DOI: https://doi.org/10.52756/ ijerr.2024.v39spl.002 *Int. J. Exp. Res. Rev.*, Vol. 39: 15-38 (2024)

Original Article | *Peer Reviewed* | *Open Access*

International Journal of Experimental Research and Review (IJERR)

ISSN: 2455-4855 (Online)
www.iaph.in
ISSN 2455-4855

Integrated Bioinformatics Analysis and Transcriptomics Analysis Predict Jumonji and AT Rich Interacting Domain2 (JARID2) as a Therapeutic Target in Human Cancers

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Article History:

Received: 09th Feb., 2024
Accepted: 12th May, 2024
Published: 30th May, 2024

Keywords:
Bioinformatics, cancer, JARID2, polycomb repressive complex2, transcriptomics

How to cite this Article:
Bhuvanadas Sreeshma, Habeeb Shaik Mohideen and Arikketh Devi (2024). Integrated Bioinformatics Analysis and Transcriptomics Analysis Predict Jumonji and AT Rich Interacting Domain2 (JARID2) as a Therapeutic Target in Human Cancers. *International Journal of Experimental Research and Review*, *39*(spl.) 15-38.
DOI:
https://doi.org/10.52756/ijerr.2024.v39spl.002

Abstract: Jumonji and AT Rich Interacting Domain2 (JARID2) protein is recognized as a pivotal gene among the Polycomb Repressive Complex2 (PRC2) components. Nevertheless, the systematic assessment of JARID2 in cancers will enable us to understand its possible role and mechanism. Therefore, in this study, a pan-cancer analysis of JARID2 in cancers using The Cancer Genome Atlas (TCGA) database was performed. We observed an increased expression of JARID2 mRNA and protein in multiple cancer tissues in comparison to the control. In addition, we showed that the high JARID2 expression was closely associated to the poor overall survival and disease-free survival rate of cancer patients. Moreover, upregulated JARID2 has been observed to be involved in triggering the tumor immune response. To supplement the findings, a differential expression profiling was performed using datasets of RNA-Seq of OSCC tissues, which were obtained from NCBI SRA database. In line with the previous findings, JARID2 was observed to be upregulated in OSCC tissues. The expression pattern was validated in various cancer cell lines using qRT-PCR analysis. Altogether, this study comprehensively demonstrates JARID2 as a possible oncogene in human cancer.

Introduction

Cancer is the most common disease, with high morbidity and excessive rates of mortality worldwide (Kesavan et al., 2023; Madhu et al., 2022, 2023). According to the cancer statistical reports by the World Health Organization, Asia is the most affected continent in 2020 (GCO, 2020). Each type of cancer possesses a unique molecular signature, which could be any alterations in genetic and epigenetic events (Li et al., 2011; Randall et al., 2014; Takeshima and Ushijima, 2019; Ghosh et al., 2024; Yadav et al., 2024). Apart from genetic aberrations, epigenetic changes play an inevitable role in promoting malignancy (You and Jones, 2012; Das et al., 2024; Halder, 2024). The cancer cells proliferate and perform metastasis via a trigger regulated by certain driver genes, which stimulate tumor growth. In cancer, the epigenetic aberrations are complicated, which include chromatin structure-based alterations involving DNA methylation, histone variants, and modifications, nucleosome remodeling, small non-coding regulatory RNA, etc. (Sharma et al., 2009). Polycomb Repressive Complex 2 (PRC2) components are well-known epigenetic molecules that maintain a repressed transcriptional state in embryonic chromatin landscapes. These molecules are responsible for several dynamic biological processes, such as cell cycle progression, stem cell plasticity, cell differentiation, proliferation, etc. Of these, Jumonji and AT Rich Interacting Domain2 (JARID2) are among the substantial entities of PRC2.

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JARID2 is one of a vital JARID family of proteins possessing the Jumonji C (JmjC) domain and AT Rich Interacting Domain (ARID), which can regulate gene transcription by altering the chromatin structure by binding to the DNA. Although JARID2 has a Jumonji domain, it lacks histone demethylase activity due to the amino acid substitutions in its conserved region (Kooistra and Helin, 2012). Though described as an accessory component of PRC2, it controls the expression pattern of sundry crucial genes during embryonic development (Herz and Shilatifard, 2010). It has been proved in mice and Drosophila melanogaster that JARID2 interacts with the PRC2 molecules to perform various functions such as cell differentiation, cell cycle regulation, organ development, and transcriptional regulation (Jung et al., 2005; Kim et al., 2003). Moreover, JARID2 has been shown to regulate PRC2 function by directing its occupancy to the chromatin and its recruitment to the target genes (Li et al., 2010; Peng et al., 2009). Additionally, EZH2, another crucial gene of PRC2, has been notably overexpressed in several cancers and is a known oncogene (Gan et al., 2018; Guan et al., 2020; Mehta et al., 2023; Solairaja et al., 2023). The knockdown of EZH2 resulted in a drastic reduction of the severity in many cancers, and therefore, numerous clinical trials are being performed to identify inhibitors targeting EZH2. Recently, JARID2 is an interacting partner of EZH2, and the colocalization of both proteins has been well studied (Adhikari et al., 2019; Li et al., 2010). However, the role and mechanism of JARID2 have not been explored in cancer. Studies have shown that JARID2 is essential for TGF-β induced Epithelial to Mesenchymal Transition (EMT) by inhibiting Cadherin1 (CDH1) and miR-200 group genes in colon and lung cancer (Tange et al., 2014a). Moreover, JARID2 coordinates to facilitate EMT through Phosphatase and TENsin homolog deleted on chromosome 10/ Protein kinase B (PTEN/Akt) signaling pathway in hepatocellular carcinoma. Knockdown of JARID2 reduced the population of tumor-initiating cells and diminished the cells' invasion capacity and sphere-forming ability (Lei et al., 2016). Cao has also studied the cancer-promoting character of JARID2 in ovarian cancer. The knockdown of JARID2 resulted in the decline of proliferation, migration, invasion, and epithelial to mesenchymal transition in human ovarian cells via regulating Phosphoinositide 3-kinases (PI3K)/Akt signaling (Cao et al., 2017). A study on breast cancer research described that JARID2 was significantly expressed in various breast cancer cells and its inhibition by small interfering RNA (siRNA) leads to the upregulation of mesenchymal markers such as Vimentin, Matrix metalloprotease 7 and 9 (MMP7 and MMP9), while the epithelial marker E-cadherin was reduced (Zhang et al., 2020). In addition, a study on the LINC00852/miR-29a-3p/JARID2 axis also confirmed the oncogenic activity of JARID2 in prostate cancer cells (Zhang et al., 2022). These studies clearly state the pro-oncogenic property of JARID2. On the contrary, it is also reported that JARID2 suppresses the self-renewal of hematopoietic progenitor cells, preventing the progression of cancer cells, thereby acting as a tumor suppressor in myeloid neoplasm (Celik et al., 2018). The studies have identified the distinguishable role of JARID2 in tumorigenesis. However, the function of JARID2 in cancer is still unacknowledged.

Fig. 1

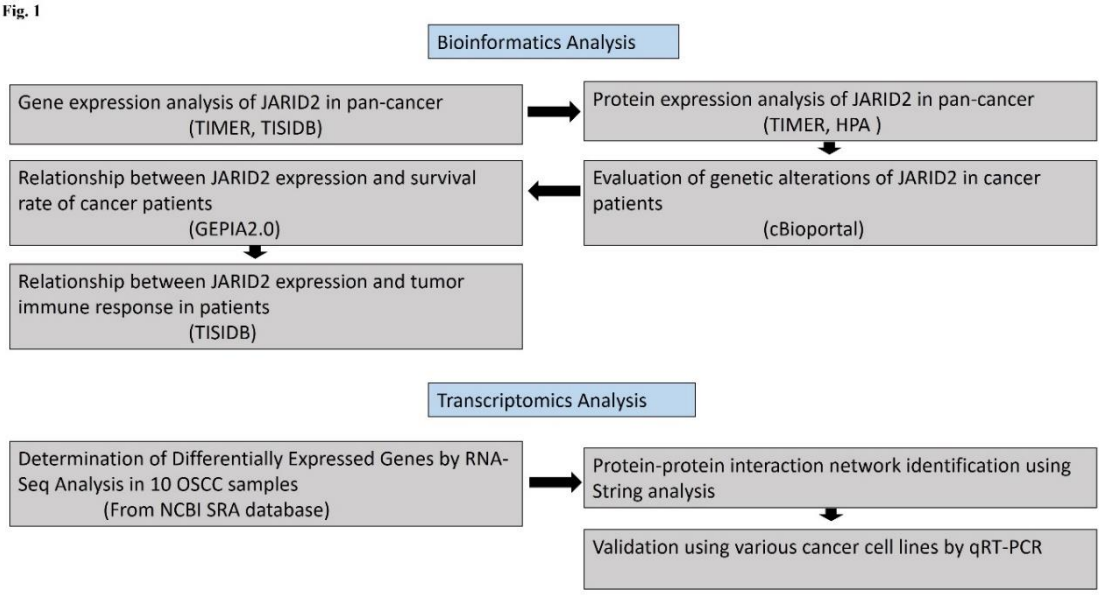

Figure 1. The study design.

Table 1. The cancer types included in the cohort of analysis are indicated.

Sl. No.	Cancer Type	Abbreviation
1	Adrenocortical Carcinoma	ACC
2	Bladder Urothelial Carcinoma	BLCA
3	Breast Invasive Carcinoma	BRCA
4	Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma	CESC
5	Cholangiocarcinoma	CHOL
6	Colon Adenocarcinoma	COAD
7	Lymphoid Neoplasm Diffuse Large B-cell Lymphoma	DLBC
8	Esophageal Carcinoma	ESCA
9	Glioblastoma Multiforme	GBM
10	Head and Neck Squamous Cell Carcinoma	HNSC
11	Kidney Chromophobe	KICH
12	Kidney Renal Clear Cell Carcinoma	KIRC
13	Kidney Renal Papillary Cell Carcinoma	KIRP
14	Acute Myeloid Leukemia	LAML
15	Brain Lower-Grade Glioma	LGG
16	Liver Hepatocellular Carcinoma	LIHC
17	Lung Adenocarcinoma	LUAD
18	Lung Squamous Cell Carcinoma	LUSC
19	Mesothelioma	MESO
20	Ovarian Serous Cystadenocarcinoma	OV
21	Pancreatic Adenocarcinoma	PAAD
22	Pheochromocytoma and Paraganglioma	PCPG
23	Prostate Adenocarcinoma	PRAD
24	Rectum Adenocarcinoma	READ
25	Sarcoma	SARC
26	Skin Cutaneous Melanoma	SKCM
27	Stomach Adenocarcinoma	STAD
28	Thyroid Carcinoma	THCA
29	Thymoma	THYM
30	Testicular Germ Cell Tumors	TGCT
31	Uterine Corpus Endometrial Carcinoma	UCEC
32	Uterine Carcinosarcoma	UCS

In this study, bioinformatics analysis of JARID2 using the TCGA database in multiple cancer types was performed to investigate the possible role of JARID2. The expression pattern of JARID2, its relationship with the overall survival analysis (OS) and disease-free survival (DFS), the effect of JARID2 expression on immune molecules in different cancers to check its immunological involvement, the co-expression network analysis and mutation analysis were determined to comprehend the potential mechanism of JARID2 in cancer prognosis or pathogenesis. Moreover, transcriptomics analysis was performed to identify and confirm the expression of JARID2 and related genes in human cancer, the study design of which is given in Figure 1. JARID2 was observed to be upregulated in cancer tissue when compared to the control. In addition, qRT-PCR in different cancer cell lines showed high expression of JARID2 which substantiated the RNA-Seq analysis, which reports JARID2 as a possible oncogene. This study further substantiates that JARID2 could be considered a promising gene to target human cancers.

Materials and methods

Gene expression analysis of JARID2

The University of California Santa Cruz (UCSC) cancer genomics browser (https://genome.ucsc.edu/) on human Dec.2013 assembly (GRCh30/hg38), the genome location of JARID2 gene was acquired (Cline et al., 2013). The subcellular localization of JARID2 was procured from Uniprot (https://www.uniprot.org/) (Bateman et al., 2021). Then, the variations between the JARID2 expression within various human cancer tissues

DOI: https://doi.org/10.52756/ijerr.2024.v39spl.002

and their adjacent normal tissues available in The Cancer Genome Atlas (TCGA) cohorts were studied by using the Tumor Immune Estimation Resource (TIMER2.0) database (http://timer.cistrome.org/) (Li et al., 2020). TIMER database provides pre-calculated RNA tissue specificity results for 10,897 tumors from 32 types of cancer. The RNA tissue specificity in tumors was compared with the control to identify the variation between them. The raw data available in the cohort was normalized using the Transcripts per Million (TPM) approach.

Comparative gene expression levels of JARID2 were also obtained from the TIMER database and are represented as box plots, as shown in Figure 1e. The statistical difference between the tumor and the control samples was calculated based on the Wilcoxon test.

