JMJD4 is a potential diagnostic and immunological biomarker in pan-cancer

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Abstract. During the development of cancer, many changes leading to a series of genetic mutations that cannot control tumor growth. Therefore, understanding the complex interactions between the intrinsic, extrinsic, and systemic mediators of tumor cells and disease progression is crucial for the rational development of effective anti-cancer treatments. In this study, we conducted a multi-omics pan-cancer exploration of the function of JMJD4 using various comprehensive analysis tools and samples collected from relevant cancer databases, to understand how JMJD4 affects pan-cancer development by identifying its clinical features and multi-omics heterogeneity. We discovered that JMJD4 was highly expressed and associated with cancer stemness, epigenetic modulations and DNA repair in pan-cancer, especially in BLCA, BRCA, CHOL, COAD, ESCA. JMJD4 was also related to most immune-infiltrating cells, which indicated that JMJD4 may serve as a diagnostic and immunological biomarker in pan-cancer. Therefore, JMJD4 can be used as an indicator and a new therapeutic target for early tumor diagnosis and a prognostic marker for many types of cancers.

Keywords: Jumonji domain containing 4 (JMJD4), pan-cancer, macrophage, stemness, epigenetic modulations, DNA damage repair.

1. Introduction

Many changes have taken place in the progression of cancer, resulting in the occurrence of a series of gene mutations, which cannot control the growth of tumors. Therefore, it often leads to a series of downstream changes, such as abnormal metabolic behavior [1], uninhibited division [2] and weakened cell-cell adhesion [3]. However, immunotherapy calls for more single gene investigation in pan-cancer for validation. With the rapid development of the TCGA database and the GEO database, it is more convenient to analyze the correlation and impact of a single gene on cancer prognosis and immune infiltration [4]. Thus, study the role of single gene in pan-cancer is an important means to discover the relationship of genes that promote tumorigenesis from a genetic perspective.

Jumonji C domain-containing 4 (JMJD4) belongs to the JMJD family, which contains a jumonji C (JmjC) domain that can demethylate multiple histone or non-histone lysine sites. Besides, JMJD4 can interacts with other proteins to form oligomers or complexes [5]. For example, JMJD4 can interacts with heatshock protein 70 (Hsp70) mechanistically to mediate the degradation of Pyruvate Kinase M2 (PKM2) through chaperone mediated autophagy to maintain a new role [6]. Moreover, JMJD4 is a histone demethylase that is closely related to cancer activity. In human colon cancer, JMJD4 protein is overexpressed in various tumors compared with normal tissues [7, 8]. Like human colon cancer, JMJD4 is also overexpressed in liver tumors [9], but very little evidence suggests JMJD4's function in breast cancer, esophageal cancer, renal cancer, lung cancer or gastric cancer [10]. However, compared with other members of the JMJD family that have been studied, the relevant experimental work on JMJD4 in pan-cancer is far behind.

Tumors represent complex ecosystems, including tumor cells, immune cells, tumor associated fibroblasts, endothelial cells, parietal cells, additional tissue resident cells, and the extracellular matrix embedded by these cells [11]. The tumor microenvironment (TME) is very important for the tumor function, which driving tumor growth, invasion, metastasis, and response to therapies [12, 13]. There might be significant differences in the composition and functional status of tumor microenvironments, intrinsic characteristics of tumors, tumor staging, and patient status refered to different types of tumors. Understanding the complex interactions between the intrinsic, extrinsic, and systemic

mediators of tumor cells and disease progression is crucial for the rational development of effective anti-cancer treatments [14].

Tumor associated macrophages (TAMs) are an important component of the tumor microenvironment, which plays a role in the coordination of angiogenesis, extracellular matrix remodeling, cancer cell proliferation, metastasis, and immune suppression, as well as resistance to chemotherapy drugs and checkpoint blockade immunotherapy [15,16,17]. Macrophages are highly plastic cells that undergo various forms of functional activation based on different signals, which can be divided into two forms of macrophage activation, commonly referred to as classically activated macrophage (M1) and alternatively activated macrophage (M2). M1 polarization is associated with macrophage dependent tissue damage, inflammation triggering and tumor cell killing, while M2 polarization promotes tissue repair and remodeling as well as resistance to chronic inflammation [18]. Macrophages can mediate the phagocytosis of cancer cells and cytotoxic tumor killing, and interact with the innate and adaptive immune systems [19]. Therefore, TAM have become increasingly clear in several experimental tumor models.

There is an inseparable relationship between JMJD4 and cancers. JMJD4 protein has long been identified as having demethylase activity, and play an epigenetic regulatory role in physiological processes and diseases. Importantly, increasing evidence suggests that the abnormal expression of JMJD4 protein in cancer and inflammatory diseases may be a potential mechanism for the occurrence and development of such diseases [20,21,22]. Therefore, JMJD4 is a new oncogene that is associated with immune suppression and DNA repair, which might be a biomarker of M2 macrophages in many cancers.

In this study, we conducted a multi-omics pan-cancer exploration of JMJD4 function by using a variety of integrated analysis tools and samples collected from relevant cancer databases, in order to find how JMJD4 influences pan-cancer development through identify its clinical characteristics and multi-omics heterogeneity, especially its effect on macrophage and immune infiltration. This study comprehensively analyzed and discussed the role of JMJD4 in pan-cancer, which laid a certain foundation for the future research on targeted therapy.

2. Methods

2.1. Data access and Procession

The batch-corrected and normalized pan-cancer and normal tissue datasets were acquired from the University of California, Santa Cruz (UCSC) datasets, which include The Cancer Genome Atlas (TCGA; encompassing 33 cancer types), Therapeutically Applicable Research to Generate Effective Treatments (TARGET; consisting of 7 pediatric cancers), and the Genotype Tissue Expression (GTEx), comprising 54 normal tissues. Cancer types with fewer than 3 samples were excluded. The simple-nucleotide variation (SNV) data processed using the cBioPortal for Cancer Genomics web interface (http://cbioportal.org) [23] were retrieved from the GDC data portal (https://portal.gdc.cancer.gov/).

2.2. JMJD4 expression

JMJD4 related diseases or phenotypes were first used from Open Target Platform (https://platform.opentargets.org/). TIMER2.0 [24] (http://timer.cistrome.org/) was used to analyze the differential JMJD4 mRNA expression between tumor tissue and adjacent normal tissue in the Gene_DE module. For cancer types lacking sufficient normal neighboring samples, GTEx and TCGA data were used in Box Plot module and TCGA data from GEPIA2.0 [25]. Expression DIY function was used for analysis (http://gepia2.cancer-pku.cn/); the absolute log2FC cutoff and p-value cutoff are set to 1 and 0.01, respectively. In addition, mRNA expression differences in cancer stages also appear in the Stage Plot module. The data of Clinical Proteomics Oncology Analysis Alliance (UPTAC) from UALCAN [26] (http://ualcan.path.uab.edu/), were used to compare the differences in JMJD4 protein expression between cancer and normal tissues. Sangerbox [27] were used to analyze

the pan-cancer prognostic risk of JMJD4, "survival" function was used to analyze survival differences, and visualization was achieved through Kaplan-Meier curves.

