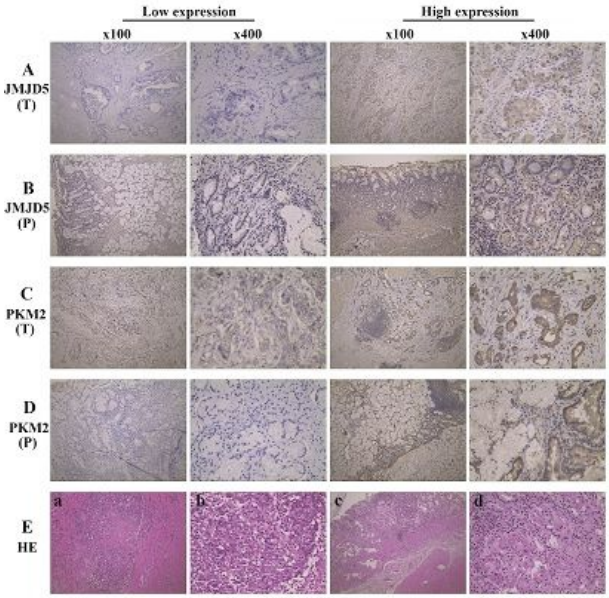

Figure 2

Immunohistochemical staining of JMJD5 and PKM2 protein in STAD. (A, C) The first two figures show representative low expression of JMJD5 and PKM2 protein in STAD. The last two figures show representative high expression of JMJD5 and PKM2 protein in STAD. (B, D) The first two figures show representative low expression of JMJD5 and PKM2 protein in para-carcinoma tissue. The last two figures show representative high expression of JMJD5 and PKM2 protein in para-carcinoma tissue. (E a, b) HE staining in STAD. (E c, d) HE staining in para-carcinoma tissue.

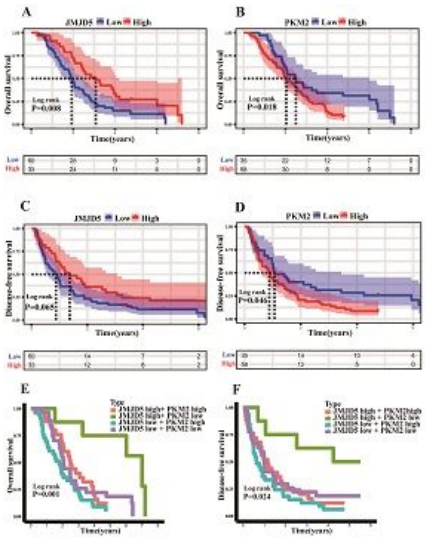

Figure 3

Kaplan Meier analysis for OS and DFS based on JMJD5 and PKM2 expression in STAD patients. (A) Kaplan-Meier analysis for OS based on JMJD5 expression (log-rank test, P=0.008); (B) Kaplan-Meier analysis for OS based on PKM2 expression (log-rank test, P=0.018); (C) Kaplan-Meier analysis for DFS based on JMJD5 expression (log-rank test, P=0.065); (D) Kaplan-Meier analysis for DFS based on PKM2 expression (log-rank test, P=0.046); (E) Kaplan-Meier analysis for OS based on both JMJD5 and PKM2 expression (log-rank test, P=0.001); (F) Kaplan-Meier analysis for DFS based on both JMJD5 and PKM2 expression (log-rank test, P=0.024).