The repository web portal for tumor-immune system interactions TISIDB (http://cis.hku.hk/TISIDB/) offers reports based on the PubMed database, high throughput sequencing data, exome and RNA sequencing database on patients who underwent immunotherapy and another public database such as TCGA database, UniProt, GO, DrugBank, etc. (Ru et al., 2019). From TISIDB, the molecular subtypes of tumors showing high expression of JARID2 were selected.

Protein Expression Analysis of JARID2

Based on the data available from The Human Protein Atlas version 22.0 and Ensembl version 103.38, the online Human Protein Atlas (HPA) database (https://www.proteinatlas.org/) provided the information related to the input, which was utilized to obtain the JARID2 protein expression data from cancer tissues and cancer cell lines (Thul and Lindskog, 2018). To acquire this, the ‘gene of interest’ was given as input and selected ‘subcellular’ and ‘pathology’ modules. The expression data thus obtained was TPM normalized (nTPM). The subcellular localization image was obtained from a ‘subcellular’ atlas consisting of immunofluorescence images of JARID2 in various cancer cell lines. The immunohistochemical staining of various tissue sections of colorectal, breast, prostate, and lung cancer was retrieved from the ‘pathology’ module.

Survival and prognosis analysis

Gene Expression Profiling Interactive Analysis (GEPIA 2) Database Analysis (http://gepia2.cancer-pku.cn/#index) developed by Peking University contains sequence expression data of 8587 normal tissues and 9736 tumor tissues (Tang et al., 2019). In our study, the “Survival Analysis” system was followed to plot the Kaplan Meier curve on overall survival analysis (OS) and disease-free survival (DFS)/ relapse-free survival (RFS) analysis based on the expression level of JARID2 in various cancers.

GEPIA 2 identifies the hazard ratio by using the Mantel-Cox test/ Log-rank test for hypothesis testing. The threshold expression was set to the cut-off high (50%) and cut-off low (50%) values to achieve high expression and low expression groups. The expression of JARID2 in different cancers was presented in percent survival. The 95% Confidence Interval (CI) curve for worst-case survival was represented as dotted lines in Figure 3. A survival map of hazardous ratio according to OS and DFS was procured from the database to categorize cancer with high and low OS and DFS with respect to the JARID2 expression, which was also calculated by the Mantel-Cox test.

Gene alteration analysis

The cBioPortal website (https://www.cbioportal.org/) is an open database source that Memorial Sloan Kettering Cancer Center developed for multidimensional cancer genome data analysis (Unberath et al., 2022). On the cBioportal website, the gene of interest was entered. The characteristics of genetic alterations of JARID2 were acquired from the “Mutations” module of cBioPortal, which provides data from the public TCGA database. This offered the details of mutations occurring in different domains of the protein as lollipop plots provided in Fig 4b. Then, the association between the genetic alterations of JARID2, Overall Survival (OS), and Disease Free Survival (DFS) analysis of patients was interpreted. The OS and DFS of all TCGA tumor samples with and without JARID2 genetic alterations were demonstrated in the ‘comparison/survival module’ available in the database. A log-rank test P-value <0.05 was considered statistically significant.

Immune infiltration analysis

The integrated repository portal for tumor immune system interactions known as TISIDB was employed to check the immune interactions. There were different modules available to analyze further. The gene symbol was entered in the ‘immune subtype’ module to examine each type of cancer and its immune subtypes where the expression of the gene of interest was associated (Su et al., 2022). The relation between the expression of JARID2 and various tumor immune subtypes is represented as C1 (wound healing), C2 (IFN-gamma dominant), C3 (inflammatory), C4 (lymphocyte depleted), C5 (immunologically), C6 (TGF-β dominant). Additionally, TISIDB was utilized to explore the correlation between JARID2 expression level and chemokines, receptors, and immunomodulators such as immune inhibitors, immune stimulators, and major

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histocompatibility complexes (MHCs) throughout human cancers. Also, the correlation between the tumor-infiltrating lymphocytes (TILs) and JARID2 expression was determined from the 'lymphocyte' module of the TISIDB database.

Acquisition of transcriptomics data

Illumina (HiSeq X Ten) PE100 sequenced human tissue samples, which included eight Oral Squamous Cell Carcinoma (OSCC) tissue (SRR8503813, SRR8503815, SRR8503816, SRR8503819, SRR8503821, SRR8503822, SRR8503824 and SRR8503826) and two non-cancerous normal oral tissue (SRR8503827 and SRR8503828) datasets were selected from the study conducted by Zhou et al. (2021). The datasets were downloaded using the National Centre for Biotechnology Information Sequence Read Archive (NCBI SRA) toolkit and were prepared using the Fastq-dump command in the Anaconda environment on a Linux platform. Pre-quality FastQC was performed for all the samples and the adapter sequences were removed as per the required protocols using the Fastp tool and post-trimming read quality was assessed with the help of FastQC.

Identification of DEGs, functional enrichment, and network mapping

Trimmed and high-quality reads were mapped with the human reference genome hg38 using HISAT2 by maintaining default parameters. Indexes for the mapping were built using the HISAT2-build command. The resultant data in SAM format was then converted into BAM format using SamTools. Further, we generated the count matrix for all the samples by mapping it against the reference genome mentioned earlier using the SubRead package of R. A separate matrix was prepared by extracting the counts from the earlier resulting files manually and this was normalized using Fragments Per Kilobase of Transcript per Million mapped reads (FPKM) approach. The normalized data was used to perform differential gene expression using the DESeq2 package of Bioconductor. The threshold set was p-value < 0.05 and |log2 fold change (FC)| >1 to shortlist the DEGs.

Heatmap and volcano plots were plotted using the ggplot2 package of R. All the DEGs were further mapped onto the Kyoto Encyclopedia for Genes and Genomes (KEGG) pathway enrichment to identify the crucial pathways that the DEG pattern has impacted. P value was set <0.05 as the cut-off value for the study. The complete analysis was performed on a Linux environment-based powerful High-performance computing facility installed at SRM IST.

JARID2 protein network analysis and functional enrichment

The protein of interest in this study is JARID2, and we generated a network involving all the proteins known to interact with JARID2. This was achieved by implementing the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) version 11.5 web service (https://string-db.org/), which dissected the interacting protein network (Szklarczyk et al., 2021). A medium confidence score of 0.40 and a minimum of 20 interactors were set to study the active interactions with JARID2.

Cell lines and cell culture

Different cancer cell lines such as Hep-G2 (Liver cancer), ACHN (Renal adenocarcinoma), A-498 (Kidney cancer), A-431 (Epidermoid carcinoma), A549 (Lung cancer), HeLa (Cervical cancer), ME180 (Cervical cancer) were compared with HEPM (Normal epithelial) for the study. All cell lines were maintained in Dulbecco's Modified Eagle's Medium (DMEM) (Himedia), which was supplemented with 10% fetal bovine serum (FBS) (Gibco) and 1% penicillin-streptomycin solution (Himedia). All cultures were grown in a humidified atmosphere at 37°C with 5% carbon dioxide.

RNA isolation and Real-time PCR

Total RNA was isolated from the cell lines using RNAiso Plus (Takara Bio, USA) as per the manufacturer's protocol and then quantified using Nanodrop (Biotek). After the qualitative and quantitative analysis, 2 µg RNA was used for synthesizing cDNA by reverse transcription PCR (RT-PCR) using Murine Leukaemia Virus Reverse Transcriptase (MuLV-RT) (New England Biolabs) and oligo (dT) (New England Biolabs). The primer sets 5'-ACCAGTCTAAGGGATTAGGACC-3', 5'-TGCTGGGACTATTCGGCTGA-3' were used to amplify JARID2. Subsequently, the relative quantification for the gene was determined using SYBR-green qRT-PCR Master Mix (Promega) and Applied Biosystems Quantstudio5 Real-Time PCR System. Relative mRNA expression levels were analyzed based on the $2^{-\blacktriangle\blacktriangle CT}$ method by normalizing the raw data with the β-actin.

Statistical Analysis

The survival curves for categorical variable prognostic analysis were generated using the Kaplan-Meier method, whereas the log-rank test P-value was utilized to calculate the statistical significance. The significance level was set at $p<0.05$. All statistical analyses were indicated as two-sided.

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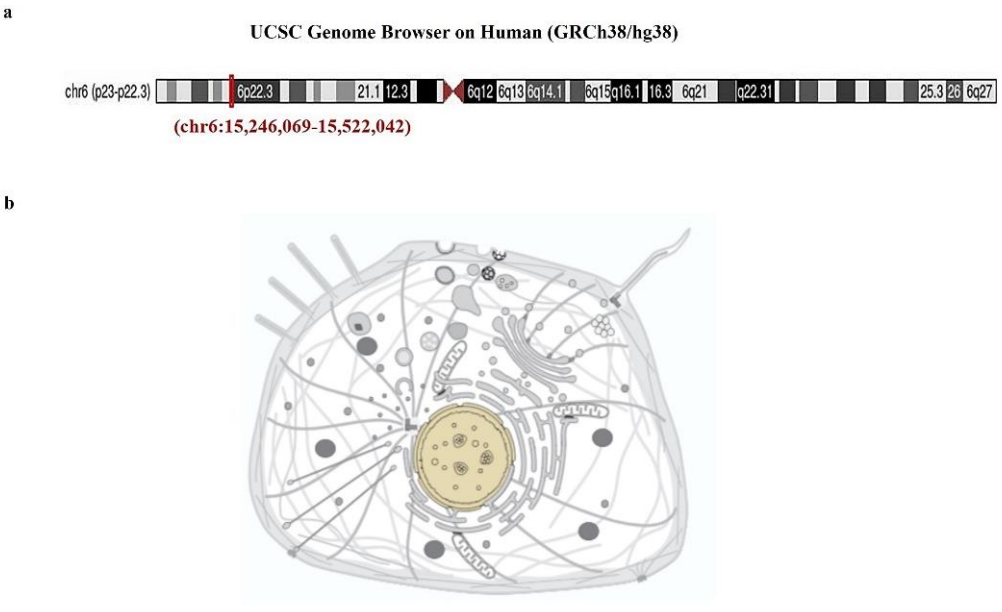
Results

Analysis of JARID2 localization and its expression levels in multiple cancers

Initially, the location of JARID2 in the human genome was analyzed from the UCSC genome browser. JARID2 was identified in chromosome 6 and its exact position was shown as 15,246,069-15,522,042 (Fig. 2a). The subcellular localization was detected to be along with the PRC2 components in the nucleus, which was determined from UniProt website (Fig. 2b). Then, the expression level of JARID2 in normal tissues was determined based on the consensus data from TIMER2.0 database as illustrated in Fig. 2c. The plots reveal the upregulation/ downregulation of JARID2 in the tumor samples concerning the control. JARID2 consensus Normalized eXpression (NX) levels of 54 normal tissue types were generated by combining the information of three transcriptomics datasets such as The Human Protein Atlas,((HPA), Genotype Tissue Expression (GTEx) and Functional Annotation of the Mammalian Genome (FANTOM5)). High expression levels of JARID2 mRNA were observed in bone marrow, cerebellum, retina, thymus, and testis. Though JARID2 mRNA expression was low in almost all normal tissues, it was evident in all of them with NX>1. This was due to the low tissue specificity. The analysis was performed in various cancer cell lines to understand the expression level of JARID2 in human cancers. The data revealed high expression in a few cancer cell lines such as testis, leukemia, bone cancer, skin cancer, and neuroblastoma (Fig. 2d). In contrast to the distribution in normal tissues, JARID2 mRNA level showed moderate RNA specificity in cancer cell lines. This has to be further analyzed to understand its role in cancer progression.

To further explore the differential expression of JARID2, its mRNA expression status in normal and cancer tissues from TCGA datasets were simultaneously studied by the TIMER2.0 database (Fig. 2e). The tumor tissues of BLCA ($p<0.05$), BRCA ($p<0.0005$), CESC ($p<0.05$), COAD ($p<0.0005$), ESCA ($p<0.005$), HNSC ($p<0.0005$), LIHC ($p<0.0005$), LUAD ($p<0.005$), LUSC ($p<0.0005$), PRAD ($p<0.05$), READ ($p<0.05$), STAD ($p<0.05$), UCEC ($p<0.0005$) had significantly higher JARID2 expression than its adjacent normal tissues. JARID2 showed elevated expression in all types of BRCA tumors, such as Basal, Her2, LumA, and LumB when compared to its control. Though HNSC tumor with HPV positive and HPV negative both shows higher expression than the normal adjacent tissues, JARID2 is higher in HPV-negative HNSC tissue. The white background columns in Fig. 2e imply the unavailability of the data for normal tissues.