2.3. Genomic Alteration

The Cancer Type Summary module was used to analyzed the frequency of three types of genomic changes (mutations, amplifications, and deep deletions) in pan-cancer through the online network tool cBioPortal [28] (https://www.cbioportal.org/). The collected and processed pan-cancer SNV data were compared with JMJD8 and JMJD7 to demonstrate the mutation landscape by using GSCA [29] (http://bioinfo.life.hust.edu.cn/GSCA/). The Kaplan-Meier curve was obtained from the Copy_Number module of Tumor Immune Dysfunction and Exclusion (TIDE). cBioPortal is a network tool for studying the correlation between cancer genome or transcriptional changes and immunotherapy response, to explore the prognostic significance of JMJD4 copy number variations (CNVs). In addition, the genome heterogeneity and gene expression analysis module analysis were conducted using TCGA data and Sangerbox (http://www.sangerbox.com/home.html). The data source was set to TMB, and the data transformation was set to log2(X+0.001) with Pearson correlation analysis. The correlation between Tumor mutation burden (TMB), Microsatellite facility (MSI), Mutant-allele tumor (MATH), Homologous recombination disease (HRD), Neoantigen (NEO), ploidy and JMJD4 expression was presented.

2.4. DNA repair, Cancer stemness and Epigenetic modification

TIMER2.0 [30] (http://timer.cistrome.org/) were used to analyze the correlation between 5 mismatched repair genes [31] and the expression of JMJD4 in pan-cancer. To further analyze the homologous recombination repair (HRR) characteristics in pan-cancer, we retrieved 30 HRR related genes from the ARIEL3 clinical trial [32] and inputted them into GEPIA2.0 (http://gepia2.cancer-pku.cn/#index) to calculate their correlation with JMJD4. The differential methylation probes-based stemness index (DMPsi) for each type of cancer was also obtained from previous studies [33] and analyzed through GSCA (http://bioinfo.life.hust.edu.cn/GSCA/#/). The relationship between JMJD4 promoter methylation and cytotoxic T lymphocytes (CTLs) and patients' survival was subsequently investigated in TIDE (http://tide.dfci.harvard.edu/). The correlation between JMJD4 and genes modified with N1-methyladenosine (m1A), 5-methylcytosine (m5C), and N6-methyladenosine (m6A) [34,35,36] heatmap was drawn through Sanger box (http://www.sangerbox.com/home.html) methylation module.

2.5. Alternative splicing

To identify clinically significant alternative splicing (AS) events, the ClinicalAS module of the tumor splicing server [37] (http://www.Oncosplicing.com/) was employed to search for AS events related to JMJD4, which were included in both the SplAdder and SpliceSeq projects. PanPlot was utilized to visualize the splicing percentage (PSI) across TCGA cancer and GTEx tissues. Additionally, the PanDiff plot was utilized to compare the PSI differences of AS events (detected in three or more types of cancer) between cancerous tissues and their adjacent or GTEx normal tissues. Finally, Kaplan-Meier curves were generated to demonstrate the prognostic implications of AS events in pan-cancer.

2.6. Functional Enrichment Analyses

The current JMJD4 Protein-Protein Interaction network with known experiment validations was searched via the online webtool String [38] (https://www.string-db.org/). The pan-cancer pathway-level somatic alterations of several vital pathways were explored on UALCAN, and the expression correlations between pathway-related signature [39] and JMJD4 were investigated on GEPIA2.0. Also, the top 100 co-expression genes of JMJD4 in pan-cancer were obtained from the Similar Gene Detection function of GEPIA2.0. The top 100 genes were adopted for functional analysis of Gene Ontology (GO) with a false discovery rate (FDR) < 0.05 using Sangerbox. The correlation between

the top 5 co-expression genes and JMJD4 were visualized by the heatmap plotted in TIMER2.0 and the scatter plots in GEPIA2.0. The quantification results of JMJD4 functional enrichment were further investigated by Gene Set Enrichment Analysis (GSEA) using the GSEA software. According to the median expression of JMJD4, all cancer samples were divided into low- and high-JMJD4 groups, and the gene sets were downloaded from the Molecular Signatures Database [40] of (http://www.gsea-msigdb.org/gsea/downloads.jsp) for Kyoto Encyclopedia of Genes and Genomes (KEGG) and hallmark pathways GSEAs ($p<0.05$).

2.7. Immune response

The roles of JMJD4 in pan-cancer microenvironment infiltration and immune cells in MAlignant Tumour tissues using Expression data (ESTIMATE), stromal, and immune scores were analyzed in Sangerbox [27] (http://www.sangerbox.com/home.html). The previously studied immune checkpoint markers [41] were used to analyze their correlation with JMJD4. The correlation between expression and immune subtypes was analyzed, and the levels of TISDB subtype modules, tumor and immune system interactions were compared in pan-cancer patients [42] (http://cis.hku.hk/TISIDB/). In addition, heatmap was drawn to demonstrate the relationship between JMJD4 expression and chemokines, with chemokine receptors and immune stimulators as chemokine and immune modulator modules, respectively. To investigate the effect of cytokine therapy on the expression of JMJD4, the Tumor Immune Sco gene MOuse [43] (TISMO, http://tismo.cistrome.org/) web tools are used to compare gene expression levels in different cell line between pre- and post-cytokine treatment.

TIMER2.0 was used for immune check to analyze the correlation between M2 macrophages and JMJD4. In addition, SpatialDB online tool [44], a database transcriptome for spatial parsing (https://www.spatialomics.org/SpatialDB/) is applied to analyze spatial expression levels and overlap of JMJD4 and M2 macrophage markers CD68 and CD163. The expression of JMJD4 in single cell resolution from different subtypes of pan-cancer was collected from GEO and tumor immune single cell hub (TISCH) [45] (http://tisch.comp-genomics.org/). Subsequently, the correlation between immune cell infiltration and JMJD4 was calculated using the CIBERSORT algorithm [46].

TIMER2.0 were used to analyze the relationship between JMJD4, regulatory T cells (Tregs), cancer-associated fibroblasts (CAFs), myeloid-derived suppressor cells (MDSCs), and CD8+ T cells. Furthermore, the function of JMJD4 in T-cell dysfunction and the CTL-associated prognosis across various cancer subtypes were explored at TIDE.

2.8. Statistic

All bioinformatics analyses are conducted on various data analysis platforms and software mentioned above. The significance of survival rate was evaluated using logarithmic rank test. Pearson or Spearman correlation coefficients were used in quantify the correlation. P-value less than 0.05 (* $p<0.05$) were considered significant.

3. Results

3.1. JMJD4 is differentially expressed in pan-cancer and can predicted patients survivial

To examing the function of JMJD4 in pan-cancer, we first analyze the JMJD4-related diseases on the OpenTarget and observed that several cancers are associated with JMJD4. The bubble graph illustrates the association of JMJD4 with nonpapillary renal cell carcinoma, renal cell carcinoma, hepatocellular carcinoma, and colon adenocarcinoma (Figure 1A). Subsequently, we utilized TIMER2.0 to analyze the differences in JMJD4 mRNA levels between pan-cancer and corresponding normal tissues. We found that JMJD4 mRNA was significantly upregulated in 14 cancer types (BLCA, BRCA, CHOL, COAD, ESCA, HNSC, KIRC, LIHC, LUAD, LUSC, PRAD, READ, STAD, UCEC) and downregulated in KICH, PCPG, and THCA (Figure 1B). Box plots revealed that JMJD4 mRNA was highly expressed in THYM, BRCA, and GBM but poorly expressed in SKCM, KIRC, and ACC (Figure 1C). Furthermore, JMJD4 was negatively correlated with advanced stages in ACC and UCEC,

and positively correlated with advanced stages in UCS (Figure 1D). UALCAN were used to compare protein expression differences between normal and primary tumor tissues in cancers. The results showed that the JMJD4 protein was upregulated in ccRCC and UCEC cancers and downregulated in HNSC (Figure 1E). We also noted that high expression of JMJD4 was associated with lower overall survival (OS) percentages in UVM, THYM, STAD, PRAD, PCPG, LAML, BLCA, and ACC (Figure 1F). These findings suggest that JMJD4 is differentially expressed across various cancers and can be serve as a predictor of patient survival.

