Bromodomain containing 4 (BRD4) as a potential prognostic marker in a pan-cancer analysis of human tumors

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Abstract

There is some evidence supporting an association between Bromodomain containing 4 (BRD4) and cancer, but no research using pan-cancer analysis has been conducted previously. We therefore investigated the oncogenic role of BRD4 in 33 tumors from the Gene Expression Omnibus and The Cancer Genome Atlas databases. BRD4 is highly expressed in many tumors, and the prognosis of certain cancers is vitally linked with BRD4 expression. BRD4 expression is associated with CD8+ T-cell infiltration levels in testicular germ cell tumors and head and neck squamous cell carcinomas, and we observed a positive relationship between BRD4 and Tcm (T central memory) and Th(T helper) cells, and a negative relationship pDC (plasmacytoid DC). BRD4 had negative associations with marophages, iDC, Treg, cytotoxic cells, and Th17 cells in multiple tumors. The top 100 genes that are most strongly related to BRD4 were identified, and enrichment analysis indicated that the biological process with the closest relationship was chromatin modifying enzymes, related pathways included the signaling pathways of intracellular receptor, cytokine Signaling in Immune system and regulation of TP53 activity through acetylation. BRD4 is related to biological cell behaviors such as DNA-templated transcription, regulation of histone modification, protein modification by small protein removal and mitotic
sister chromatid segregation. As the first study to perform a pan-cancer analysis of BRD4, the present findings will improve the understanding of the oncogenic role of BRD4 in different tumors.

**Keywords**: BRD4; cancer; prognosis; immune infiltration

1. Introduction

The intricacy of tumors means that they require complex regulation. It is therefore necessary to analyze the genes relating to pan-cancer expression and determine the correlation between pre- and post-evaluations and the potential molecular mechanism[1]. The Cancer Genome Atlas (TCGA) public database and the Gene Expression Omnibus (GEO) project provide data on the functional genomics of different tumors[2-4], which we can use for pan-cancer analysis.

Bromodomain and extra-terminal domain (BET) family is a class of proteins that can specifically recognize acetylated lysine and regulate gene transcription, which plays an important role in the occurrence and development of many diseases. BRD4 is a member of bet family, which can bind with acetylated histone or non histone proteins, and then regulate gene replication and transcription, affecting cell cycle, cell differentiation, apoptosis[5].

Other research groups have suggested a functional association between the multifunctional BRD4 protein and the occurrence of ovarian[6], lung[7,8], and breast[9,10] cancers. The current evidence from animal and cell studies supports correlations between different cancer types and BRD4. Nevertheless, despite extensive clinical data, no evidence is available on the pan-cancer associations
between BRD4 and different tumor types. The present study is the first to use TCGA database and the GEO project to conduct a pan-cancer investigation of BRD4. Many aspects such as survival condition, gene expression, immune infiltration, genetic changes, and related cellular pathways are summarized in order to determine the possible molecular mechanisms of BRD4 in clinical prognoses or the pathogenesis of different cancers.

We hypothesized that BRD4 mutation alters its expression level, causes changes in the body’s immune system, changes the expression levels of various immune cells, and further influences tumor prognosis and survival time, affecting some pathways in vivo. Our goal was to determine these changes, explore how BRD4 influences changes in immune cells and prognoses, and identify the affected molecular pathways in vivo to provide direction and guidance for clinical and drug treatments.