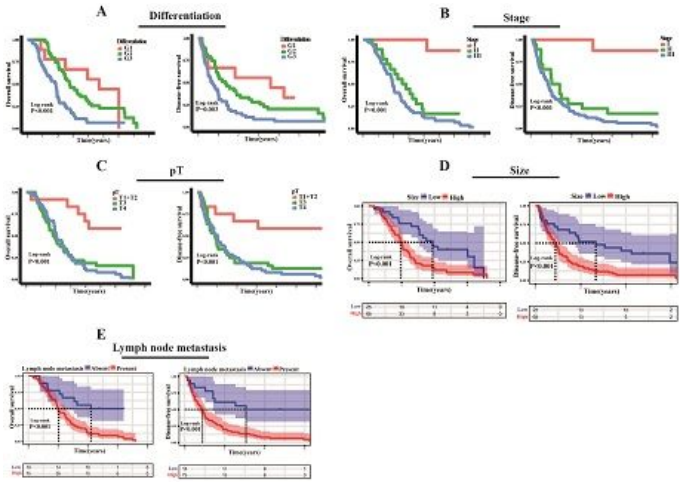

Figure 4

Kaplan Meier analysis for OS and DFS based on differentiation, stage, Pt, size and lymph node metastasis in STAD patients. (A) Kaplan-Meier analysis for OS and DFS based on differentiation (log-rank test, P<0.001, P=0.003); (B) Kaplan-Meier analysis for OS and DFS based on stage (log-rank test, P<0.001, P<0.001); (C) Kaplan-Meier analysis for OS and DFS based on pT (log-rank test, P<0.001, P<0.001); (D) Kaplan-Meier analysis for OS and DFS based on size (log-rank test, P<0.001, P<0.001); (E) Kaplan-Meier analysis for OS and DFS based on lymph node metastasis (log-rank test, P<0.001, P<0.001).

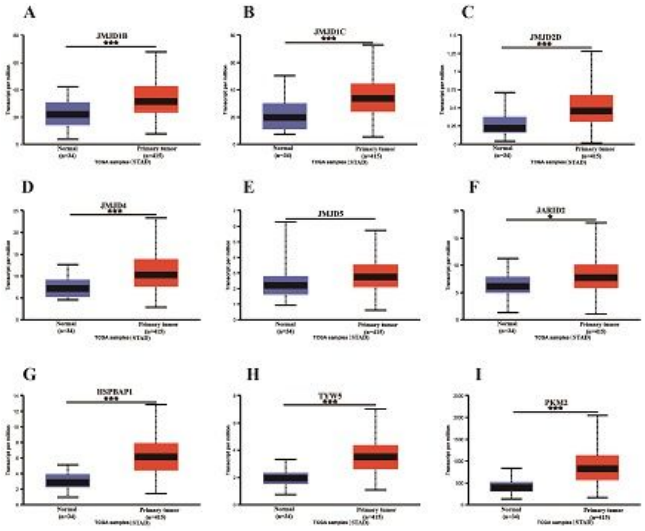

Figure 5

Univariate and multivariate analysis of prognostic factors for OS and DFS in STAD. (A) Univariate analysis of prognostic factors for OS; (B) Multivariate analysis of prognostic factors for OS; (C) Univariate analysis of prognostic factors for DFS; (D) Multivariate analysis of prognostic factors for DFS.

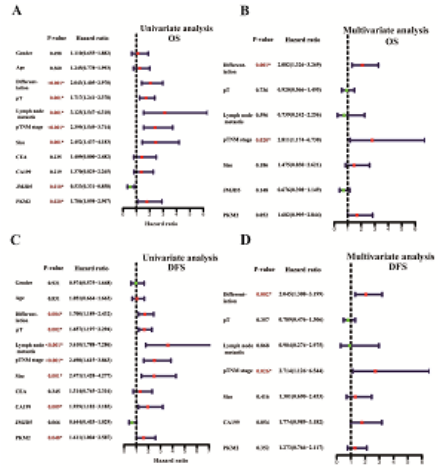

Figure 6

The mRNA expression of JMJD family members and PKM2 in STAD and normal tissues (UALCAN). Box plots of JMJD family members and PKM2 mRNA expression. Red: tumor tissue; blue: normal tissue; *, P<0.05; **, P<0.01; ***, P<0.001.