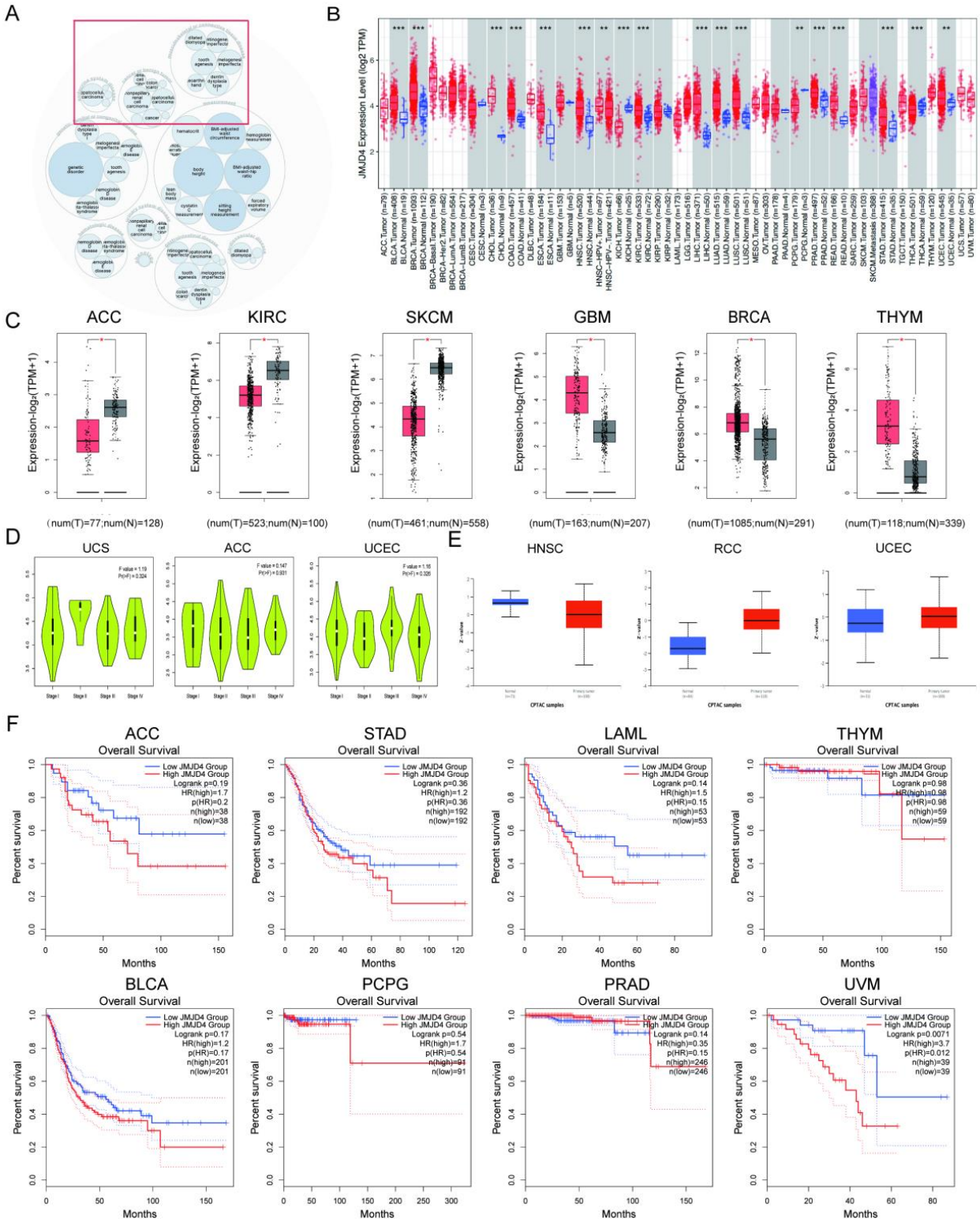


Figure 1. JMJD4 is differentially expressed in pan-cancer and can predicted patients survivial

A) Analyzed disease web tools related to JMJD4 on Open Target. The red dashed line indicates JMJD4 related cancer. B) The expression levels of JMJD4 mRNA in pan-cancer, and their corresponding control tissues were analyzed on TIMER2.0. Tumors and normal tissues are colored in red and blue, respectively, the transfer organization appears purple. C) The box plots of JMJD4 mRNA log2 expression levels between tumors and normal tissues in 6 cancer types on GEPIA2.0. T and N represant tumors and nomal tissues, respectively. D) JMJD4 expression levels in 4 different stages of 3 cancers were analyzed on GEPIA2.0. E) The protein expression differences between normal and primary tumor tissues in 3 cancers were compared on UALCAN. F) Kaplan Meier curve was drawn to predict patient OS. Red indicate high JMJD4 group, blue line indicated low JMJD4 group. *, **, and *** represent $p<0.05$, $p<0.01$, and $p<0.001$, respectively.

3.2. JMJD4 associated with genomic instability in pan-cancer

The genomic alterations of JMJD4 in TCGA pan-cancer were analyzed, including mutation, amplification, and deep deletion. To seek whether JMJD4 gene is altered at the genome level, we displayed the JMJD4 pan-cancer CNV and SNV analysis results and found that high JMJD4 amplification in BRCA, CHOL, LIHC and OV and high JMJD4 mutation in CHOL, UCEC and SKCM (>2%) (Figure 2A). To seek whether JMJD4 gene is altered at the genome level, we displayed the JMJD4 SNV analysis results and compared with JMJD8 and JMJD7 on GSCA and find that high SNV rates were observed in many cancers, especially, the deleterious mutation of JMJD4 reached 3.39% in UCEC (Figure 2B, C, and D). Moreover, high JMJD4 CNV group patients showed higher survival rates in LIHC and BRCA, and lower survival rates in READ, KIRP and ACC by the Kaplan-Meier plots from GSCA web tool (Figure 2E). JMJD4 was positively correlated with HRD in 4 cancers (KIRP, KICH, HNSC and STAD) and was negatively in 2 cancers (SARC and CHOL). We can also find that JMJD4 was positively correlated with MATH in 4 cancers (THYM, MESO, ACC and KICH) and was negatively in READ, LAML and UCS. As for MSI, JMJD4 was positively in THYM, TGCT and UCEC and was negatively in COAD and UCS. Moreover, JMJD4 was positively correlated with NEO in 3 cancers (BRCA, CESC and LIHC) and was negatively in KICH, THCA and CHOL. For Ploidy, JMJD4 was positively correlated with 3 cancers (STAD, THYM and STES) and was negatively associated with SARC and TGCT. 4 cancers (THYM, MESO, ACC and KICH) showed positive correlation with TMB while 3 cancers (READ, LAML and UCS) present a negative correlation (Figure 2F). These results strongly suggested that JMJD4 can serve as a marker for genome instability.