2. Materials and Methods

2.1 Analysis of gene expression

We used the website http://timer.cistrome.org/ to obtain TIMER2.0 (Tumor Immune Estimation Resource 2nd edition), entered BRD4 into the “Gene_DE” module, and observed the differences in BRD4 expression between tumors of specific subtypes and adjacent normal tissues or different tumors in TCGA database. We did not analyze some highly restricted tissues such as TCGA diffuse
large-B-cell lymphomas (DLBCs) and THYMs (thymomas). We used the website http://GEPIA2.cancer-pku.cn/#Analysis to obtain GEPIA2 (Gene Expression Profiling Interactive Analysis 2nd Edition)\cite{11} and its “Expression-Analysis Box Plots” that can be used to create box plots of the expression differences between tumor tissues and the corresponding normal tissues from the GTEx (Genotype-Tissue Expression) database. We set the log$_2$ relative change cutoff at 1 and a P-value cutoff of 0.01, expressed as “Match TCGA normal and the GTEx data.” We also used GEPIA2 to obtain a violin plot of BRD4 expression in all TCGA tumors at different pathological stages (stage I to stage IV) using the “Pathological Stage Plot” module. Log$_2$ TPM (transcripts per million) + 1 was used to transform expression data from the violin plot or box.

2.2 Survival prognostic analysis

GEPIA2 is an online tool for TCGA gene expression and survival analysis. The GEPIA2\cite{11} “Survival Map” module was applied to all TCGA tumors to identify the DFS (disease-free survival) and OS (overall survival) due to high and low expression of BRD4. Expression thresholds were applied to the low (50%) and high (50%) cutoff values to divide into low- and high- expression cohorts of BRD4. The log-rank test in the “Survival Analysis” module were used for hypothesis testing and the survival plots, respectively. TCGA data were then extracted, and a receiver operating characteristics (ROC) curve was plotted using the “Survival ROC” software package. In the ROC curve image, the abscissa and the vertical
axis indicate the false- and true-positive rates, respectively. A larger area under the ROC curve (AUC) indicates greater prognostic accuracy.

2.3 Analysis of genetic alteration

We selected the “TCGA Pan-Cancer Atlas Studies” section on the cBioPortal website (https://www.cbioportal.org/)[12, 13], and entered “BRD4” to investigate the genetic alteration characteristics of BRD4. We obtained information on copy-number alterations (CNA), mutation types, and frequencies of all tumors in the “Cancer Type Summary” TCGA module. We used the “Comparison” module on TCGA cancer cases to obtain data on differences in OS, progression-free survival, disease-specific survival, and DFS rates with and without BRD4 gene changes. Log-rank P values were used to construct the Kaplan-Meier graph, with a P value of <0.05 considered significant.

2.4 Analysis of immune infiltration

We chose the “Immune Gene” module from the TIMER2 web server to determine the relationship between all TCGA tumor immune infiltrations and BRD4 expression. CD8+ T cells immune infiltration data was obtained using the MCPCOUNTER, QUANTISEQ, CIBERSORT, CIBERSORT-ABS, TIMER, EPIC, and XCELL algorithms. P values and sectional correlation values were obtained using Spearman’s rank correlation test with purity adjustment. We used these data to construct scatter and maps. RNA-seq data and clinical data were then extracted in the Level 3 HTSeq-FPKM format from TCGA database, and the correlations between BRD4 and various immune cells were analyzed using the Gene Set Variation Analysis package of R software. The following cell types were
analyzed: aDC (activated Dendritic cells), B cells, CD8+ T cells, cytotoxic cells, DC(Dendritic cells), eosinophils, immature DC, macrophages, mast cells, neutrophils, NK CD56bright cells, NK CD56dim cells, NK cells, pDC(plasmacytoid Dendritic cells), T cells, T helper (Th) cells, Tcm (T central memory)cells, T effector memory cells, follicular Tfh (T follicular helper cells), T gamma delta (Tgd) cells, Th1 cells, Th17 cells, Th2 cells, and Treg cells.