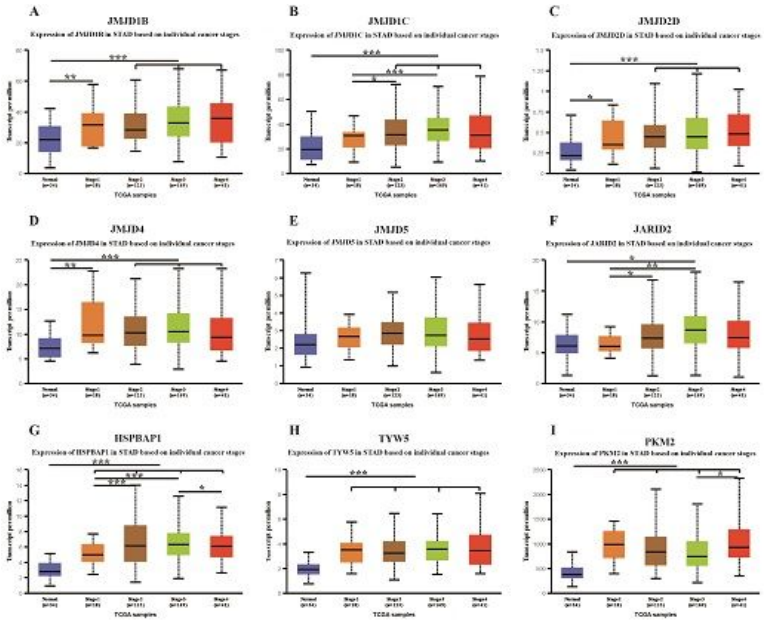

Figure 7

Association of JMJD family members and PKM2 transcript levels with cancer stage (UALCAN). Relationship of the mRNA expression levels of JMJD family members and PKM2 with cancer stage, (*, P<0.05; **, P<0.01, ***, P<0.001).

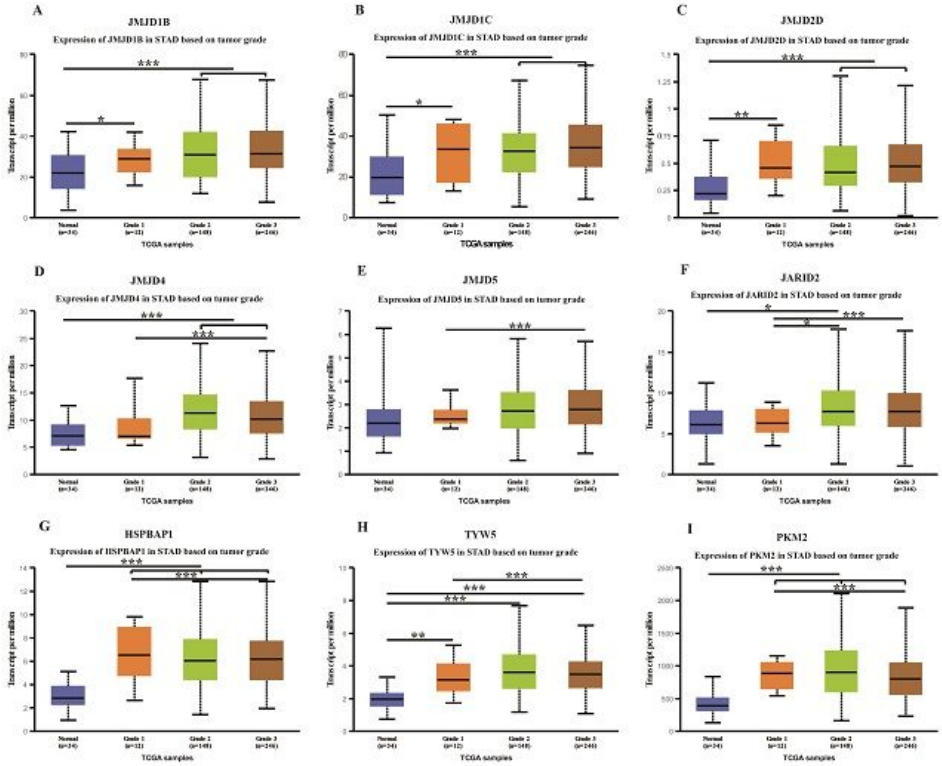

Figure 8

Association of JMJD family members and PKM2 transcript levels with cancer grade (UALCAN). Relationship of mRNA expression levels of JMJD family members and PKM2 with cancer grade, (*, P<0.05; **, P<0.01, ***, P<0.001).

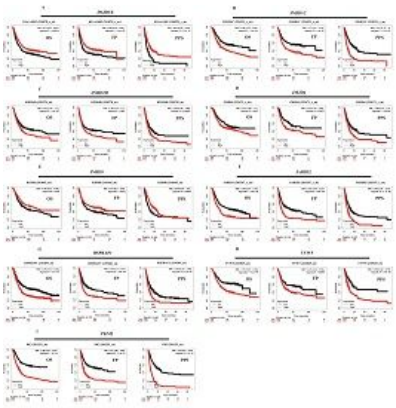

Figure 9

The Prognostic value of mRNA expression of JMJD family members and PKM2 in STAD (Kaplan-Meier plotter). Association of the mRNA expression of JMJD family members and PKM2 with OS, FP and PPS. Red: high expression; Black: low expression. The samples of gastric cancer patients were obtained from the datasets of GSE14210 (n=145), GSE15459 (n=200), GSE22377 (n=43), GSE29272 (n=268), GSE51105 (n=94), and GSE62254 (n=300) in GEO database which were processed by the same standard.