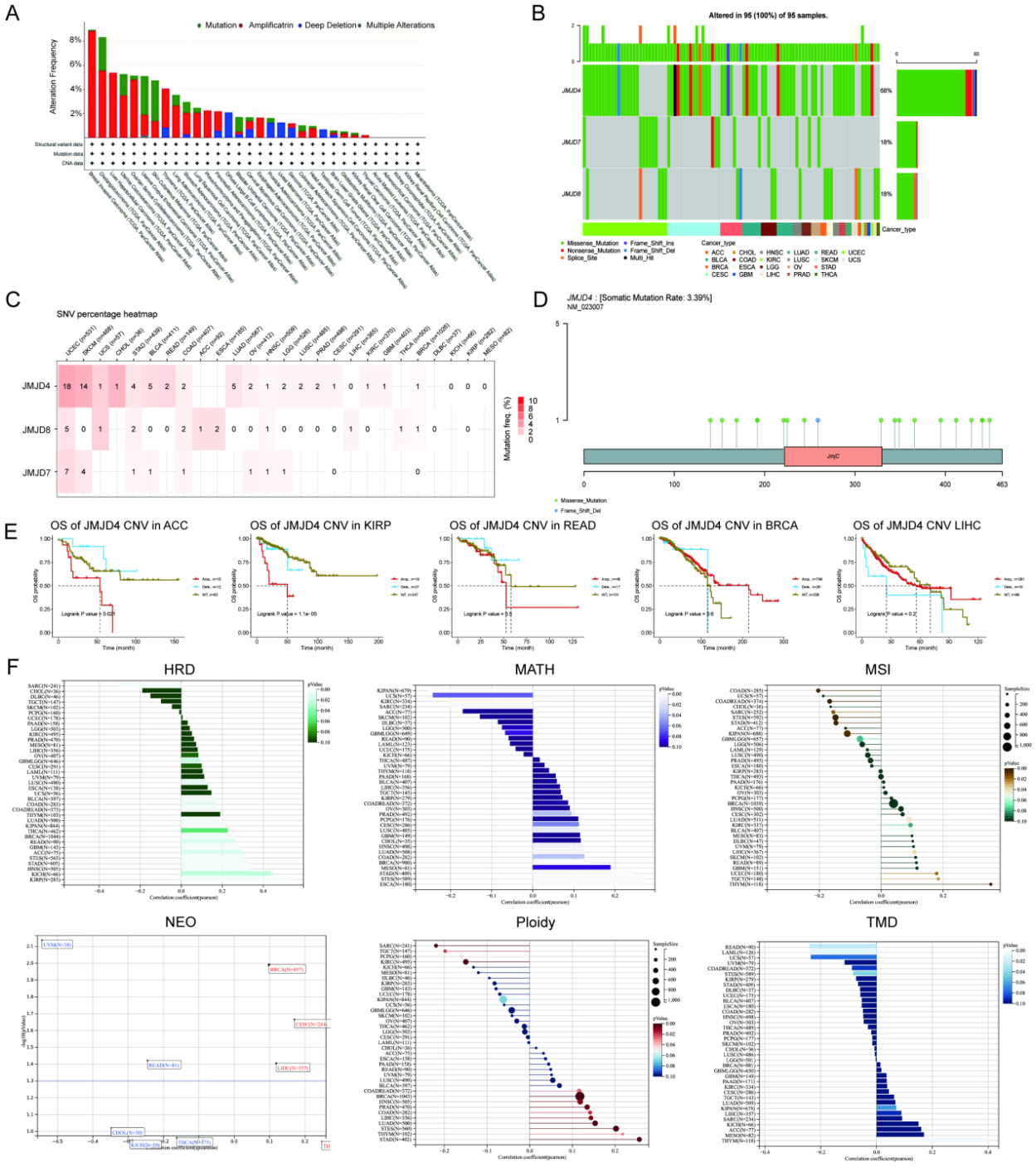


Figure 2. JMJD4 associated with genomic instability in pan-cancer

A) JMJD4 is associated with genomic instability in cancer. The green dot indicated the mutation, red dot indicated the amplification, blue dot indicated the deep deletion, gray dot indicated multiple alterations. B) The genome alteration landscape of JMJD4, JMJD7, and JMJD8 on GSCA. C) The prospects of JMJD4, JMJD7 and JMJD8 SNV in pan-cancer. D) The somatic mutation rate of JMJD4 in UCEC. E) Kaplan-Meier diagrams of JMJD4 CNVs in pan-cancer. F) The correlation coefficients in HRD, MATH, MSI, NEO, Ploidy, TMD and JMJD4 expression.

3.3. JMJD4 involved in cancer DNA repair, stemness, and epigenetic modulations

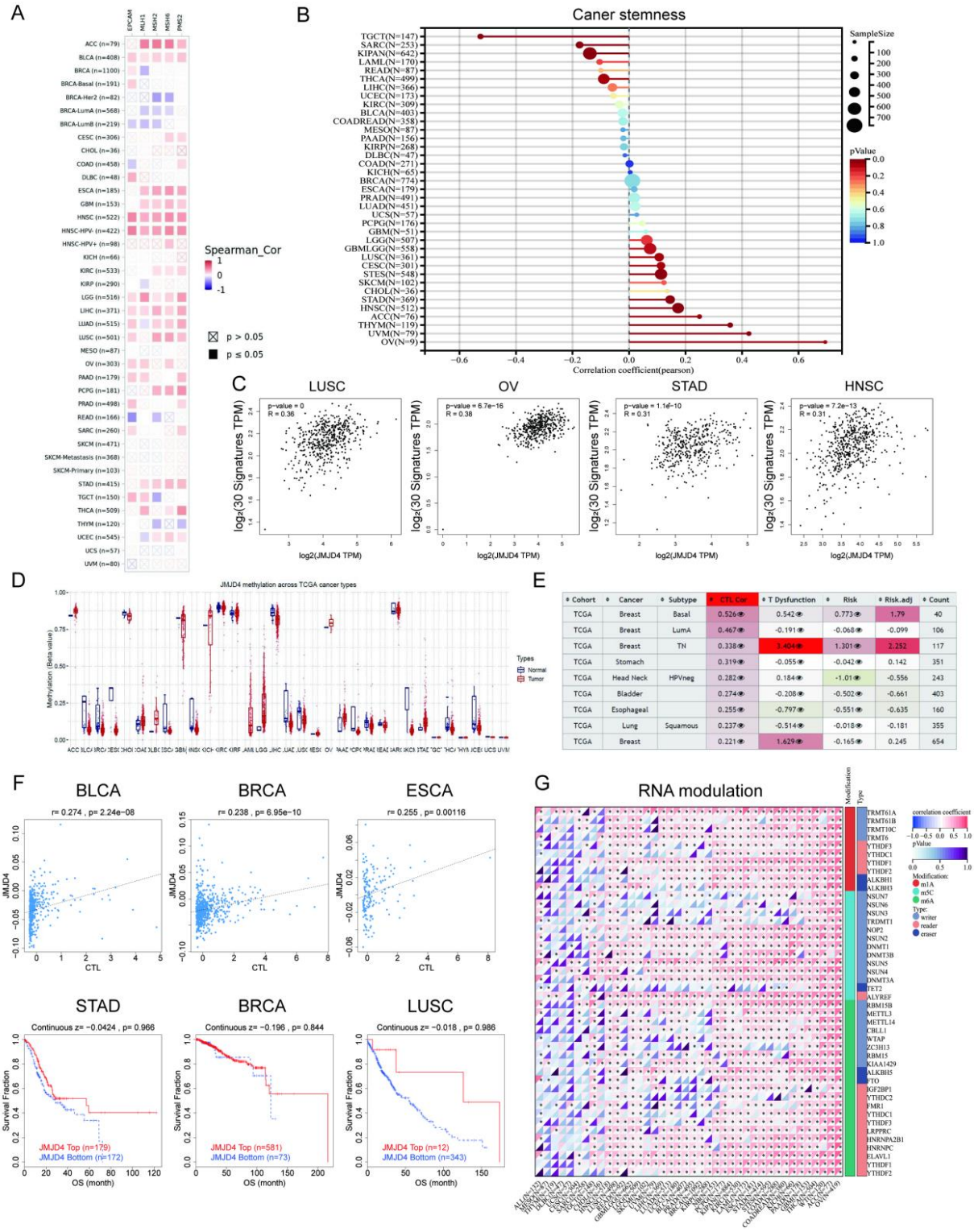
To determine whether JMJD4 is involved in cancer genetic modulations, we analyzed the correlation between JMJD4 and DNA repair genes, cancer stemness and DNA methylations. We discovered that JMJD4 was positively correlated with multiple mismatch repair (MMR) genes in most cancers, including ACC, BLCA, ESCA, GBM, LGG, LIHC, LUSC, PCPG, STAD, and especially