2.5 Analysis of BRD4-related gene enrichment

We chose the organism “Homo sapiens” from the STRING\textsuperscript{[12]} website (https://string-db.org/) and the single protein name “BRD4.” The main parameters were then set as follows: “low confidence [0.150]” as the minimum interaction point, maximum number of displayed interaction factors (“no more than 50 interactors in the first shell”), meaning of the network edge (“evidence”), and active interaction sources (“experiments”). Furthermore, we obtained BRD4-binding proteins that had been determined experimentally. We use the data of TCGA tumors and normal tissues to identify the 100 genes most strongly associated with BRD4 in the GEPIA2 “Similar Gene Detection” module. Selected genes and Pearson’s correlation analysis of BRD4 paired genes were used in the “Correlation Analysis” GEPIA2 module. The dot plot used log2 TPM + 1, to determine correlation coefficients (R Values) and P values. The P values and partial correlation heat map data from the Spearman’s rank correlation test were determined using selected genes in the TIMER2 “Gene_Corr” module after purity adjustment.
We also performed pathway analysis by combining the two data sets. We uploaded the lists of genes to the Metascape[13] (http://metascape.org/gp/index.html#main/step1) website, and chose “Homosapiens (147)” and “Express Analysis.” The flow chart of analysis is shown in Figure 7.

3. Results

We hypothesized that BRD4 mutation alters its expression level, alters the body’s immune system, alters the expression of various immune cells, influences tumor prognosis and survival time, and affects some pathways in vivo. Our goal was to analyze these changes, determine the influence of BRD4 changes on immune cells and prognoses, and identify the affected molecular pathways in vivo, with an overall aim of providing direction and guidance for clinical treatment and drug transformation. Our results indicated that BRD4 expression affects the prognosis of many tumors, including at different stages. There are several ways via which BRD4 genes can be altered, with the most common being mutation, which also affects the prognosis of adrenocortical carcinoma (ACC). The immune environment is also affected by BRD4, changes in which play a role in the changes in different immune cells in various tumors. The 100 genes most closely related to BRD4 were identified, and enrichment analysis indicated that the most closely related biological process was chromatin modifying enzymes, related pathways includes the signaling pathways of intracellular receptor, cytokine Signaling in Immune system and regulation of TP53 activity through acetylation.
3.1 Analysis of gene expression

In order to determine the effects of BRD4 on cancer in humans, we used the TIMER2 website to explore BRD4 expression in various types of cancer from TCGA. As demonstrated in Figure 1A, expression differences in levels between tumor and normal tissues were found in Breast invasive carcinoma (BRCA), cholangiocarcinoma (CHOL), Colon adenocarcinoma(COAD), Esophageal carcinoma (ESCA), Head and Neck squamous cell carcinoma(HNSC), Kidney renal papillary cell carcinoma (KIRP), liver hepatocellular carcinoma (LIHC), Lung squamous cell carcinoma (LUSC), stomach adenocarcinoma (STAD), thyroid carcinoma (THCA) (all P<0.001), rectum adenocarcinoma (READ) (P<0.01), and Uterine Corpus Endometrial Carcinoma (UCEC) ( P<0.05).

Because some tumors did not have enough samples of normal tissue in TCGA (those tumors are shown with a white background in Figure 1A), we used normal tissues from the GTEx data set as a control, and evaluated the BRD4 expression difference between tumor and normal tissues of sarcoma (SARC), acute myeloid leukemia (LAML), brain lower grade glioma (LGG) (P<0.05, Figure 1B). No differences were apparent in other tumors, including adrenocortical carcinoma (ACC), cholangiocarcinoma (CHOL), lymphoid neoplasm diffuse large B-cell lymphoma (DLBC).

The Clinical Proteomic Tumor Analysis Consortium (CPTAC) integrates genomic and proteomic data in order to identify and describe all proteins within tumor and normal tissues, and explores candidate proteins that can be used as
tumor biomarkers. Data from the CPTAC data set indicated that BRD4 total protein expression was higher in breast cancer (P<0.01), colon cancer, lung adenocarcinoma, clear renal cell carcinoma, ovarian cancer and uterine corpus endometrial carcinoma than in normal tissues (P<0.001, Figure 1C), whereas its expression was lower in Uterine Corpus Endometrial Carcinoma than in normal tissues. (P<0.001, Figure 1C).