To explore the role of JARID2 in prominent cancers, the JARID2 expression was analyzed with the molecular subtypes of cancers with high incidence and mortality rates using the TISIDB database. The results suggested that JARID2 expression was significantly associated with the subtypes of different cancers such as LUSC (Basal, classical, primitive, secretory), READ (Chromosomal instability (CIN), genome stable (GS), hyper-mutated-single-nucleotide variants (HM-SNV), hyper-mutated-indel (HM-Indel), LIHC (icluster:1, icluster:2, icluster:3), COAD (Chromosomal instability (CIN), genome stable (GS), hyper-mutated -single-nucleotide variants (HM-SNV), hyper-mutated- indel (HM-Indel)), BRCA (Basal, Her2, LumA, LumB), specific gene fusions of PRAD (ERG, ETV1/4, FLI1 or mutations such as SPOP, FOXA1, IDH1), OV (Differentiated, immunoreactive, mesenchymal, proliferative) and HNSC (Atypical, basal, classical, mesenchymal) (Fig. 2f). Taken together, these results showed high expression of JARID2 in pan-cancer tissues concerning normal tissues, which could be due to its oncogenic behavior in these cancers.



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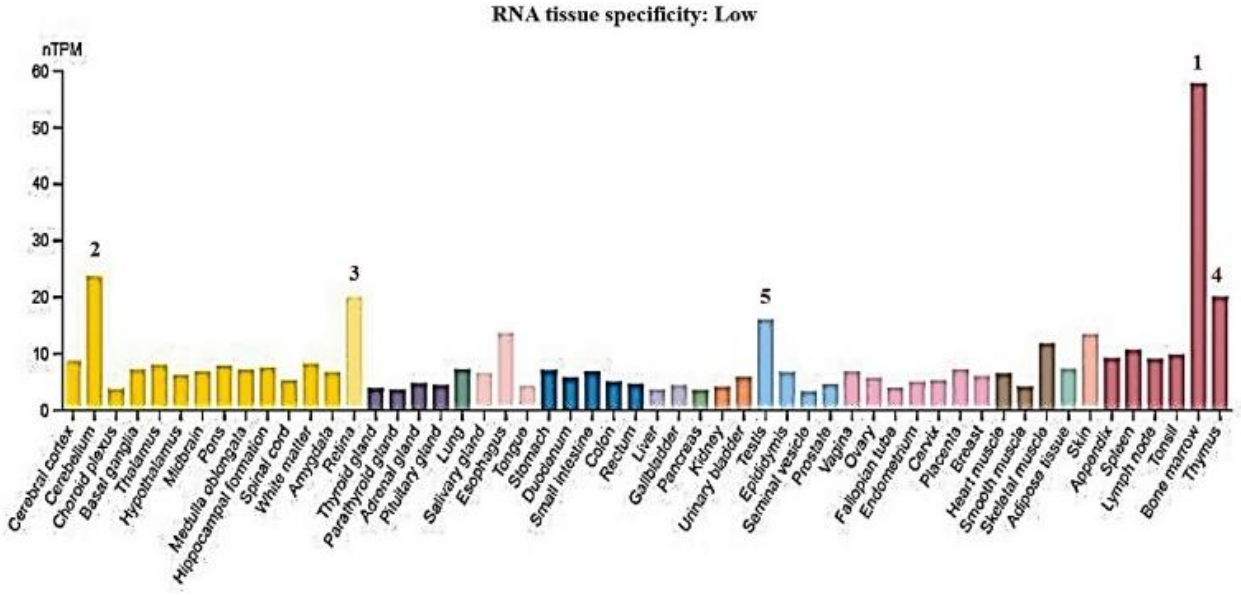

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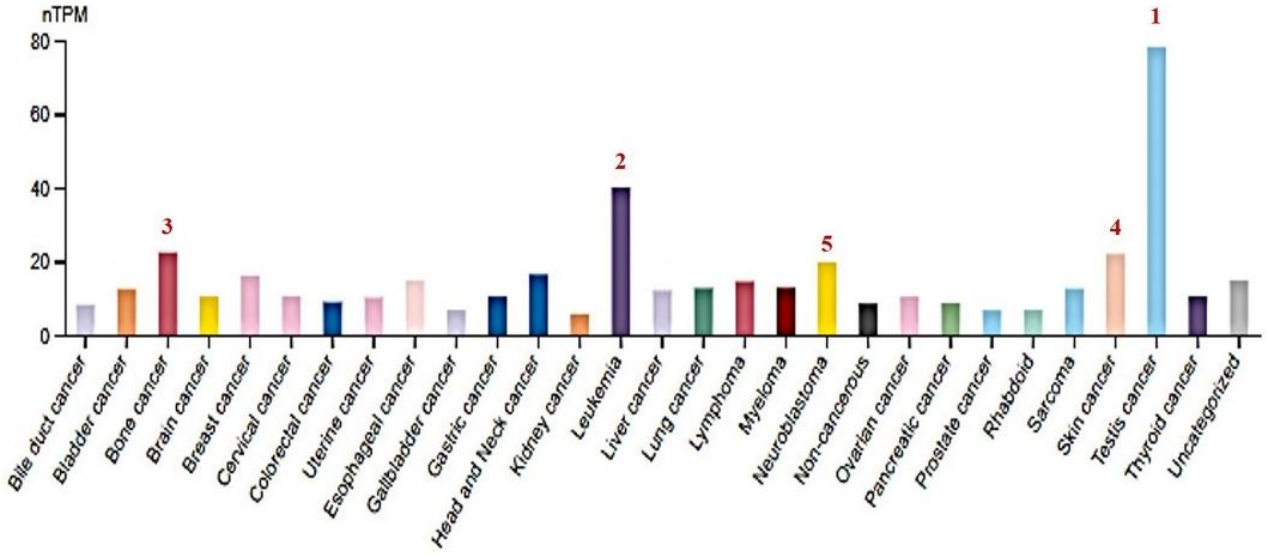

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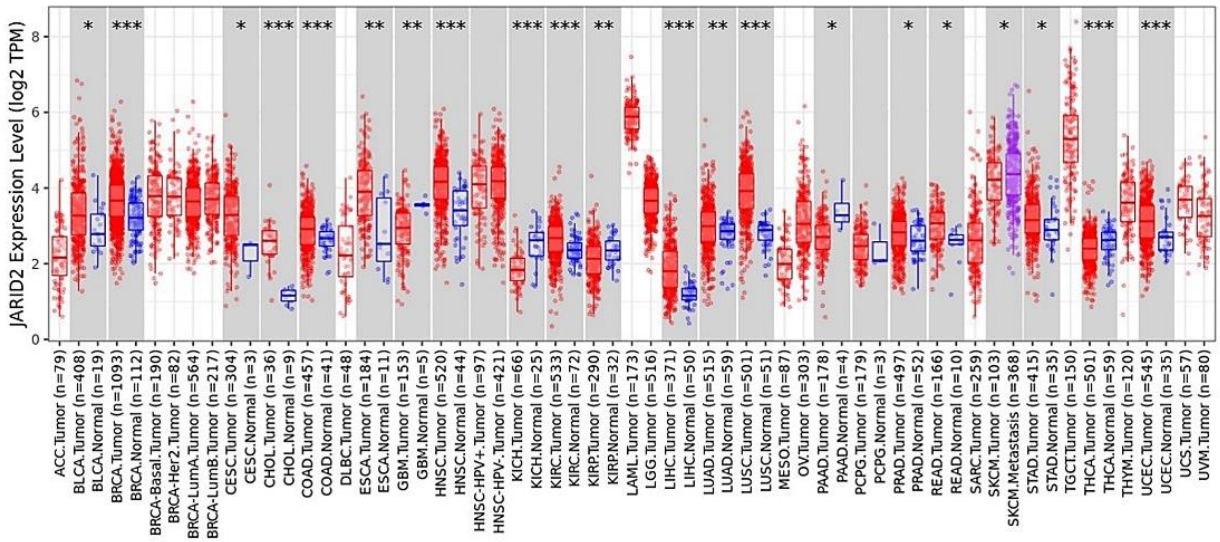

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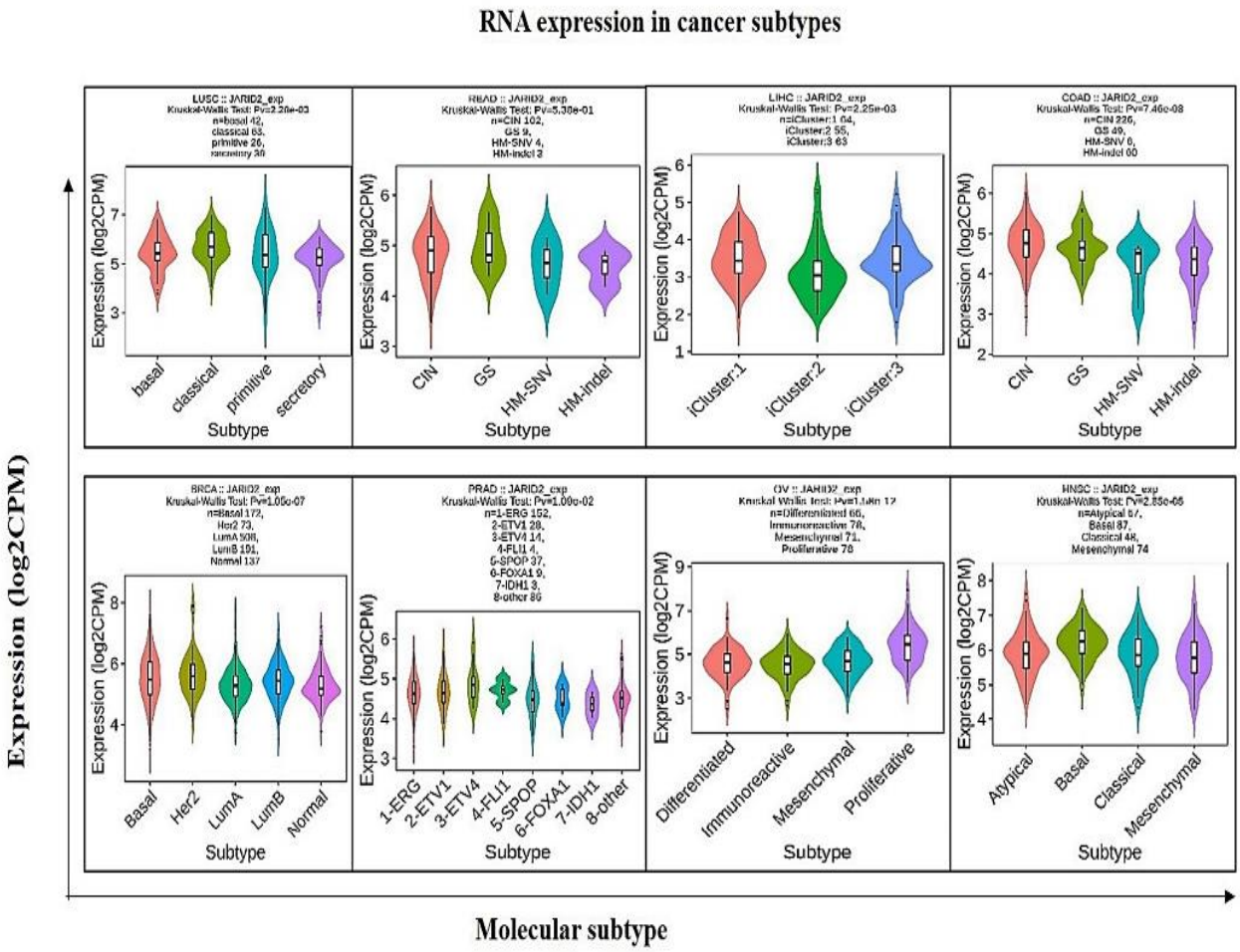

Figure 2. JARID2 location, expression in normal and cancer tissues. (a) Genomic location of human JARID2 gene; (b) JARID2 is detected in the nucleus along with the PRC2 genes; (c) RNA specificity in normal tissues of JARID2; (d) RNA tissue specificity in different cancers; (e) Comparative JARID2 expression level in normal and cancer tissues; (f) Analysis of JARID2 expression level in various molecular subtypes of cancers which has high JARID2 expression. *p<0.5, **p<0.05, ***p<0.005.

Analysis of JARID2 protein expression in human cancers

The previous findings demonstrate the abnormal expression of JARID2 mRNA in various cancers. The protein expression of JARID2 in these cancers was analyzed to substantiate the mRNA expression results obtained. Primarily, TIMER2.0 was utilized to determine the protein expression of JARID2 in normal tissues (Fig. 3a). Cerebral cortex, thyroid gland, adrenal gland, nasopharynx, bronchus, stomach, rectum, kidney, epididymis, endometrium, cervix, placenta, heart muscle, smooth muscle, skeletal muscle and bone marrow showed high JARID2 expression, whereas soft tissues and adipose tissues exhibited low expression and unveiled no expression in tissues such as oral mucosa, salivary gland, esophagus, liver and gall bladder. The same experiment was performed in cancer tissues, which helps in comparing the expression status with the normal tissues (Fig. 3b). The results revealed that more than 90% of the patients with skin cancer, testicular cancer, pancreatic cancer, urothelial cancer, and head and neck cancer had strong JARID2 positivity. Moreover, other cancers such as thyroid, lung, colorectal, carcinoid, renal, prostate, cervical, and melanoma also showed strong JARID2 expression (>80%). The immunohistochemistry results also demonstrated higher protein expression of JARID2 in cancer tissues such as colorectal, breast, prostate, and lung than in its normal tissues (Fig. 3c). The immunofluorescence results revealed nuclear and mitochondrial localization of JARID2 in a metastatic neuroblastoma cell line (SH-SY5Y) (Fig. 3d).