HNSC and HNSC-HPV (Figure 3A). The intercorrelation between cancer stemness and JMJD4 expression are visualized in Figure 3B, we noticed that JMJD4 obtained strong correlation with OV, followed by UVM, THYM and ACC, but with negative correlation in TGCT. LUSC, OV, STAD and HNSC showed positive correlations between stemness and JMJD4, which also presented consistent trends in HRR signature, demonstrating that JMJD4 interplayed with DNA repair-mediated cancer stemness (Figure 3C).

Since the DMPsi reflexed the DNA methylation status of cancer, we subsequently sought JMJD4's influences on cancer epigenetic modulations. As depicted in the Figure 3D, JMJD4 showed significant negative correlations with methyltransferases in ESCA, GBM, HNSC, BLCA, BRCA, CESC, CHOL, LIHC, UCEC, SKCM, THCA, LUAD, LUSC, PCPG, and PRAD. Inversely, their positive association was exhibited in 9 cancers, including LAML, LGG, ACC, COAD, DLBC, KICH, OV, PAAD, and STAD.

We also searched TIDE for the interplays between JMJD4 promoter methylation and cancer subtypes, CTL, and risks. Figure 3E presents the list of the top 9 CTL-correlated cancer subtypes. JMJD4 promoter methylation was positively correlated with CTL infiltration in BRCA, STAD, HNSC, BLCA, ESCA, and LUSC. The scatter plots and the Kaplan–Meier curves demonstrated that JMJD4 promoter methylation was associated with CTL infiltration and predicted more prolonged survival in BRCA, STAD and LUSC (Figure 3F).

In addition to DNA methylation, we also investigated the correlation between JMJD4 and RNA modulator gene expression. The heatmap shows the correlation between JMJD4 expression and RNA modulations in pan-cancer (Figure 3G). Surprisingly, we discovered that high JMJD4 was associated with many RNA modulator genes in many cancers, including m1A, m5C, and m6A, indicating that JMJD4 was involved in RNA modifications. These results showed that JMJD4 has a close relationship with cancer DNA repair, stemness, and epigenetic regulation.



Cohort	Cancer	Subtype	CTL Cor	T Dysfunction	Risk	Risk.adj	Count
TCGA	Breast	Basal	0.526	0.542	0.773	1.79	40
TCGA	Breast	LumA	0.467	-0.191	-0.068	-0.099	106
TCGA	Breast	TN	0.338	3.404	1.301	2.252	117
TCGA	Stomach		0.319	-0.055	-0.042	0.142	351
TCGA	Head Neck	HPVneg	0.282	0.184	-1.01	-0.556	243
TCGA	Bladder		0.274	-0.208	-0.502	-0.661	403
TCGA	Esophageal		0.255	-0.797	-0.551	-0.635	160
TCGA	Lung	Squamous	0.237	-0.514	-0.018	-0.181	355
TCGA	Breast		0.221	1.629	-0.165	0.245	654

Figure 3. JMJD4 involved in cancer DNA repair, stemness, and epigenetic modulations

A) The association between JMJD4 and five MMR genes in pan-cancer cells. B) The correlation between cancer stemness and JMJD4, where point size represents sample size and color represents p-value. C) The correlation between HRR characteristics of 30 genes and JMJD4 expression (R≥0.3) of 4 types of cancer. D) JMJD4 and methyltransferase between tumors and normal tissues. E) The correlation table between JMJD4 methylation level and CTL related factors was retrieved from TIDE network tool. The fourth column represents CTL correlation, and the fifth column represents the CTL dysfunction z-score of the interaction item. F) The levels of JMJD4 methylation and CTL markers were shown in scatter plots, while Kaplan-Meier plots showed survival analyses grouped by JMJD4

high methylation and low methylation. G) The relationship between JMJD4 expression and RNA regulation in correlated pan-cancer cells. * Indicates p<0.05.

3.4. JMJD4 alternative splicing were correlated to patient survival

Alternative splicing is a process in which the exons of RNA produced by the transcription of major genes or mRNA precursors are reconnected through RNA splicing in multiple ways. The resulting different mRNA may be translated into different protein structures; therefore, a gene may encode multiple proteins. Thus, we analyzed the splicing mode and the PSI of Intron_Retention_13400 in pan-cancer. Figure 4A showed that cancers such as KICH and THCA exhibit higher PSI than the normal samples. Those with prognostic values were presented in Figure 4C by the Kaplan–Meier curves. High PSI predicted higher xxx (DSS) in BRCA and OS in ACC. Similarly, high PSI also predicted higher disease-free interval (DFI) in LGG, and PFI in KIRC and UVM. These results indicate that the selective splicing of JMJD4 is closely related to patient survival rate and can serve as an effective marker for predicting patient survival rate.