Using the GEPIA2 “Pathological Stage Plot” module identified correlations between cancer pathological stages and BRD4 expression including COAD, KICH (P<0.001) and SKCM (P<0.01), ACC, PAAD, OV (P<0.05, Figure 1D).

3.2 Analysis of survival

Cancer cases were divided into high- and low- BRD4 expression groups, and TCGA and GEO data were mainly used, respectively, to investigate the correlations between BRD4 expression and the prognoses of different tumors. As shown in Figure 2A, high BRD4 expression was linked to poor OS for ACC (P=0.0066), LGG (P=0.00038), MESO (P=0.0033) and SKCM (P=0.017) in TCGA database.

In the DFS analysis of TCGA COAD cases (P=0.0091) and HNSC cases (P=0.029), a correlation was indicated between poor prognosis and low BRD4 expression. The poor DFS for ACC (P=3.9e-05), BLCA (P=0.027), KICH (P=0.029), LGG (P=0.0024), was linked to high BRD4 expression (Figure 2B).
As shown in Figure 2C, the AUC values for ACC (AUC=0.848), LGG (AUC=0.813), COAD (AUC=0.805) and HNSC (AUC=0.821) all exceeded 0.7, indicating that BRD4 is a highly reliable predictor.

3.3 Analysis of genetic alteration

We used TCGA cohort to analyze different tumor samples and their genetic alteration status with BRD4. As displayed in Figure 3A, the highest alteration frequency of BRD4 (>12%) was in patients with uterine corpus endometrial carcinoma with “mutation” as the primary type.

The dominant type of ovarian serous cystadenocarcinoma cases and all adrenocortical carcinoma, brain lower grade glioma, uveal melanoma, mesothelioma with genetic changes were specified as CNA “amplification” in Figure 3A, showing an alteration frequency of about 12%, 3%, nearly 2%, 1%, 1% respectively. It is worth noting that all genetically altered diffuse large B-cell Lymphoma, thymoma, lung adenocarcinoma, pancreatic adenocarcinoma, kidney renal papillary cell carcinoma, kidney renal papillary cell carcinoma, acute myeloid leukemia, and kidney renal clear cell carcinoma had BRD4 copy-number mutation (Figure 3A).

Figure 3B displays the sites, types, and case numbers of the BRD4 genetic alterations. We suggest that the primary type of genetic change is BRD4 mutations. 236 gene mutation data were obtained, including 206 missense, 24 truncating, 3 splice and 3 inframe data. Furthermore, in the database, the alteration of the R1256Q gene was discovered in two cervical squamous cell
carcinoma cases and one instance of uterine endometrioid carcinoma, the alteration of the L1361I and L1361F gene were discovered in two uterine endometrioid carcinoma cases and one instance of cutaneous melanoma. (Figure 3B), which induced missense mutations of BRD4, and the alteration of the P46S and P46Rfs*47 gene were discovered in two cutaneous melanoma cases and one instance of uterine endometrioid carcinoma. We also detected that different types of cancer had potential associations between clinical survival prognosis and BRD4 gene alteration.

Figure 3C indicates that compared with cases without BRD4 alteration, ACC patients with alteration had better outcomes for OS (P= 6.996e-4), disease-specific survival (P= 4.300e-4), and progression-free survival (P= 0.0159); however, there were insufficient data on DFS to draw any conclusions.

3.4 Analysis of immune infiltration

As an essential part of the tumor microenvironment, the occurrence, development, and metastasis of cancer are closely related to tumor-infiltrating immune cells[20,21]. It has been reported that the tumor stromal microenvironment aims to regulate the effect of tumor-infiltrating immune cells[22,23]. In this study we used the XCELL, CIBERSORT, CIBERSORT-ABS, TIMER, QUANTISEQ, EPIC, and MCPCOUNTER algorithms to investigate various cancer types from TCGA in order to identify potential relationships between BRD4 expression and the infiltration levels of different immune cells.
Analysis performed using all or most of the selected algorithms revealed significant negative correlation between BRD4 expression and immune infiltration of CD8+ T cells and the tumors cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), HNSC, HNSC-HPV−, HNSC-HPV+. This analysis also indicated that there were positive correlations among the above-mentioned indicators and DLBC (lymphoid neoplasm diffuse large B-cell lymphoma), KICH (kidney chromophobe), PRAD (prostate adenocarcinoma) and TGCT (testicular germ cell tumors) (Figure 4).