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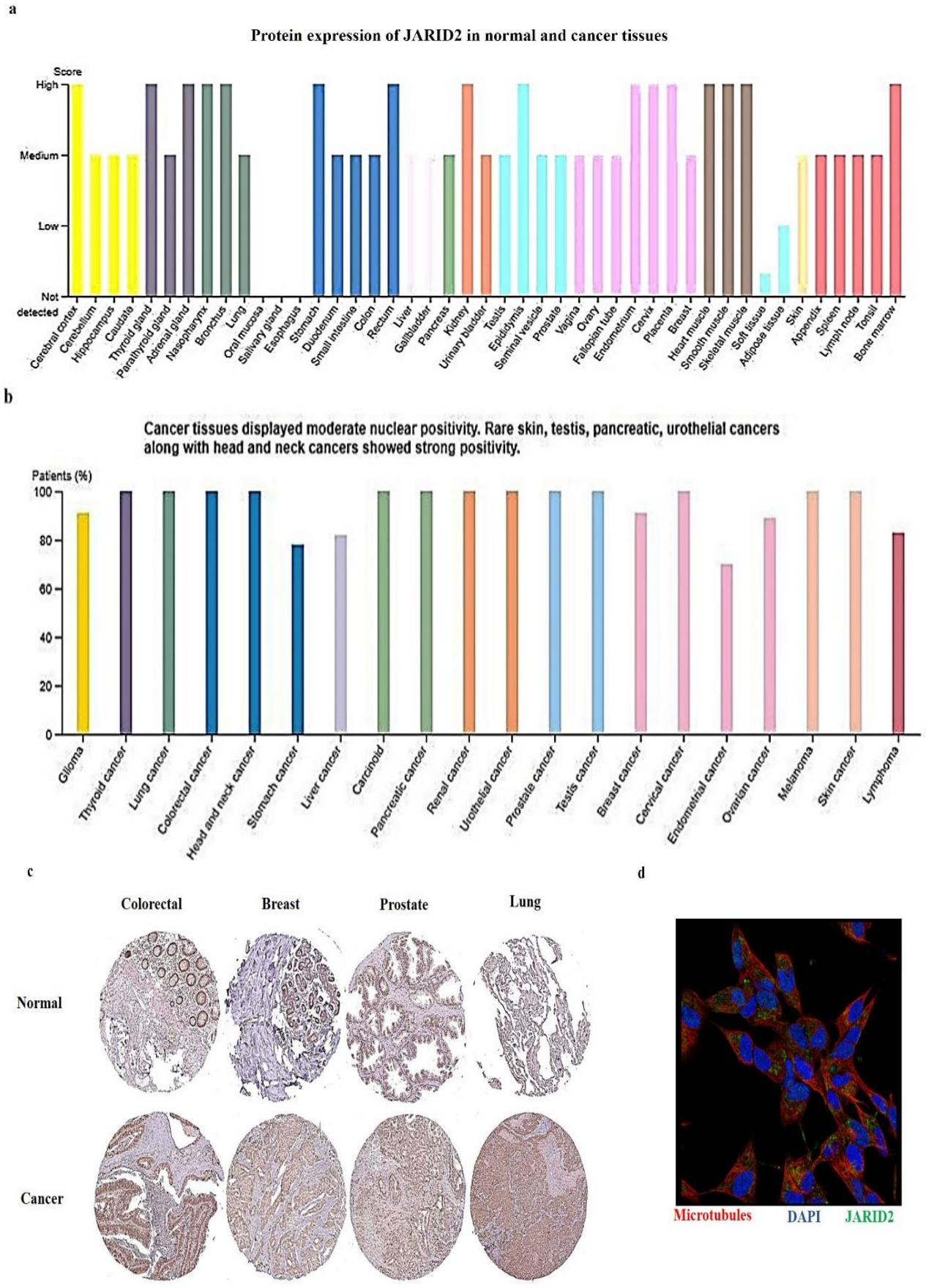

Figure 3. JARID2 protein expression and localization in normal and cancer tissues (a) Protein expression level of JARID2 in various normal tissues; (b) JARID2 protein expression in different cancer tissues; (c) Immunohistochemical staining of JARID2 in normal and prominent cancer tissues; (d) Immunofluorescence staining of JARID2 in metastatic neuroblastoma cell line (SH-SY5Y)[Red- microtubules, Blue- DAPI, Green- JARID2].

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Analysis of the association between the JARID2 expression and prognosis of cancer patients

To reveal the potential prognostic role of JARID2, we executed the analysis of the correlation between OS and DFS of pan-cancer cohorts, with the expression of JARID2 using the GEPIA2.0 database. The results suggested that high JARID2 expression was associated with poor overall survival rate in cancers (Fig. 4a), exclusively in ACC ($p<0.00021$), SARC ($p<0.018$), SKCM ($p<0.033$), TGCT ($p<0.052$) and THCA ($p<0.02$) (Fig. 4b and 4c). Similarly, the disease-free survival rate was also verified to conclude the involvement of JARID2 expression in cancer prognosis. The results disclosed that high JARID2 expression denoted an unfavorable disease-free survival rate in patients with ACC ($p<6.7e-06$) and LIHC ($p<0.015$) (Fig. 4d, 4e and 4f). Together, these data indicate that high JARID2 expression correlates to poor cancer prognosis.

Fig. 4

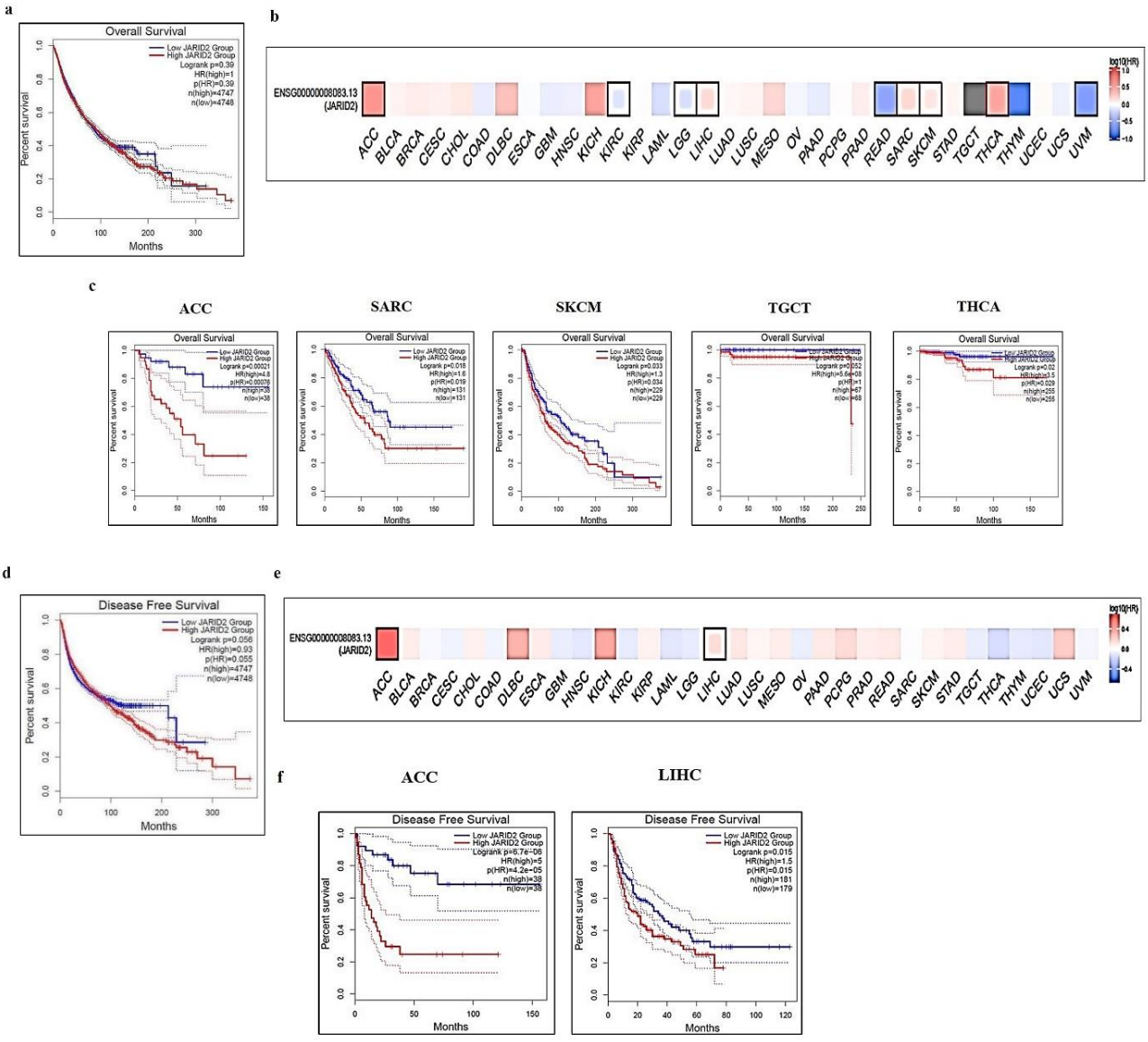


Figure 4. Correlation between survival and JARID2 expression in different cancer types. (a) Correlation between JARID2 gene expression and overall survival in different cancer types in TCGA were assessed using GEPIA2.0; (b) High JARID2 expression was observed to be associated with poor overall survival rate in various cancers; (c) Cancers such as ACC, SARC, SKCM, TGCT and THCA shows poor OS associated with high JARID2 expression; (d) Correlation between JARID2 gene expression and disease-free survival in different cancer types in TCGA were assessed using GEPIA2.0; (e) High JARID2 expression was associated with poor disease-free survival; (f) Cancer types such as ACC and LIHC shows poor disease-free survival correlated to high JARID2 expression. *p<0.5, **p<0.05, ***p<0.005, ****p<0.0005.

DOI: https://doi.org/10.52756/ijerr.2024.v39spl.002

Determination of genetic alterations of JARID2 in various cancers

By utilizing the cBioPortal database, the genetic alterations of JARID2 in TCGA datasets were performed. From the results, JARID2 had multiple genetic alterations commonly observed in cancers, primarily dominated by mutations (<12%) followed by amplifications (<8%) and deep deletions (<6%) (Fig. 5a). Out of all cases, UCEC patients showed higher frequency of JARID2 mutations as the predominant alteration type (~12%) followed by STAD (~8%) and SKCM (~5%). Amplification was observed as the second common alteration, which is observed in OV (~8%). Deep deletions are one among the alterations observed in cancers with a higher percent in TGCT (<6%) and in also identified in STAD, CESC, and ESCA, whereas structural variations and other alterations are also noticed in TGCT, SKCM, LUAD, and BLCA with less than 1%.

Sequentially, we investigated the types of genetic alterations occurring in JARID2 by using the cBioPortal database. The lollipop plot results revealed that the principal genetic alteration occurring across the domains of JARID2 was a missense mutation (~216) (Fig. 5b). The second leading type of alteration is described to be truncating mutations (~46), which was also noticed throughout JARID2 domains except zf domain. The fusion of other genes with JARID2 has been reported in LUAD, BRCA, CESC, BLCA, HNSC and SKCM. In addition, splice alterations and inframe mutations were also detected.

Determining the role of JARID2 in tumor immunological response

The anti-tumor immune response is meticulously coordinated by a series of mechanisms initiated by Step1: cancer cell antigen release, Step2: presentation of cancer antigen, Step3: priming and activation, Step4: trafficking of immune cells to cancer cells, Step5: immune cell infiltration to the tumor, Step6: cancer cell recognition by T-cells and finally Step7: killing of cancer cells. We utilized the TISIDB database to study the contribution of JARID2 expression in immune activation events. Initially, the association between the JARID2 expression and quantity of tumor-infiltrating lymphocytes (TILs) was studied with a heatmap (Fig. 6a). The chemokine receptors, immune-inhibitors, immunostimulators, major histocompatibility complexes and receptors that correspond to JARID2 expression are shown in Supplementary file 1.

Furthermore, to understand the underlying correlation between JARID2 expression and Cancer-associated Fibroblasts (CAFs), TISIDB database analysis was performed. The results suggested that JARID2 expression was significantly associated with the immune subtypes, which included C1 (Wound healing), C2 (IFN-γ dominant), C3 (Inflammatory), C4 (Lymphocyte depleted), C5 (Immunologically quiet), and C6 (TGF-β dominant) across pan-cancer. LGG, KIRC, KIRP, COAD, LUSC, OV, PCPG, STAD, and LUAD showed a significant correlation between JARID2 expression and immune subtypes (Fig. 6b). Based on these results obtained, it can be speculated that JARID2 expression in these cancers could act as a vital role in triggering tumor immune responses. Further studies are required to understand JARID2.