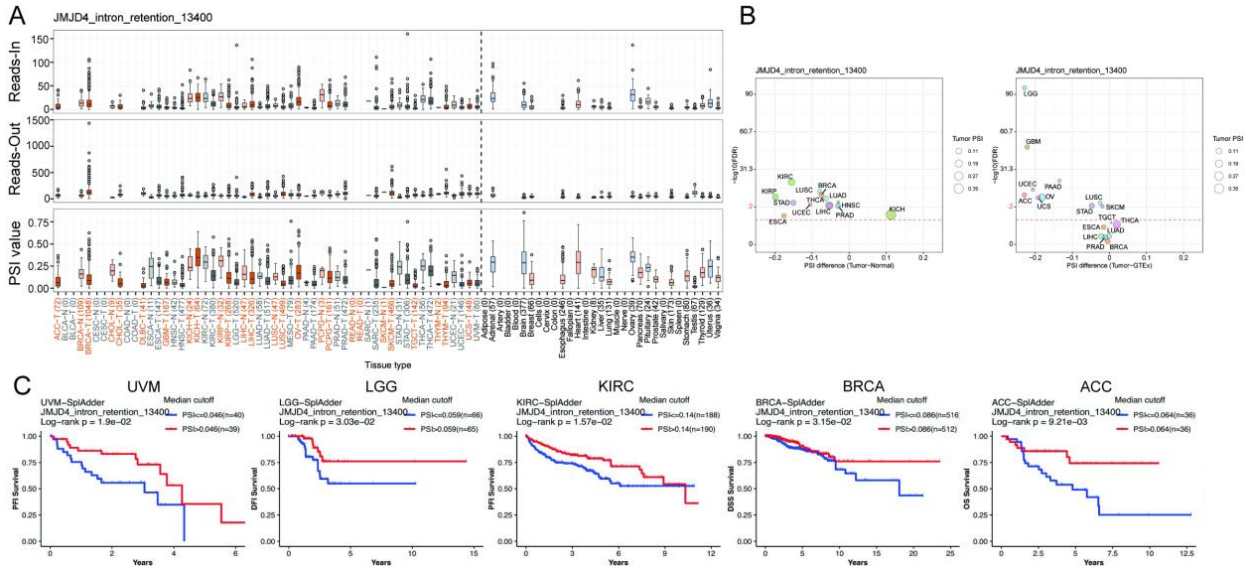


Figure 4. JMJD4 alternative splicing were correlated to patient survival

A) The reads-in, reads-out, and PSI values of JMJD4_ Intro Retention_13400 in all cancer tissues, adjacent tissues, and normal tissues. Color labels represent cancer and its corresponding adjacent tissues, while black labels represent non tumor tissues. B) PSI differences between tumors, adjacent normal tissues (upper) and tumors, GTEx normal tissues (lower); The red dashed line represents 0.05 of FDR, the dot size represents tumor PSI, and different cancers are marked with different colors. C) Kaplan-Meier curves were plotted for predicting patient OS, DSS, DFI, and PFI. All data comes from the OncoSplitting online network tool. * Indicates p<0.05.

3.5. JMJD4 was involved in demethylation, chromatin modification, protein transportation, cell cycle and immune activities

To further investigated the function of JMJD4 in cancers, the interactive proteins with experimental validations were obtained from String web tool, and 10 proteins were displayed (Figure 5A). We then compared the JMJD4 expression between altered and non-altered pathways on UALCAN and noticed that JMJD4 was elevated in altered chromatin modifiers status, SWI/SNF complex, p53/Rb-related pathway in GBM while poorly expressed in lung cancer (Figure 5B). The top 100 JMJD4 expressed genes in pan-cancer were analyzed on GEPIA2.0, and the top 5 genes (SNAP47, ARF1, DAP3, MTX1, and MRPL55) showed high correlations with JMJD4 in many cancer types (Figure 5C). The functional enrichment of GO terms exhibited multiply protein regulation (Figure 5D). The GSEA results of GO and KEGG suggested the close association between

immune activities and JMJD4. These results indicated that JMJD4 has a high correlation in demethylation, chromatin modification, protein transport, cell cycle, and immune activity.

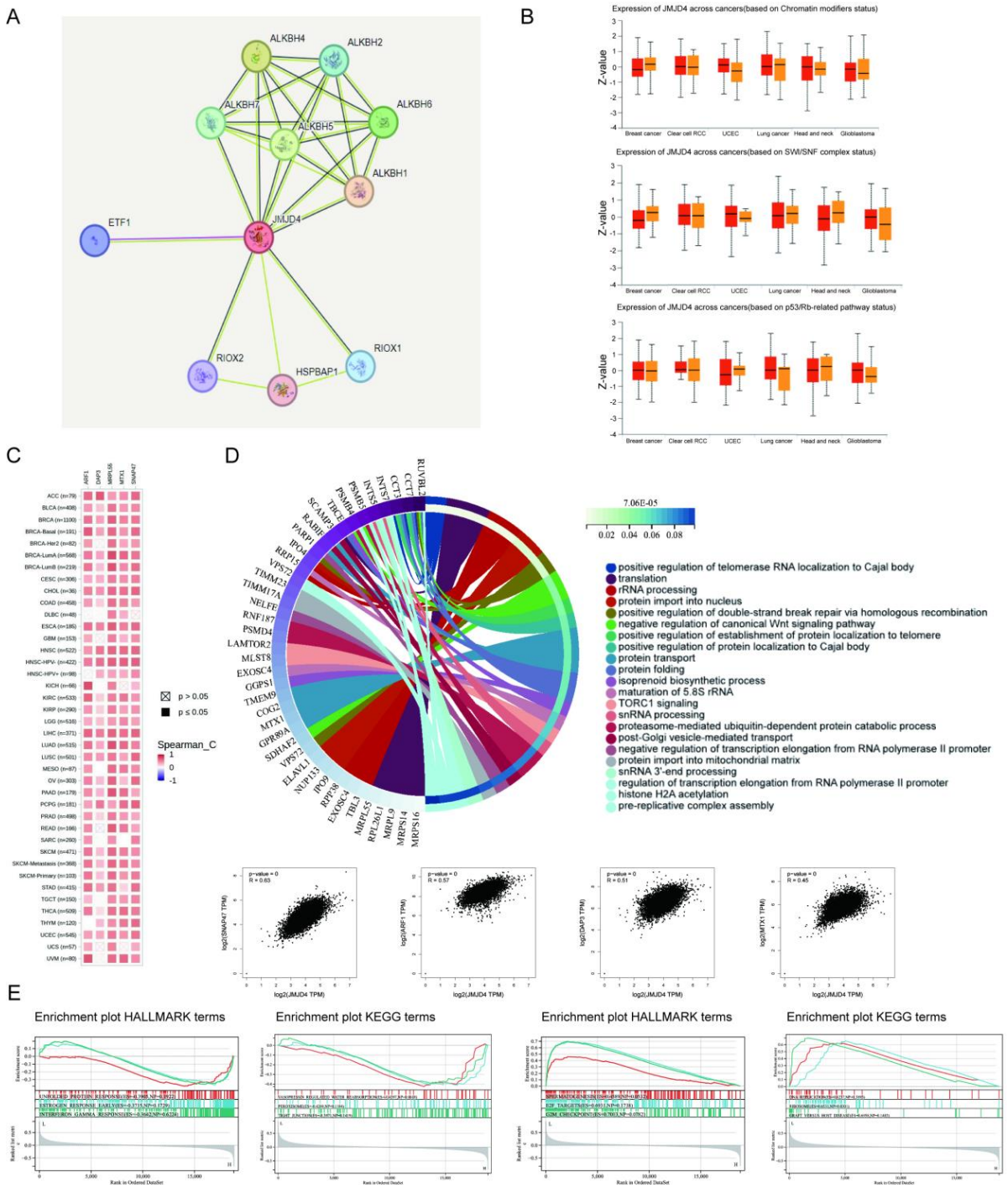


Figure 5. JMJD4 was involved in demethylation, chromatin modification, protein transportation, cell cycle and immune activities

A) 10 types of proteins were selected and displayed from the String web tool. B) The box plot of JMJD4 expression at the pathway level between somatic changes and non-changes was obtained from the UALCAN network tool for 6 cancer groups. C) The top 100 genes co-expressed with JMJD4 in whole cancer cells were analyzed on GEPIA2.0, and in most cancer types, the top 4 genes showed a high correlation with JMJD4. D) The circular graph of the top 100 JMJD4 co-expressed genes enriched GO pathways were identified on GEPIA2.0. Only the top genes of each pathway are listed at the left end of the corresponding color band. E) The enrichment maps of KEGG and HALLMARK terms in the entire cancer were analyzed using GSEA.