BRD4 was correlated with multiple immune cells in different tumor environments, suggesting that BRD4 affects the tumor microenvironment, progression, and prognosis. The main enrichment sources of BRCA, DLBC, KICH, KIRP, LIHC, MESO, OV, PAAD, PCPG, PRAD, READ, SKCM, STAD, THCA, THYM, UCEC, UCS and BRD4 were the Tcm and Th cells. Positive enrichment was significant in BRCA, READ, SARC, SKCM, STAD, and Tgd. pDC was negatively correlated with BRCA, DLBC, KICH, KIRP, LIHC, OV, PRAD, SARC, SKCM, TGCT, THCA, THYM, UCEC, UVM and BRD4. BRD4 was negatively correlated with marophages, iDC, Treg, cytotoxic cells, and Th17 cells in multiple tumors (Figure 5A-B).

3.5 Enrichment analysis of BRD4-correlated protein

To study the molecular mechanism of BRD4 during tumorigenesis, we conducted various pathway enrichment analyses to identify targeted BRD4-combining proteins and their corresponding expression-related genes. We used
previous experimental evidence to identify the top 50 BRD4-binding proteins on the STRING web. The network of interactions between these proteins is shown in Figure 6A. Combining the GEPIA2 tool with TCGA tumor expression data revealed the top 100 genes associated with BRD4 expression.

Figure 6B demonstrates a positive link between BRD4 expression levels and the following genes: WIZ (Widely Interspaced Zinc Finger) \( R=0.79 \), CHERP (Calcium homeostasis endoplasmic reticulum protein) \( R=0.71 \), SETD1A (SET domain protein 1A) \( R=0.68 \), MAML1 (mastermind-like 1) \( R=0.65 \), CREBBP (CREB-binding protein) \( R=0.62 \), SRCAP (SNF2-related CREBBP activator protein) \( R=0.64 \), POM121C \( R=0.62 \), ZNF142 (zinc finger protein 142) \( R=0.64 \), UBAP2L (ubiquitin-associated protein 2-like) \( R=0.59 \), and GATAD2A (GATA zinc finger domain-containing protein 2A) \( R=0.66 \) (all \( P<0.001 \)). Most specific cancer types showed positive relationships between BRD4 and the above ten genes, as indicated by the corresponding heat map data (Figure 6C).

The above two results were combined in Metascape to determine the Gene Ontology (GO) annotation results. The data in Figure 6D suggest that during tumor pathogenesis, chromatin modifying enzymes might be correlated with the effects of BRD4, as similarly suggested from a previous study\(^{[14]}\). This analysis also indicated that most of the above genes are related to biological cell behaviors such as DNA-templated transcription, regulation of histone modification, protein modification by small protein removal, DNA methylation or demethylation and
mitotic sister chromatid segregation. This may be relevant to the signaling pathways of intracellular receptor, cytokine signaling in immune system and regulation of TP53 activity through acetylation (Figure 6D).