Identification of DEGs in cancer

To confirm the expression of JARID2 in cancers, we analyzed the transcriptomic datasets of an HNSC type, Oral Squamous Cell Carcinoma (OSCC), with 8 cancer tissues and 2 normal oral tissues. After the data processing, Principle Component Analysis (PCA) was performed for all the samples and the plot is given in supplementary file 1. We identified 46,735 genes that are differentially expressed between OSCC and control based on false discovery rate <0.05 and |log2 fold change (FC)| >1. The Differentially Expressed Genes (DEGs) obtained are provided as supplementary file 2. The number of DEGs in OSCC was represented as a pie chart (Fig. 7a). We observed 8,510 upregulated genes, including JARID2 (Ensemble ID – ENSG00000008083) and 38,225 downregulated genes in the OSCC. The DEGs were plotted using the heatmap function from the ggplot2 package. The heatmap represents DEGs in OSCC when comparing it with the normal oral samples (Fig. 7b). The volcano plot for the DEG is given in Fig. 7c, where each dot depicts a gene. The red dots denote significantly upregulated genes and green dots correspond to significantly downregulated genes. KEGG pathway analysis disclosed that the DEGs were associated with pathways related to cancers, as shown in Fig. 7d. The significant genes associated with the analysis were represented in supplementary file 3. JARID2 was observed as one of the significant genes that adds relevance to the contribution of this gene in OSCC tumorigenesis. To uncover the molecular mechanism of JARID2 in the progression of cancers, the co-expression networks and the interaction between them need to be well analyzed. STRING, a protein-protein interaction information database, was performed and the results were visualized using Cytoscape (https://cytoscape.org/, V3.10.1). The results showed a strong association of JARID2 with PRC2 components, which indicated a persuasive relation among them (Fig. 7e). The functional enrichment was also performed and is given as supplementary file 4.

DOI: https://doi.org/10.52756/ijerr.2024.v39spl.002

Fig. 5

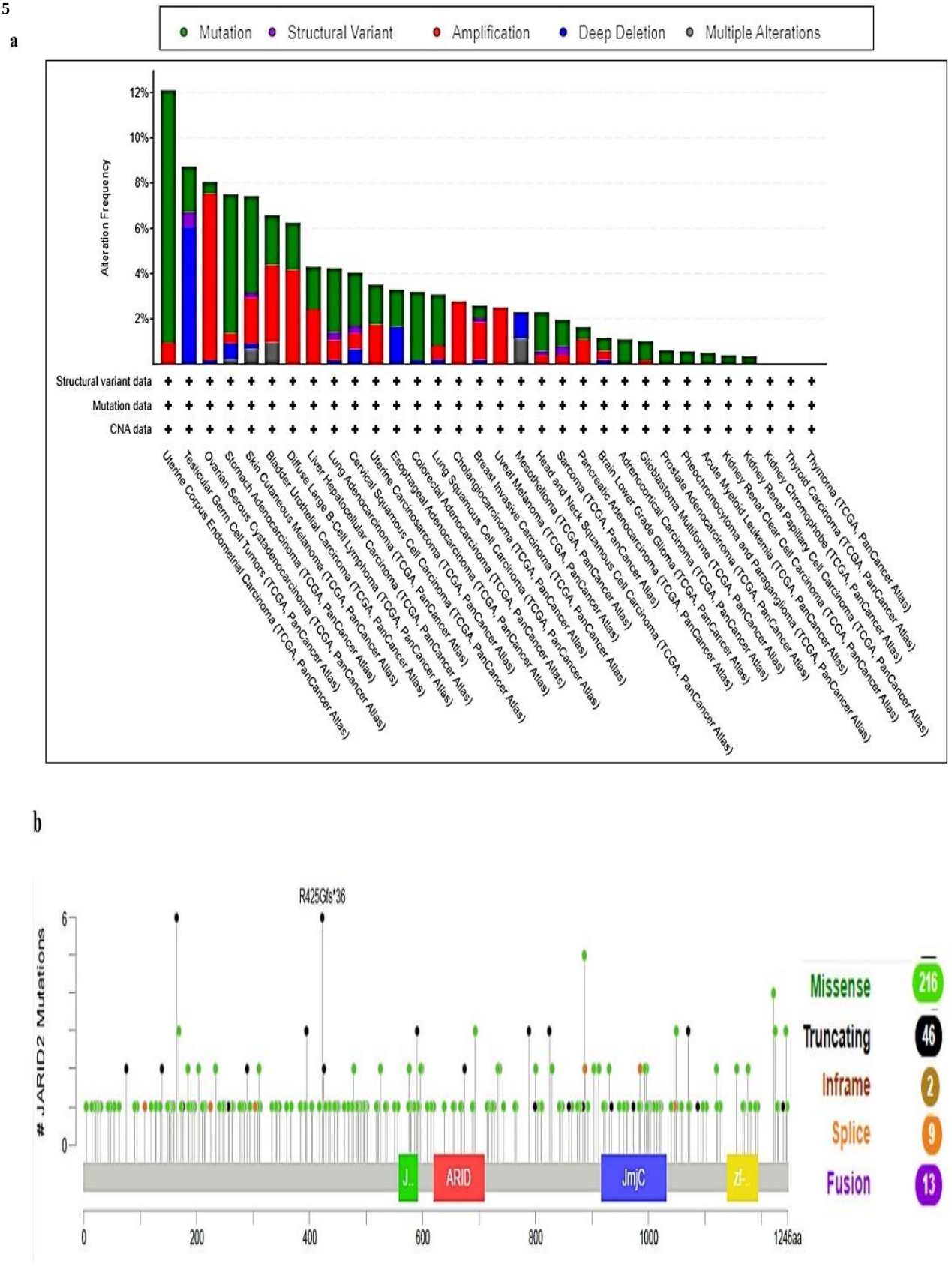


Figure 5. Genetic alterations of JARID2 in different cancer types. (a) The distribution of various types of mutations of JARID2 in different types of cancers were demonstrated using cBioportal tool; (b) The possible mutation types occurring in JARID2 domains were represented as lollipop plot.

DOI: https://doi.org/10.52756/ijerr.2024.v39spl.002

Fig. 6

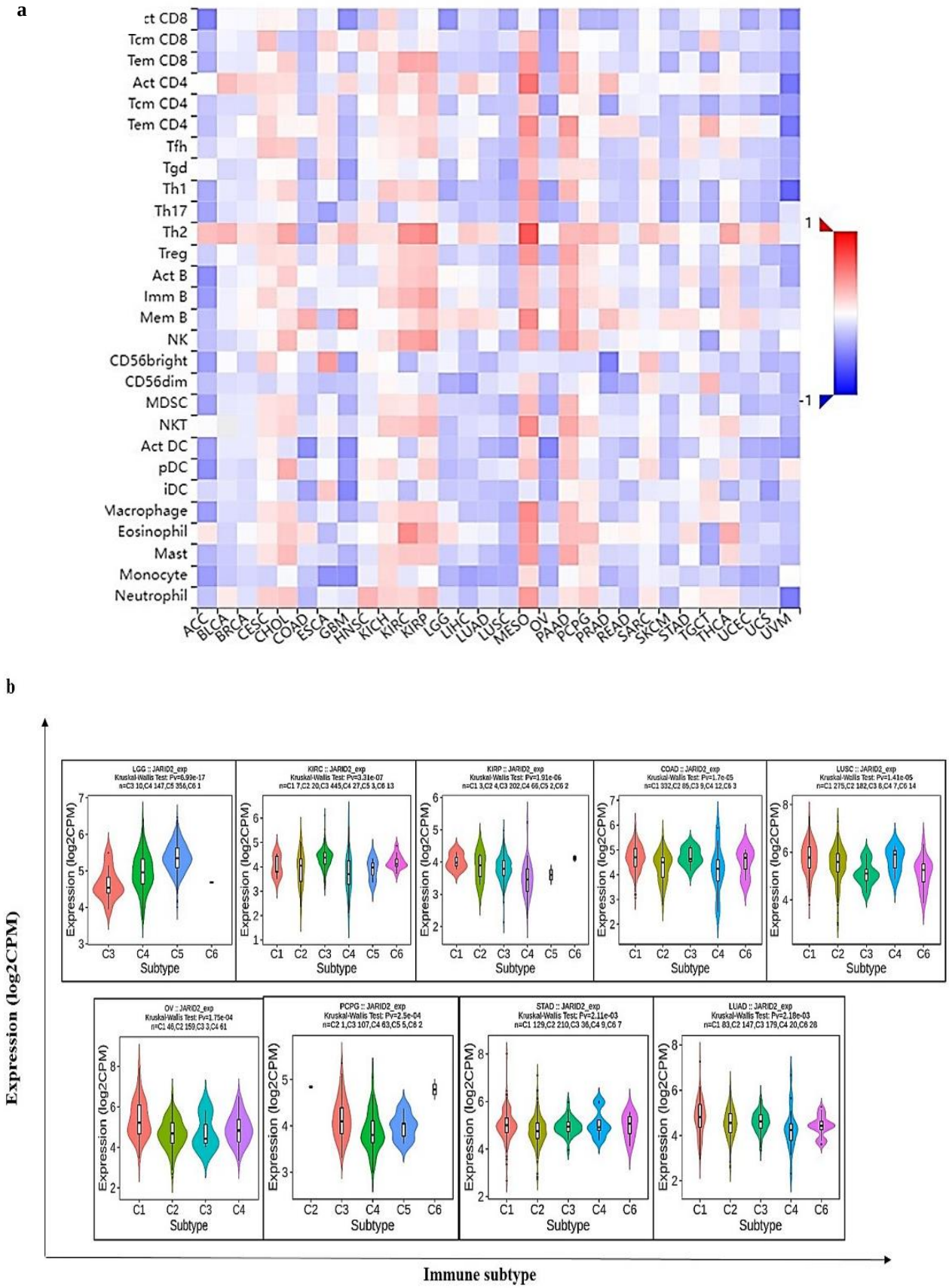
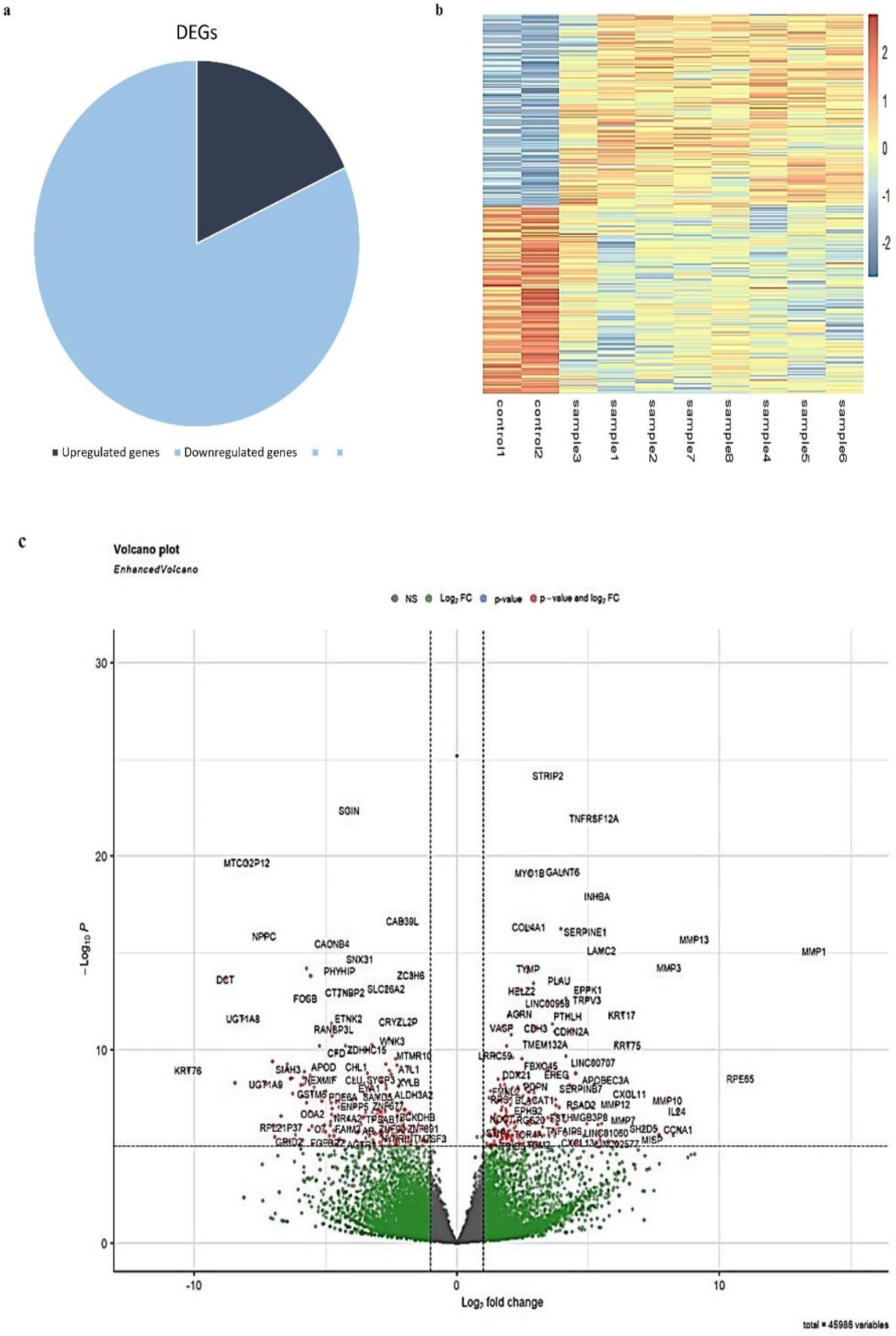