3.6. JMJD4 associated with immune infiltration and cytokine expression

To illustrate the association of JMJD4 and immune responses, the bar charts of the correlations between JMJD4 and ESTIMATEScore, ImmuneScore, and StromalScore were displayed, and the top 6 correlations were exhibited for each Score (Figure 6A). As we can noticed that JMJD4 was reversely correlated to ESTIMATEScore and ImmuneScore in many cancers, including LUAD, ESCA, STES, THCA, STES and TGCT. Moreover, the cancers showing negative JMJD4-ImmuneScore correlations also harbored negative correlations with most immune checkpoint genes, like BRCA, PRAD, LIHC and LUSC (Figure 6B). We also analyzed the associations between JMJD4 and chemokines, receptors, and immunostimulatory. As showed in Figure S1A, JMJD4 was negatively associated with several chemokines (CXCL9, 10, 11, 12, and 13), several receptors, and immunostimulatory in pan-cancer. Subsequently, we explored whether JMJD4 was differentially expressed in diverse cancer immune subtypes via TISIDB. The histogram exhibits that JMJD4 was significantly associated with immune subtypes in different cancers, such as BRCA, LUAD and STAD (Figure 6C). These results indicated that JMJD4 has a strong correlation with immune infiltration and cytokine expression, which might influence immune environment in many cancers by inhibiting immunostimulatory function and influencing immune checkpoint.

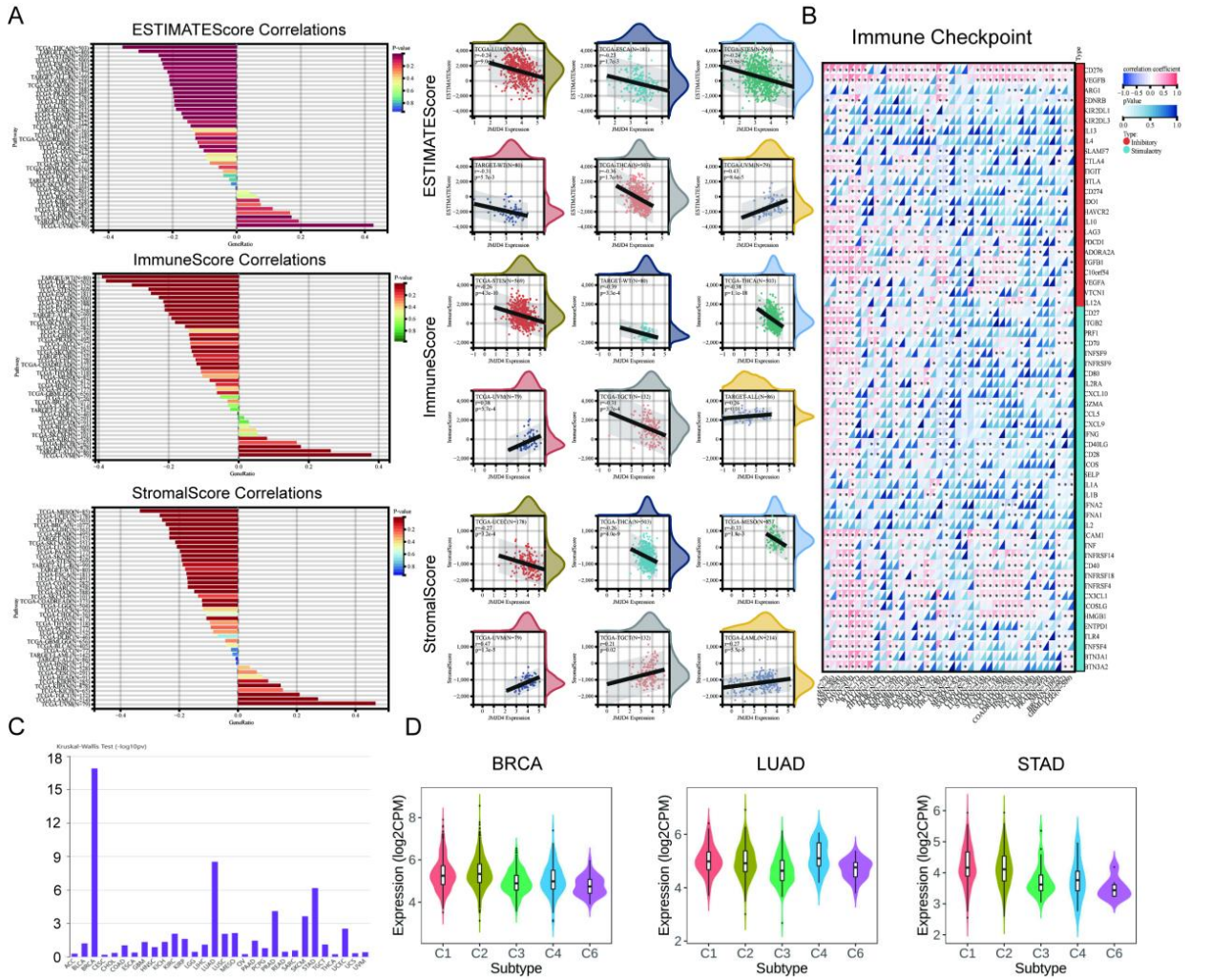


Figure 6. JMJD4 associated with immune infiltration and cytokine expression

A) The correlation between JMJD4 and ESTIMATEScore, ImmuneScore, and Stromal Score (left) were displayed, the top 6 correlated cancers for each Score were showed (right). B) Heat map of the relationship between immune checkpoints and JMJD4 expression in pan-cancer cells. C) The correlation between JMJD4 and immune subtypes was obtained from the TISIDB online tool. D) The expression of JMJD4 in 6 immune subtypes of 3 types of cancer. C1 indicates wound healing, C2

indicates IFN-gamma dominant, C3 indicates inflammatory, C4 indicates lymphocyte depleted, C5 indicates immunologically quiet, C6 indicates TGF-b dominant. * Indicates p<0.05.