4. Discussion

As a member of the bromodomain and extra terminal domain (BET) family\textsuperscript{[15]}, bromodomain 4 (BRD4) regulates cell cycle by regulating gene transcription. Therefore, BRD4 plays an important role in the physiological activities of normal cells and tumor cells. Overexpression, gene rearrangement and gene mutation of bet family proteins (especially Brd4) are often associated with many diseases, especially malignant tumors\textsuperscript{[16,17]}. BET family proteins also play an important role in promoting the abnormal expression of oncogenes such as c-myc in a variety of hematological malignancies, such as mixed lineage leukemia (MLL) and acute myeloid leukemia (AML), and promoting abnormal cell proliferation. Studies have suggested that inhibiting the expression of BRD4 can effectively inhibit the development of breast cancer, nasopharyngeal carcinoma and endometrial cancer\textsuperscript{[18-20]}. It is still unknown if BRD4 can react via specific molecular mechanisms for different tumors during pathogenesis. Pan-cancer results on whole tumors were not obtained through our literature search of other publications on BRD4.

Based on data from the CPTAC, GEO, and TCGA databases, we investigated the genetic alteration and molecular characteristics of gene expression in 33 different tumors, and comprehensively examined the BRD4 gene.
BRD4 is highly expressed in many tumors. However, apparent conclusions were found for different tumors from the BRD4 survival prognosis analysis. Our study employed the GEPIA2 tool to examine the potential relationships between high BRD4 expression and poor OS in various tumors. Updated survival information or alternative data processing may support these findings.

Many previous studies have analyzed the mechanism of high BRD4 expression in breast cancer and its metastasis\cite{21-22}. Our TCGA database analysis indicated that BRD4 expression was significantly higher in BRCA tumor tissues than in normal tissues. There were fewer than 100 CHOL cases with high BRD4 expression or low BRD4 expression. Analyses with larger samples may verify the above conclusions. Further molecular experimental data are needed to determine whether BRD4 expression plays a critical role in the occurrence of these tumors, or whether it is the consequence of antitumor transformation in normal tissues.

BRD4 expression in HNSC tumors was particularly higher than normal, and was significantly related to tumor prognosis. A negative correlation was found between BRD4 and the proportion of CD8+ T cells in HNSC. No study has analyzed the relationship between HNSC and BRD4, which provides new opportunities for scientific research.

We were able to draw conclusions regarding BRD4-uniting genes and factors associated with BRD4 expression in all tumors, and performed various enrichment analyses to identify the possible effects of DNA-templated transcription, regulation of histone modification, protein modification by small protein removal,
DNA methylation or demethylation, mitotic sister chromatid segregation for cancer pathogenesis or etiology. Various immune deconvolution methods identified significant negative correlations between immune infiltration levels and BRD4 expression of CD8+ T-cells in CESC, HNSC, HNSC-HPV−, HNSC-HPV+ tumors. The Tcm and Th cells exhibit positive enrichment in most tumors, while Tgd and eosinophils exhibit positive enrichment in only some tumors. BRD4 was negatively correlated with pDC in most tumors, and was negatively correlated with macrophages, iDC, Treg, cytotoxic cells, and Th17 cells in many tumors.

In summary, our pan-cancer analysis of BRD4 initially indicated a significant link—from the perspective of clinical tumor samples—between BRD4 expression and immune cell infiltration, and clinical prognosis or tumor mutational burden, which may improve the understanding of the molecular mechanism of BRD4 during tumorigenesis.

**FIGURE LEGENDS**

**Figure 1** We analyzed various databases to obtain BRD4 expression data. (A) TIMER2 analysis indicated that different cancers and specific cancer subtypes affect the BRD4 gene expression status. Samples with gray backgrounds represent both tumor and normal tissue samples, which can be compared statistically. Samples with white backgrounds represent only tumor samples, which cannot be compared statistically (*P<0.05; **P<0.01; ***P<0.001). (B) We used normal tissue data on SARC (sarcomav), LAML (acute myeloid leukemia), and LGG (thymoma) from the Genotype-Tissue Expression database as controls.
for comparisons with the corresponding data from The Cancer Genome Atlas (TCGA) project, which are presented as a box plot (*P<0.05). (C) Expression levels were also compared between tumor tissue and normal tissue of BRD4 proteins in breast cancer, colon cancer, LUAD (lung adenocarcinoma), UCEC (uterine corpus endometrial carcinoma), clear cell renal cell carcinoma, and ovarian cancer based on the CPTAC data set (**P<0.001). (D) We analyzed the prime pathological stages (stages I to IV) to identify BRD4 gene expression levels for ACC (adrenocortical carcinoma), COAD (colon adenocarcinoma), KICH (kidney chromophobe), PAAD (pancreatic adenocarcinoma), OV (ovarian serous cystadenocarcinoma), and SKCM (Skin Cutaneous Melanoma) based on TCGA data. The logarithmic scale was produced using log2 TPM + 1.