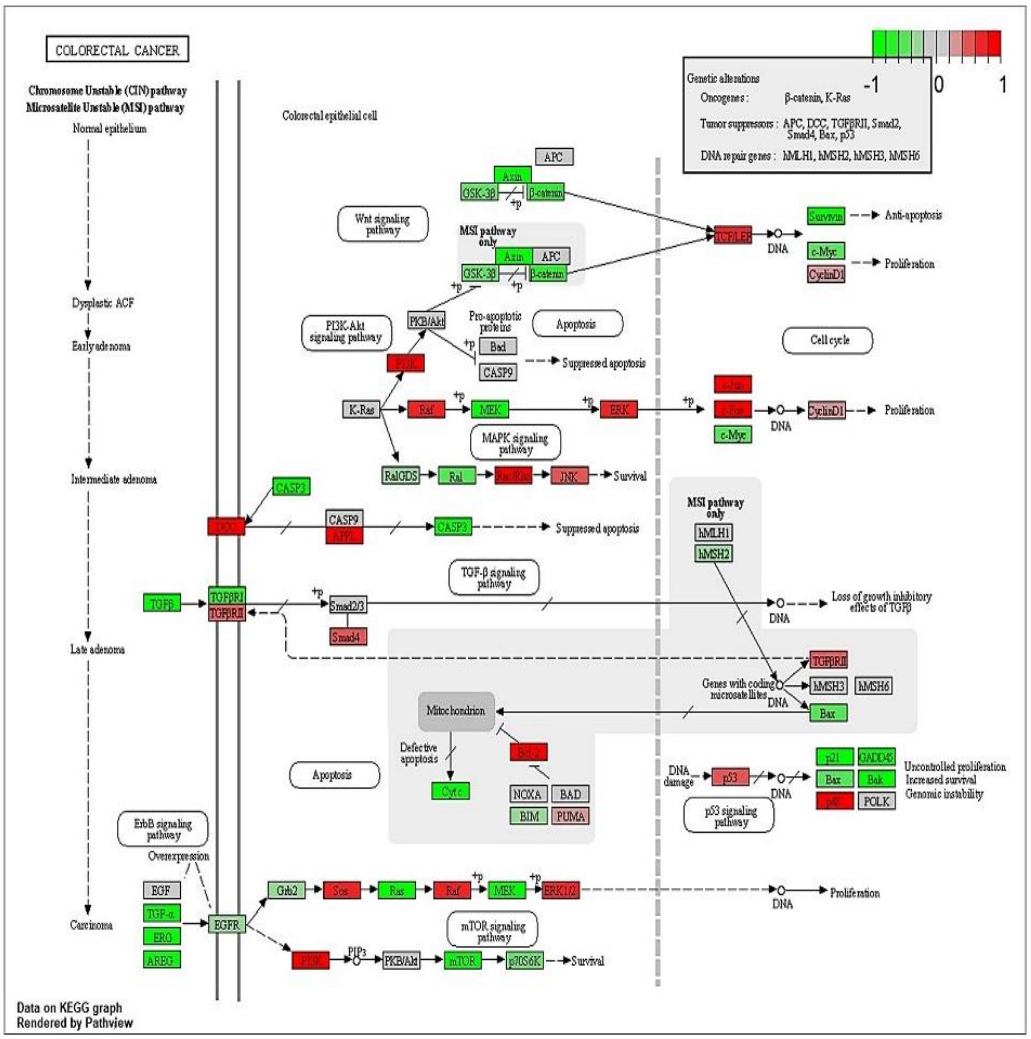
Figure 6. Examination of the effect of JARID2 on immunological status in pan-cancer. (a) The correlation of JARID2 expression with immune molecules is given as heatmap; (b) The significant correlation between JARID2 expression and immune subtypes [C1 (Wound healing), C2 (IFN-γ dominant), C3 (Inflammatory), C4 (Lymphocyte depleted), C5 (Immunologically quiet) and C6 (TGF-β dominant)] were observed in LGG, KIRC, KIRP, COAD, LUSC, OV, PCPG, STAD and LUAD.

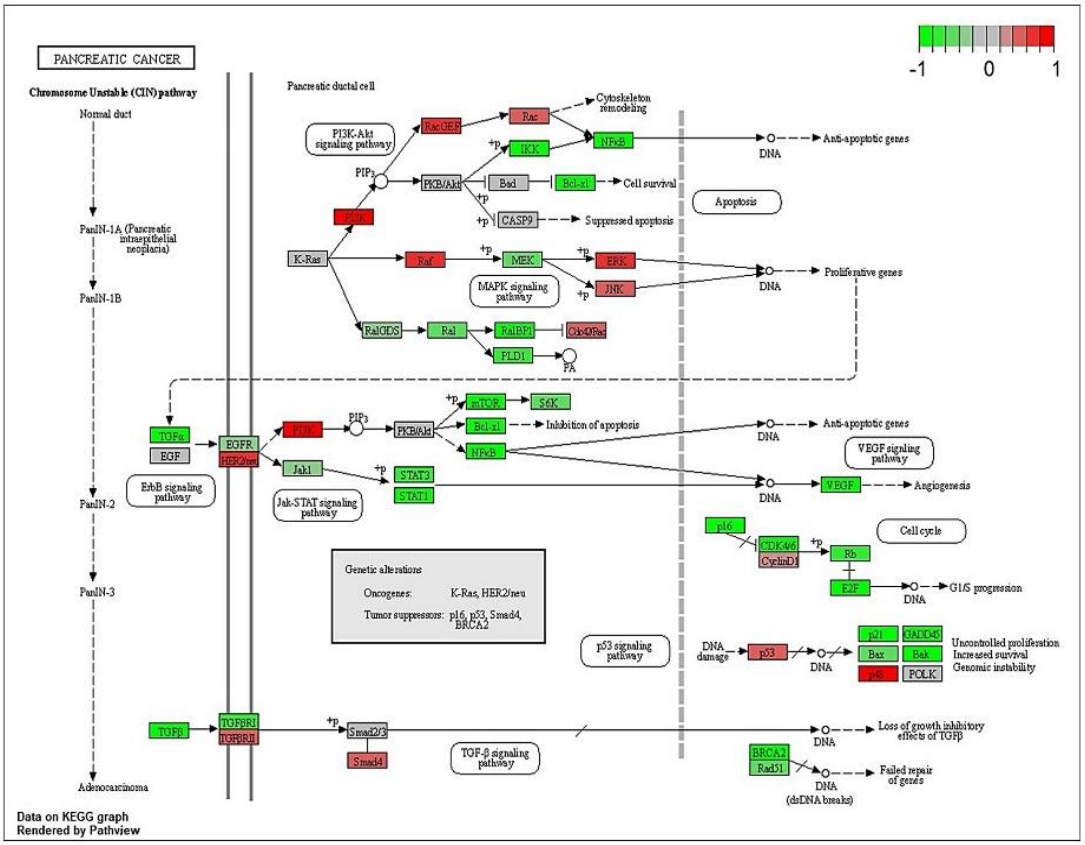
DOI: https://doi.org/10.52756/ijerr.2024.v39spl.002

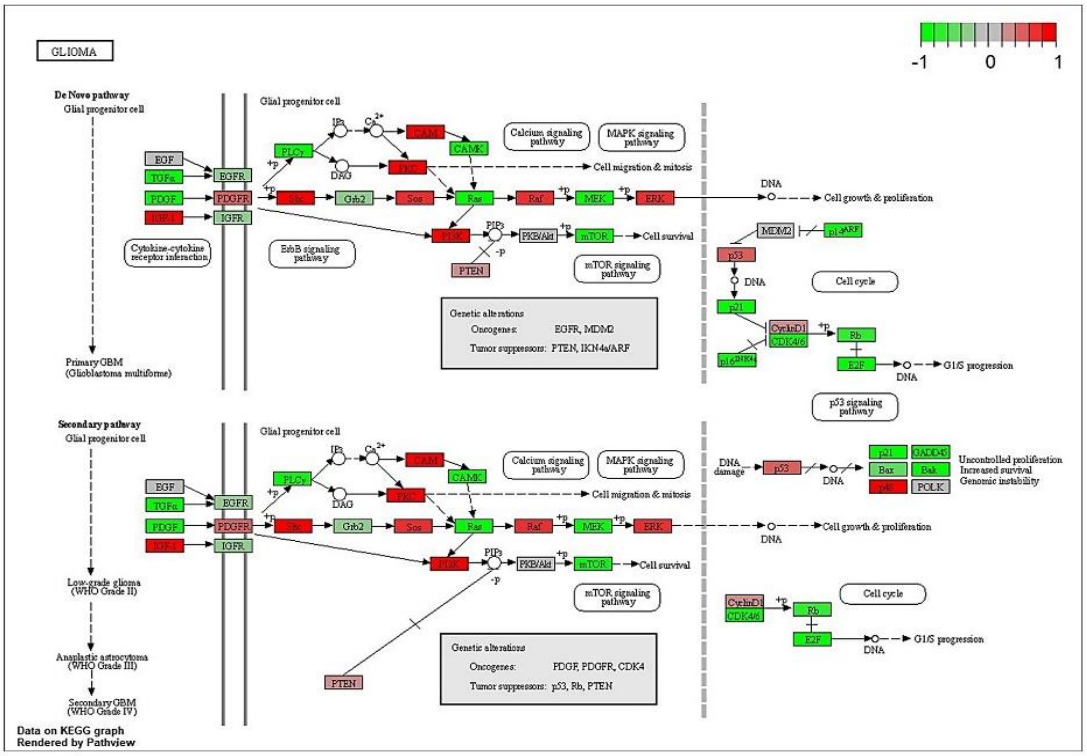
Fig. 7

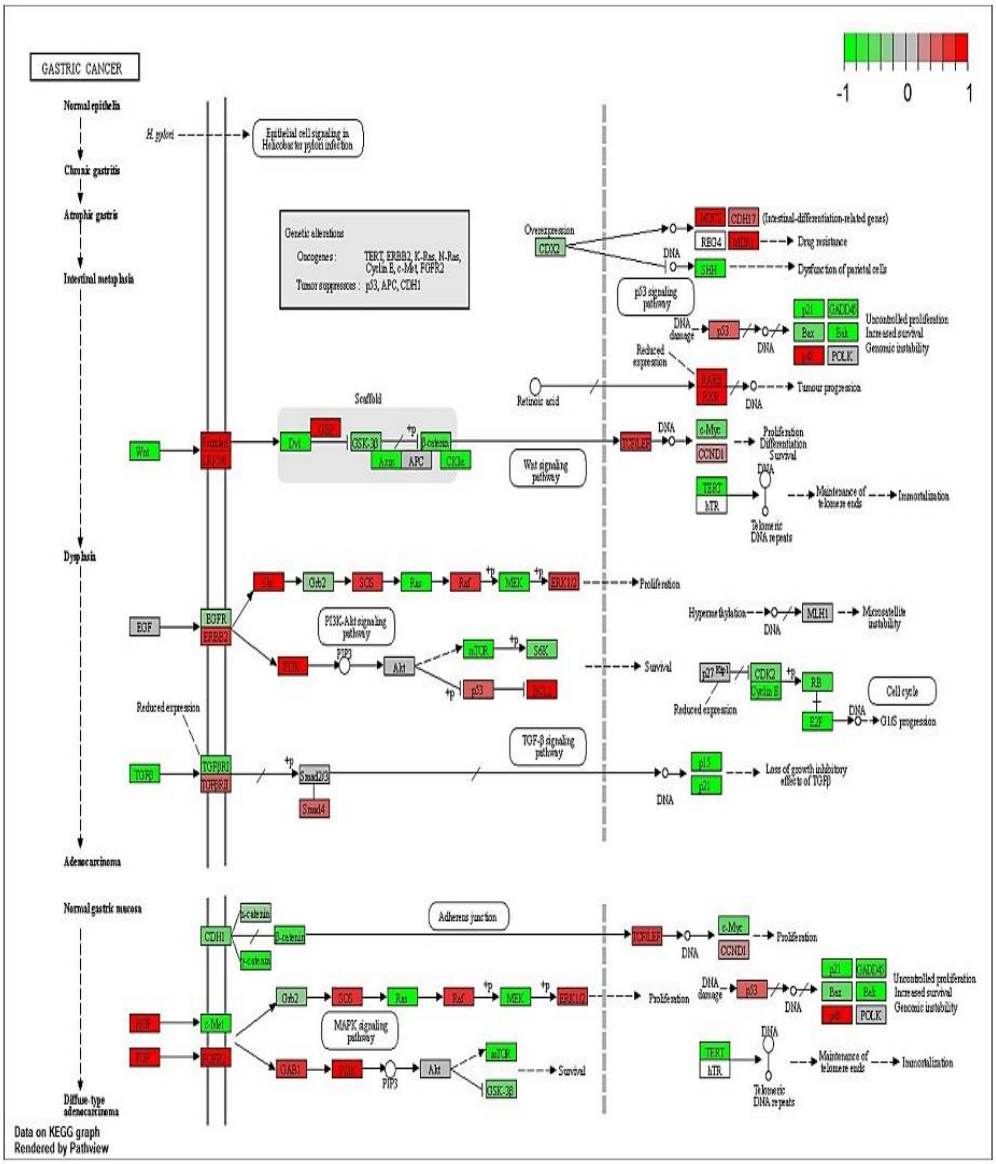

a
DEGs
Upregulated genes
Downregulated genes
b
control1
control2
sample3
sample1
sample2
sample7
sample8
sample4
sample5
sample6
c
Volcano plot
EnhancedVolcano
NS
Log2 FC
p-value
p-value and log2 FC
−Log10 P
Log2 fold change
total = 45988 variables