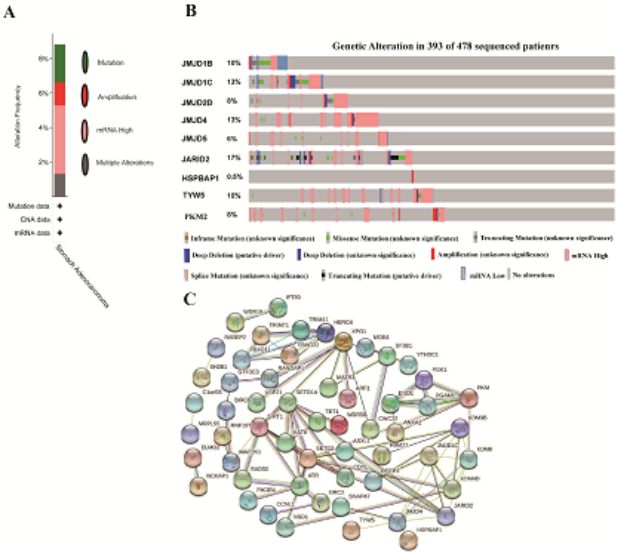

Figure 10

Gene expression and mutation analysis of JMJD family members and PKM2 in STAD (cBioPortal). (A, B) JMJD family members and PKM2 expression and mutation analysis in STAD. (C) The network for JMJD family, PKM2 and 90 co-expression genes.

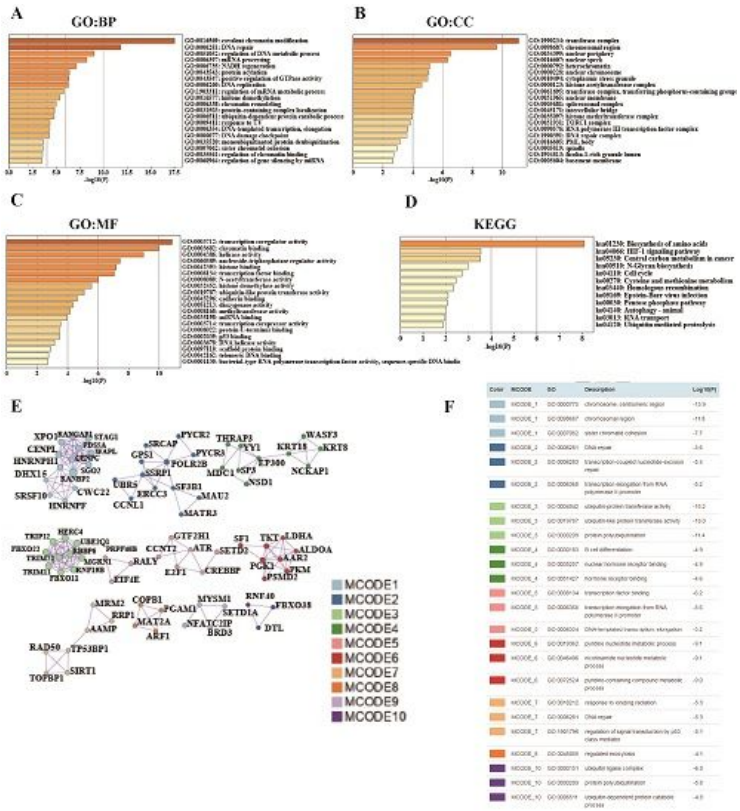

Figure 11

The predicted functions, pathways, and protein-protein interactions of JMJD family members, PKM2 and their similar genes (Metascape). (A) Gene ontology inclusion biological Process (BP), (B) cellular component (CC), (C) molecular function (MF) and (D) Kyoto Encyclopedia of Genes and Genome (KEGG) analysis are represented in the figures. (E, F) Protein-protein interaction (PPI) enrichment analysis of these genes and MCODE (Molecular Complex Detection) components identified in the gene lists.

Supplementary Files

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