Figure 2 We used TCGA database to discover the relationships between BRD4 gene expression and the prognoses in various cancers. The Gene Expression Profiling Interactive Analysis 2nd Edition (GEPIA2) database was used to analyze different tumors in TCGA project for (A) overall survival (OS) in BRD4 gene expression and (B) disease-free survival (DFS) analyses. OS is the time from the onset of a condition onset to death from any cause. DFS is the time from onset to the first tumor recurrence/metastasis or death from any cause. Progression-free survival is the time from onset to the first tumor progression or death. Blue and red square in the picture show negative and positive associations of BRD4 gene expression with the prognosis, respectively. Positive results for survival and
Kaplan-Meier curves are shown. The receiver operating characteristics (ROC) curve between BRD4 and tumor prognosis was plotted based on data from TCGA database. (C) The areas under the ROC curves for adrenocortical carcinoma (ACC), brain lower grade glioma (LGG), colon adenocarcinoma (COAD), and head and neck squamous cell carcinoma (HNSC) were 0.848, 0.813, 0.805, and 0.821, respectively, indicating a high predictive value for tumor prognosis.

**Figure 3** TCGA was used to obtain the mutation effect of BRD4 on different tumors via the cBioPortal. This figure displays (A) the alteration frequency in different mutation molds, (B) mutation sites, and (C) the potential links between mutation condition and versions of ACC survival curves, as obtained using the cBioPortal tool.

**Figure 4** Links between BRD4 expression and CD8+ T immune cells. We used various algorithms to identify any links between BRD4 expression and immune cells. Within whole cancer types in TCGA project, we explored the expression level of BRD4 and the CD8+ T-cell infiltration status (A, B).

**Figure 5** Gene Set Variation Analysis (GSVA) of BRD4 enrichment and immune cells. RNA-seq data and clinical data were extracted in the Level 3 HTSeq-FPKM format from TCGA database, and Spearman's rank correlation test was performed using the GSVA package of R software to analyze the correlation of each tumor's immune cells. (A) Enrichment analysis of the immune cell relationship between BRD4 and breast invasive carcinoma, COAD, diffuse large-B-cell lymphoma, KICH, KIRC, KIRP, LAML, liver hepatocellular carcinoma, LUAD, MESO, OV, and
pancreatic adenocarcinoma. (B) Enrichment analysis of the immune cell relationship between BRD4 and PCPG, PRAD, READ, sarcoma, SKCM, stomach adenocarcinoma, TGCT, thyroid carcinoma, THYM, UCEC, UCS, and uveal melanoma.

**Figure 6** Analysis of genes and proteins relevant to BRD4. (A) The available BRD4-binding proteins determined by the experiment, obtained using the STRING tool. (B) The top 10 genes that were most strongly related to BRD4 from TCGA database, and the relationships between BRD4 expression and the following genes: WIZ (Widely Interspaced Zinc Finger), CHERP (Calcium homeostasis endoplasmic reticulum protein), SETD1A (SET domain protein 1A), MAML1 (mastermind-like 1), CREBBP (CREB-binding protein), SRCAP (SNF2-related CREBBP activator protein), POM121C, ZNF142 (zinc finger protein 142), UBAP2L (ubiquitin-associated protein 2-like), and GATA2 (GATA zinc finger domain-containing protein 2A). Obtained from the GEPIA2. (C) Detailed cancer types and the corresponding heat map data. (D) GO annotation results obtained using the Metascape platform.