d


COLORECTAL CANCER
Chromosome Unstable (CIN) pathway
Microsatellite Unstable (MSI) pathway
Normal epithelium
Colorectal epithelial cell
Genetic alterations
Oncogenes : β-catenin, K-Ras
Tumor suppressors : APC, DCC, TGFBRII, Smad2, Smad4, Bax, p53
DNA repair genes : hMLH1, hMSH2, hMSH3, hMSH6
-1
0
1
Dysplastic ACF
Early adenoma
Intermediate adenoma
Late adenoma
Carcinoma
Wnt signaling pathway
MSI pathway only
PI3K-Akt signaling pathway
MAPK signaling pathway
TGF-β signaling pathway
ErbB signaling pathway
Overexpression
mTOR signaling pathway
p53 signaling pathway
Cell cycle
Apoptosis
Survivin
c-Myc
CyclinD1
Anti-apoptosis
Proliferation
Suppressed apoptosis
Survival
Loss of growth inhibitory effects of TGFβ
Genes with coding microsatellites
Mitochondrion
Defective apoptosis
Uncontrolled proliferation
Increased survival
Genomic instability
Data on KEGG graph
Rendered by Pathview


PANCREATIC CANCER
Chromosome Unstable (CIN) pathway
Normal duct
Pancreatic ductal cell
-1
0
1
PanIN-1A (Pancreatic intraepithelial neoplasia)
PanIN-1B
PanIN-2
PanIN-3
Adenocarcinoma
PI3K-Akt signaling pathway
MAPK signaling pathway
ErbB signaling pathway
Jak-STAT signaling pathway
TGF-β signaling pathway
p53 signaling pathway
VEGF signaling pathway
Cell cycle
Apoptosis
Genetic alterations
Oncogenes: K-Ras, HER2/neu
Tumor suppressors: p16, p53, Smad4, BRCA2
Anti-apoptotic genes
Proliferative genes
Angiogenesis
G1/S progression
Loss of growth inhibitory effects of TGFβ
Failed repair of genes
Uncontrolled proliferation
Increased survival
Genomic instability
Data on KEGG graph
Rendered by Pathview


GLIOMA
-1
0
1
De Novo pathway
Glial progenitor cell
Primary GBM
(Glioblastoma multiforme)
Secondary pathway
Low-grade glioma
(WHO Grade II)
Anaplastic astrocytoma
(WHO Grade III)
Secondary GBM
(WHO Grade IV)
Calcium signaling pathway
MAPK signaling pathway
Cytokine-cytokine receptor interaction
ErbB signaling pathway
mTOR signaling pathway
Cell migration & mitosis
Cell growth & proliferation
Cell survival
Cell cycle
p53 signaling pathway
Genetic alterations
Oncogenes: EGFR, MDM2
Tumor suppressors: PTEN, INK4a/ARF
Oncogenes: PDGF, PDGFR, CDK4
Tumor suppressors: p53, Rb, PTEN
Uncontrolled proliferation
Increased survival
Genomic instability
G1/S progression
Data on KEGG graph
Rendered by Pathview


GASTRIC CANCER
-1
0
1
Normal epithelia
Chronic gastritis
Atrophic gastris
Intestinal metaplasia
Dysplasia
Adenocarcinoma
Normal gastric mucosa
Diffuse-type adenocarcinoma
H.pylori
Epithelial cell signaling in Helicobacter pylori infection
Genetic alterations
Oncogenes: TERT, ERBB2, K-Ras, N-Ras, Cyclin E, c-Met, FGFR2
Tumor suppressors: p53, APC, CDH1
Scaffold
Wnt signaling pathway
PI3K-Akt signaling pathway
TGF-β signaling pathway
MAPK signaling pathway
Adherens junction
Retinoic acid
Tumor progression
Proliferation
Differentiation
Survival
Immortalization
Microsatellite instability
Loss of growth inhibitory effects of TGFβ
Uncontrolled proliferation
Increased survival
Genomic instability
Data on KEGG graph
Rendered by Pathview

e

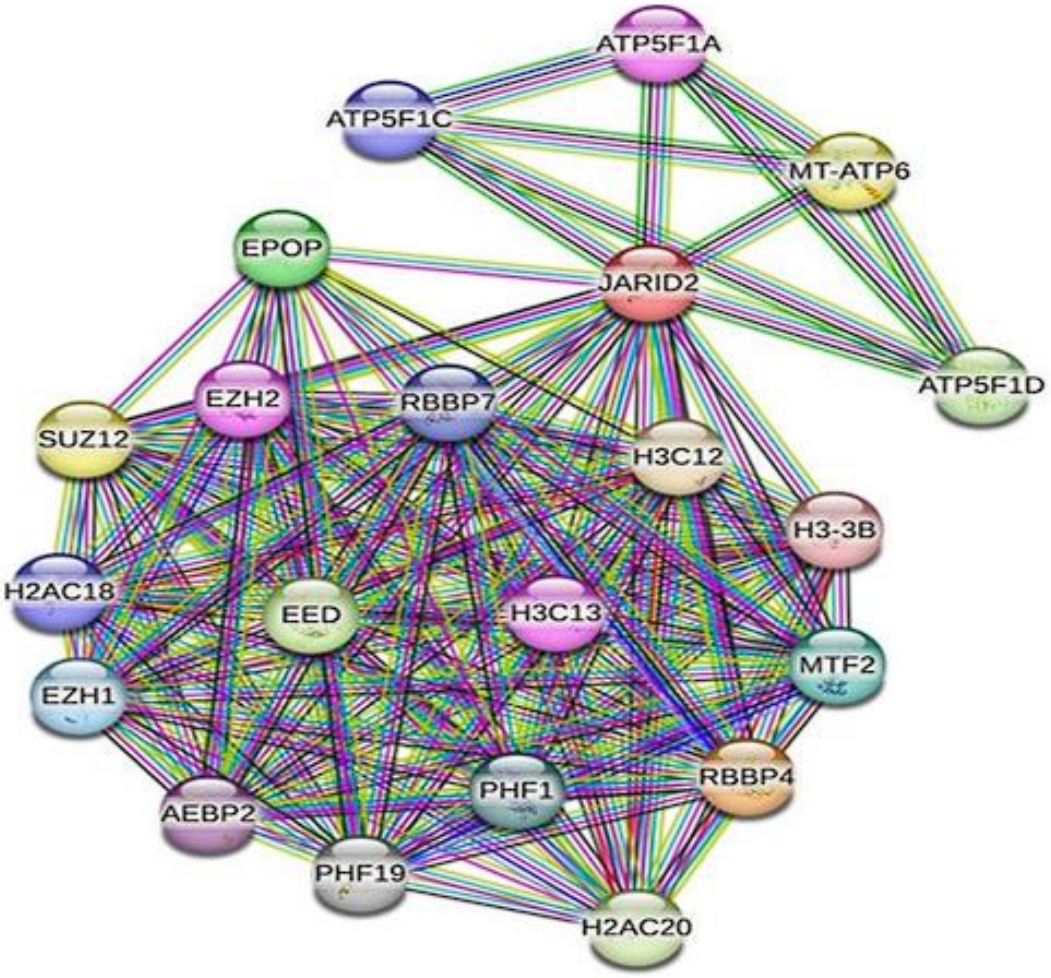


Figure 7. Transcriptomic analysis (a) Heatmap of Differentially Expressed Genes (DEGs) based on FPKM values. Each row represents DEGs and each column represents tissue samples (control 1-2 & OSCC 1-8). 46,735 genes were differentially expressed. (b) Volcano plots displaying the expression of DEGs in OSCC and control tissues. Red dots mark up-regulated genes and green dots mark down-regulated genes. Genes with high (logFC) values were labelled. (d) KEGG analysis of JARID2 showing different pathways intricated in various cancers such as hepatocellular cancer, glioma, pancreatic cancer and gastric cancer (e) String analysis showing protein-protein interaction of JARID2. Each node represents a protein and each edge represents an interaction.

Fig. 8

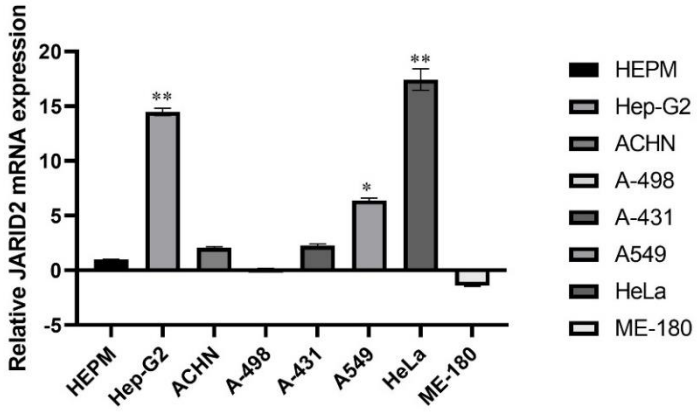


Figure 8. qRT-PCR of JARID2 in cancer cell lines. mRNA expression pattern of JARID2 in HEPM (Epithelial cell line) was compared to different cancer cell lines: Hep-G2 (Liver cancer), ACHN (Renal adenocarcinoma), A-498 (Kidney cancer), A-431 (Epidermoid carcinoma), A549 (Lung cancer), HeLa (Cervical cancer), and ME180 (Cervical cancer). JARID2 was observed to be upregulated in Hep-G2, ACHN, A-431, A549 and HeLa. Data represent mean ± SD. p-value was determined by two-tailed student's t-test. *p≤0.05, **p ≤0.005.

DOI: https://doi.org/10.52756/ijerr.2024.v39spl.002

mRNA expression of JARID2 in different cancer cell lines

To substantiate the results obtained, we performed qRT-PCR of JARID2 in various cancer cell lines. The expression of JARID2 in HEPM (Normal epithelial cell line) was used as control for comparing it with different cancer cell lines such as Hep-G2 (Liver cancer), ACHN (Renal adenocarcinoma), A-498 (Kidney cancer), A-431 (Epidermoid carcinoma), A549 (Lung cancer), HeLa (Cervical cancer) and ME180 (Cervical cancer). JARID2 was highly expressed in Hep-G2, ACHN, A-431, A549, and HeLa (Fig. 8). Hence, the qRT-PCR results were concordant with the previous findings.

Fig. 9

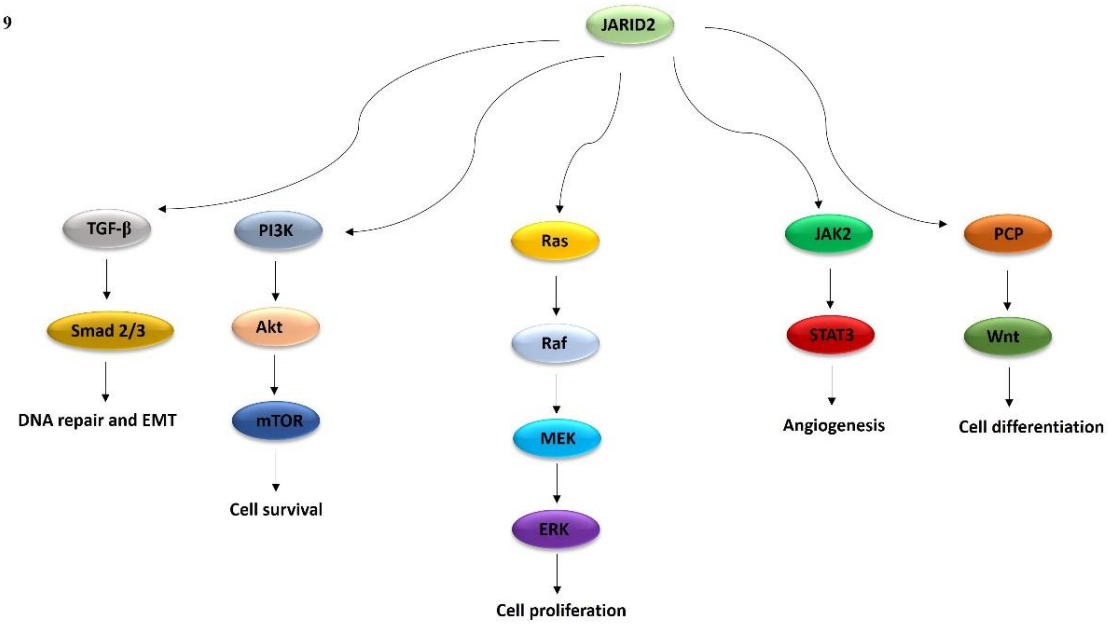


Figure 9. Involvement of JARID2 in various signaling pathways to promote cancer. JARID2 is implicated in various signaling pathways such as TGF-β, PI3K/Akt/mTOR, Ras/Raf/MEK/ERK, JAK2/STAT3, Wnt/PCP to stimulate and enhance cancer growth. According to the study, JARID2 is expected to promote cancer by various molecular signaling pathways, which are represented in the figure. These observations added to the identification of JARID2 as a vital entity that could be utilized to target cancer.