3.7. JMJD4 is highly correlated with M2 macrophage infiltration in pan-cancer

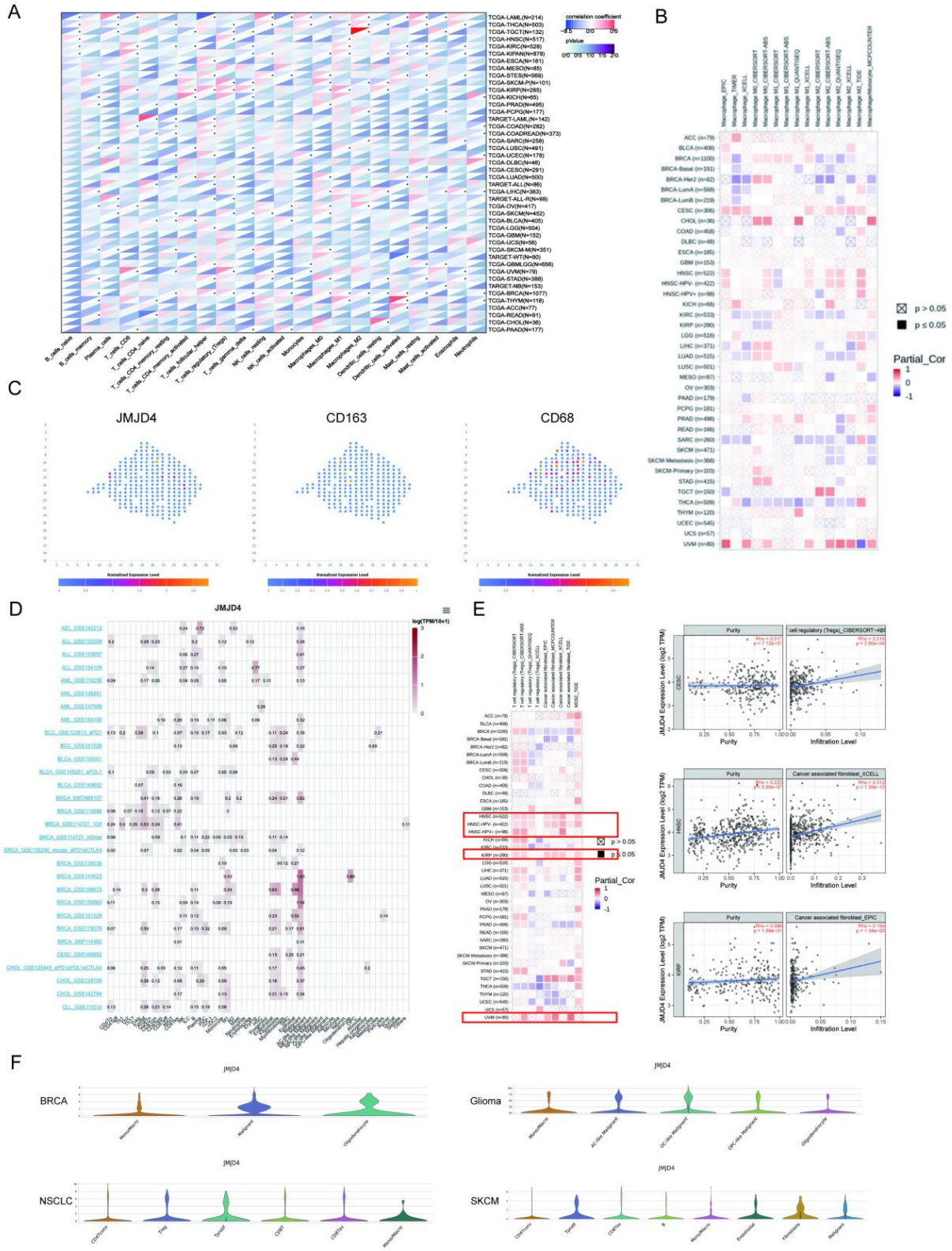


Figure 7. JMJD4 is highly correlated with M2 macrophage infiltration in pan-cancer

To investigate the immune role of JMJD4 in pan-cancer, we first ran the CIBERSORT algorithm to obtain 22 immunocyte correlations with JMJD4. We discovered that JMJD4 presented strong positive correlations with M2 macrophages in TGCT, LAML, THYM and CHOL (Figure 7A). Also,

B-cells-naive were negatively associated with JMJD4 in 22 cancers. By focusing on M2 macrophages, we conducted multiple algorithms on TIMER2.0 to analyze the correlation between their infiltration level and JMJD4 expression in pan-cancer, and the association was observed in KICH, KIRC, KIRP, LGG, LUSC, and UVM (Figure 7B).

Spatial transcriptional data on SpatialDB were obtained to depict the spatial overlapping of JMJD4 and M2 macrophage biomarkers CD68 and CD163 on BRCA cancer tissues. As expected, JMJD4, CD68, and CD163 markers presented similar spatial distributions, which implied their potential co-expression (Figure 7C). Additionally, we retrieved JMJD4 expression data in single-cell cellular subtypes from TISCH. As exhibited in Figure 7D, JMJD4 was expressed by M2 macrophages or malignant cells in SKCM, NSCLC, glioma and BRCA. Further, we used TIMER2.0 to display the correlations between JMJD4, Tregs, CAFs, and MDSCs. JMJD4's positive correlations with at least two cell types were observed in several cancers, including HNSC, HNSC-HPV-, HNSC-HPV+, KIRP and UVM (Figure 7E). We also compared JMJD4 expression in the collected cancer single-cell datasets and discovered that M2 or undefined macrophages were expressed it in BRCA, Glioma, NSCLC and SKCM (Figure 7F).

A) The correlation between 22 immune cells and JMJD4. B) The correlation between M2 macrophage infiltration level and the expression of JMJD4 in pan-cancer cells. C) The spatial transcription section displays the expression of JMJD4, CD68, and CD163 markers. The dot color represents the expression level of the marker. D) The expression of JMJD4 in cancer single cell clusters from TISCH online tool. E) The correlation heatmap between JMJD4 level and other immune cells penetration were calculated on TIMER 2.0. The red box highlights two or more types of cancer cells with consistent trends. F) Violin plot of JMJD4 expression in GEO pan-cancer single cell clusters. * Indicates $p<0.05$.

4. Discussion

The causes of cancer including genomic changes, transcriptional abnormalities, and epigenetic regulation. Until recent years, there have been fewer studies indicating that JMJD4 affects the progression of tumor cells. Therefore, in this article, we comprehensively analyzed and elaborated on the recently discovered cancer-related gene JMJD4 to conduct a multi-analysis from clinical significance, multi-omics characteristics, and its role in cancer immunity. Our research findings indicate that JMJD4 is associated with several cancers and can serve as a genetic marker to providing new insights into the clinical diagnosis and treatment of cancer.

Our research clearly indicates that JMJD4 is crucial in MMR and HHR in various cancer type. We found that JMJD4 reduce TMD and MSI in STAD through the MMR and HRR pathways, which is also examined in THYM. In addition, the importance of JMJD4 was further confirmed through its function on p53/RB, SWI/SNF, chromatin modification marker correlation, and reverse enriched G2M checkpoint pathways results. In addition, other members of the JMJD family may also participate in regulating the DNA repair process. For example, the HRR pathway requires JMJD5 [47], while the NHEJ pathway will be inhibited by JMJD1A [48]. The opposite effect of JMJD5 and JMJD1A on DNA repair can be achieved through H3K36 demethylation [49]. Therefore, JMJD4, JMJD5, and JMJD1A may also manipulate the DNA repair process through the HRR pathway.

Previously, Liang et al. [50] reported that JMJD8 is closely related to pan-cancer in various aspects. Since JMJD protein family have certain function similarity, but we still found different members may display different roles. We have noticed that the KEGG signaling pathways of JMJD4 is closely related to metabolism and immune activity, as well as highly correlated in demethylation, chromatin modification, cell cycle, and protein transport, which is somehow different from JMJD8. However, the strong correlation between JMJD4 and immune infiltration and cytokine expression is highly consistent with JMJD8. Especially for M2 macrophage, JMJD4 expression is strongly correlated with M2 macrophage infiltration in pan-cancer. In addition, JMJD4 is also associated with high permeability of Tregs, CAFs, and MDSCs. Therefore, the relationship between JMJD4 and tumor

immune microenvironment is very close, which might confirm to be the feature of JMJD family function.

Since there is relatively few research on JMJD family's impact on pan-cancer at present, our research fills the gap and provide new enlightenment for cancer patients with poor therapeutic outcomes. However, our research is limited to the online platforms and software for data analysis and calculations, further experiments might be needed to validates our primary results. Moreover, the sample size we used may change with the persistent updates of various cancer databases around the world, which may lead to more precise conclusion in the future. Given the important immune regulation role of JMJD4 in pan-cancer, we are looking forward to screening potential compounds as new therapeutic strategies and developed as new drug targets which could be applied for diagnostics, prognostics and therapeutics in the era of personalized medicine.

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