Discussion

The pan-cancer analysis offers a comprehensive knowledge of molecular attributes and their aberrations in various cancers. This vast platform aids in identifying biomarkers, which are crucial for prognosis, diagnosis, and treatment. Leiserson et al. (2015) characterized 3281 samples from 12 types of cancers by pan-cancer analysis using TCGA databases. They identified 16 significantly mutated networks, including less studied signaling pathways (Leiserson et al., 2015). Another comprehensive pan-cancer study suggested 142 driver genes in 1699 types of pediatric leukemias and solid tumors by examining their genomic landscape and mutational signatures (Ma et al., 2018). Priestley et al. (2019) provided new insights into pan-cancer analysis, where the mutational landscape and driver genes in the metastatic tumor genome and primary tumor genome showed similarity, further identifying the difference in characteristics between them that contribute to the treatment resistance. These studies promoted pan-cancer analysis to identify pivotal genes associated with cancers. In addition, these types of studies distinguish oncogenesis's correlation with genome, transcriptome, proteome, and epigenome in pan-cancer.

Polycomb Repressive Complex2 (PRC2) promotes transcriptional repression mandatory for proper embryonic development. Li et al. (2010) observed that JARID2 associates with PRC2 to promote its enzymatic activity. Additionally, JARID2 plays a vital character in stimulating PRC2 components to perform its function. In several researches, JARID2 has been reported to be a crucial molecule in regulating cancers. In oral cancer, JARID2 is involved in signaling pathways regulating epithelial-to-mesenchymal transition (EMT) and maintenance of tumor-initiating cells (TICs), which promotes cancer progression (Zhu et al., 2017). Recent research demonstrated that the LINC00852/miR29a-3p/JARID2 axis promoted the progression of prostate cancer and thus, they suggest that this axis could be employed for targeted therapy (Zhang et al., 2022). Moreover, JARID2 was upregulated in cisplatin-resistant non-small cell lung cancer (NSCLC), which was then associated with Notch1 and was proved to advance stemness and cisplatin resistance. The increased expression of JARID2 imparts a cancer stem cell-like property to the cells, which increases cancer progression by enhancing therapeutical resistance (Wang et al., 2021).

DOI: https://doi.org/10.52756/ijerr.2024.v39spl.002

JARID2 was identified to be intricated in EMT mediated by TGF-β in lung and colon cancer cell lines (Tange et al., 2014a). These findings prove the oncogenic or pro-tumorigenic role of JARID2 in cancers. In contrast, researchers have reported that JARID2 also acts as a tumor-suppressor in the advancement of myeloid neoplasms, namely non-malignant myeloproliferative neoplasms (MPNs) and myelodysplastic syndromes (MDS) to secondary Acute Myeloid Leukemia (sAML). In this study, the genetic deletion of JARID2 in animals with MPNs either showed an extensive reduction of its overall survival rate or promoted the progression to sAML. In addition, this study established the fact that JARID2 recruits PRC2 to inhibit its target genes to modulate self-renewal pathways in hematopoietic progenitor cells (Celik et al., 2018). Additionally, researchers have identified that JARID2 restricts the self-renewal ability of long-term hematopoietic stem cells and that the dysregulation of JARID2 results in hematopoietic malignancies. This study further confirmed the tumor-suppressive role of JARID2 in myeloid neoplasms (Celik et al., 2017). A study by Martin and Moorehead (2020) revealed that JARID2 is a crucial accessory subunit that helps in recruiting PRC2 to chromatin (Martin and Moorehead, 2020). It was reported in another study that during leukemia, colon, and uterine adenocarcinoma, the mutations occurring in SUZ12 lead to the depletion of JARID2, which results in an enhanced PRC2 chromatin occupancy (Parreno et al., 2022). These studies altogether speculated the different aspects of JARID2 in cancers. Therefore, it is necessary to understand the role of JARID2 in pan-cancer to comprehend its possible mechanism. To the best of our knowledge, this is the first comprehensive bioinformatics study of JARID2, unveiling its potential character and probable mechanism in human malignancy.

JARID2 was observed in chromosome 6, the localization of which was identified in the nucleus as per the HPA database. Primarily, the expression of the JARID2 gene in 54 types of normal tissues, 29 cancer cell lines, and 33 tumor tissues from the TCGA dataset was investigated. The RNA expression in normal tissues showed low RNA specificity, while the cancer cell lines such as testis, leukemia, bone, skin, and neuroblastoma showed moderate specificity. However, the cancer tissues of BLCA, BRCA, CESC, COAD, ESCA, HNSC, LIHC, LUAD, LUSC, PRAD, READ, STAD, and UCEC exhibited higher JARID2 expression indicating its pro-carcinogenic role. Moreover, the results from the TISIDB database demonstrated the JARID2 expression was significantly related to the molecular subtypes of LUSC, READ, LIHC, COAD, BRCA, PRAD, OV, and HNSC. Furthermore, the protein expression from the TIMER2.0 database revealed more than 90% of the cancer patients with skin, testis, pancreatic, urothelial, and head and neck cancers revealed strong JARID2 expression. In addition, thyroid, lung, colorectal, renal, carcinoid, prostate, cervical, and melanoma revealed more than 80% expression. Immunohistochemical staining of JARID2 in the available normal and cancer tissues was obtained to substantiate these results. The breast, colorectal, prostate, and lung tissues showed higher expression, which correlated to the results retrieved from the TISIDB database.

A non-small cell lung cancer (NSCLC) study observed a homozygous deletion of two genes JARID2 and ARID2, in a set of samples. Their further analysis revealed that the loss of function mutation of ARID2 was detected in 5% of NSCLC, which identifies ARID2 as one of the most frequently mutated genes in NSCLC (Manceau et al., 2013). Additionally, a group of researchers has observed the genetic mutations occurring in locally advanced triple-negative breast cancer (TNBC) patients who did not attain pathologic complete response (non-pCR) post neoadjuvant chemotherapy, possessing rapid tumor metastasis. They identified mutations in a few genes, including 42.9% JARID2 alteration, which was correlated to the patients with a shorter DFS group. Their *in-vitro* experiments proved the knockdown of JARID2 resulted in enhancing mesenchymal markers Vimentin, MMP7, and MMP9 and a decrease in epithelial marker E-cadherin. This study revealed JARID2 as an impeccable tumor suppressor gene in TNBC (Zhang et al., 2020). Therefore, to check and substantiate the association of JARID2 expression level with patient prognosis in pan-cancer, the GEPIA2.0 database was used. In ACC, SARC, SKCM, TGCT, and THCA, the JARID2 expression was relatively high and is correlated to poor overall survival. The patients with THYM have high JARID2 expression but showed a favorable overall survival rate, indicating JARID2 as a protective factor in THYM. The DFS analysis verifies that high JARID2 expression correlates to the unfavorable DFS in ACC and LIHC. These findings open up the oncogenic and cell-protective role in cancer patients. This verified that JARID2 expression could be considered a predictor of tumor prognosis.

Several reports are showing that genetic alteration has a potential role in tumor progression, patient survival, and therapeutic response (Cha, Lee, and Won 2021) (Yang et al., 2011). In this study, we disclosed that the mutations occurring in JARID2 are frequent in UCEC (~12%),

DOI: https://doi.org/10.52756/ijerr.2024.v39spl.002

followed by STAD and SKCM. Additionally, amplification was observed principally in OV with ~8% alteration rate. Almost all the cancer types possessed different types of JARID2 alterations. The analysis of various alterations occurring in JARID2 disclosed that missense mutations were the leading type of alteration with 216 reports, followed by 46 truncating mutations as the second-leading genetic alteration. These results indicate that JARID2 is a promising oncogene. The tumor immune response of JARID2 was scrutinized by determining the abundance of tumor-infiltrating lymphocytes (TILs) and Cancer-Associated Fibroblasts, which was compared with JARID2 expression to identify prognosis, therapy resistance, and the possibility of disease recurrence in different cancers. The result showed that due to the high expression of JARID2, most of the TILs such as Th2, Act, CD4, Mem B, Natural Killer cells, etc., were significantly reduced in ACC, LUSC, OV, and UVM. However, few TILs were increased in CHOL, KICH, KIRP, MESO, PAAD, etc. The high JARID2 expression in ACC and LUSC correlated to a low immune cell activity, whereas low expression of JARID2 in KICH, KIRP, and PAAD corresponded to a high immune response. This shows a pro-oncogenic behavior of JARID2 by regulating tumor immune response. In addition, the JARID2 expression had a significant relationship with immune subtypes of LGG, KIRC, KIRP, COAD, LUSC, OV, PCPG, STAD, and LUAD. Taken together, our work elucidated the role and underlying effect of JARID2 in tumor immunity and its prognostic values in different tumors. Furthermore, this study possesses a limitation that all cancer types may not have the same sample size, which could lead to the improper prediction of results.

The transcriptomics analysis of OSCC tissue samples revealed 46,735 DEGs with 8,510 upregulated and 38,225 downregulated genes. Consistent with the previous data, JARID2 was upregulated in OSCC. All PRC2 genes except EZH1 and RbAp46 showed high expression in OSCC when compared to the control. Heatmap and volcano plot represent the differentially expressed genes. Moreover, KEGG analysis showed significant pathways intricated in different cancers. In addition, the STRING analysis provided the protein-protein interaction network of JARID2 and their functional enrichment. To validate the findings, qRT-PCR was performed. The results showed upregulation of JARID2 in various cancer cell lines such as Hep-G2, ACHN, A-431, A549, and HeLa, which confirmed the results obtained from the transcriptomics analysis. On the other hand, JARID2 showed minimal expression in A-498 and downregulation in ME-180, possibly due to the activity of some other genes on JARID2.

Furthermore, JARID2 has been known to regulate cancer via various molecular signaling pathways. In a study, JARID2 was observed to be highly expressed in lung and colon cancer cell lines with EMT induced by TGF-β, and the knockdown of JARID2 resulted in a decreased association of TGF-β and EMT (Tange et al., 2014b). The JARID2 knockdown also reduced proliferation and invasion in ovarian cancer cell lines through the PI3K/Akt/mTOR signaling pathway (Cao et al., 2017). The downregulation of JARID2 significantly decreased migration, invasion, proliferation, and metastasis in hepatocellular carcinoma via PTEN/Akt signaling (Lei et al., 2016) and in ovarian endometriosis (Gu et al., 2023). In addition, a study revealed that the leptin secreted by adipocytes upregulated JARID2 expression through the JAK2/STAT3 pathway. Moreover, JARID2 is involved in glycolysis, lipid metabolism, proliferation, invasion, and stemness to promote the invasion and growth of breast cancer cells (Liu et al., 2023). The KEGG analysis from our study also showed that TGF-β, PI3K, and MAPK signaling are involved in the progression and metastasis of various cancers by damaging DNA repair, enhancing cell proliferation, reducing cell differentiation, inhibiting apoptosis, increasing angiogenesis, etc. These findings support our conclusion that JARID2 might associate with this molecular mechanism to stimulate cancer progression.

Conclusion

To conclude, JARID2 is a predicted oncogene that is upregulated in various cancers, the expression and genetic alteration of which is strongly correlated to the survival of cancer patients. Moreover, these results disclose the crucial relation of JARID2 expression with tumor immunity, the mechanism of which is not known. The transcriptomics study also correlates with the bioinformatics study, where JARID2 was significantly upregulated in OSCC samples. Additionally, the qRT-PCR results confirmed both the analyses. However, *in-vitro* and *in-vivo* studies are required to substantiate these data and to confirm their therapeutical applications. This work delivers the preliminary findings predicting the plausible role of JARID2 in cancer progression.

Acknowledgment

The authors express gratitude to SRM Institute of Science and Technology for providing the study infrastructure.

Conflict of Interest

The authors declare that they have no competing interests.

Funding support

This research did not receive any specific grant from funding agencies in the public, commercial, or non-profit sectors.

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How to cite this Article:

Bhuvanadas Sreeshma, Habeeb Shaik Mohideen and Arikketh Devi (2024). Integrated Bioinformatics Analysis and Transcriptomics Analysis Predicts Jumonji and AT Rich Interacting Domain2 (JARID2) as a Therapeutic Target in Human Cancers. *International Journal of Experimental Research and Review*, *39*(spl.) 15-38.

DOI : https://doi.org/10.52756/ijerr.2024.v39spl.002