**Figure 7** The flow chart of analysis

### 5. Conclusion

This is the first research study to systematically evaluate the potential role of BRD4 in disease progression and prognosis in several types of cancer. The present finding indicates that BRD4 expression may regulate tumor prognosis by altering and regulating certain immune cells, which has positive relationships with
Tcm and Th cells, and Tgd, and negative relationships with pDC, maropahges, iDC, Treg, cytotoxic cells, and Th17 cells. Chromatin modifying enzymes may be affected by BRD4, and BRD4 may be involved in the signaling pathways of intracellular receptor, cytokine signaling in immune system and regulation of TP53 activity through acetylation. It is therefore necessary to further investigate the diagnostic and therapeutic value of BRD4 in a variety of human cancers.

**Research highlights**

This is the first pan-cancer analysis of BRD4.

Novel effects of BRD4 on tumor prognosis and immune microenvironment have been revealed.

The relationship between the BRD4 protein and gene has been displayed.

**Disclosure**

The authors report no conflicts of interest in this work.

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None

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Figure 1

Please see the Manuscript PDF file for the complete figure caption.
Figure 2

We used TCGA database to discover the relationships between BRD4 gene expression and the prognoses in various cancers. The Gene Expression Profiling Interactive Analysis 2nd Edition (GEPIA2) database was used to analyze different tumors in TCGA project for (A) overall survival (OS) in BRD4 gene expression and (B) disease-free survival (DFS) analyses. OS is the time from the onset of a condition onset to death from any cause. DFS is the time from onset to the first tumor recurrence/metastasis or death from any cause. Progression-free survival is the time from onset to the first tumor progression or death. Blue and red square in the picture show negative and positive associations of BRD4 gene expression with the prognosis, respectively. Positive results for survival and Kaplan-Meier curves are
shown. The receiver operating characteristics (ROC) curve between BRD4 and tumor prognosis was plotted based on data from TCGA database. (C) The areas under the ROC curves for adrenocortical carcinoma (ACC), brain lower grade glioma (LGG), colon adenocarcinoma (COAD), and head and neck squamous cell carcinoma (HNSC) were 0.848, 0.813, 0.805, and 0.821, respectively, indicating a high predictive value for tumor prognosis.

Figure 3
TCGA was used to obtain the mutation effect of BRD4 on different tumors via the cBioPortal. This figure displays (A) the alteration frequency in different mutation molds, (B) mutation sites, and (C) the potential links between mutation condition and versions of ACC survival curves, as obtained using the cBioPortal tool.

Figure 4

Links between BRD4 expression and CD8+ T immune cells. We used various algorithms to identify any links between BRD4 expression and immune cells. Within whole cancer types in TCGA project, we explored the expression level of BRD4 and the CD8+ T-cell infiltration status (A, B).
Figure 5

Gene Set Variation Analysis (GSVA) of BRD4 enrichment and immune cells. RNA-seq data and clinical data were extracted in the Level 3 HTSeq-FPKM format from TCGA database, and Spearman’s rank correlation test was performed using the GSVA package of R software to analyze the correlation of each tumor’s immune cells. (A) Enrichment analysis of the immune cell relationship between BRD4 and breast invasive carcinoma, COAD, diffuse large-B-cell lymphoma, KICH, KIRC, KIRP, LAML, liver hepatocellular carcinoma, LUAD, MESO, OV, and pancreatic adenocarcinoma. (B) Enrichment analysis of the immune cell relationship between BRD4 and PCPG, PRAD, READ, sarcoma, SKCM, stomach adenocarcinoma, TGCT, thyroid carcinoma, THYM, UCEC, UCS, and uveal melanoma.
Figure 6

Analysis of genes and proteins relevant to BRD4. (A) The available BRD4-binding proteins determined by the experiment, obtained using the STRING tool. (B) The top 10 genes that were most strongly related to BRD4 from TCGA database, and the relationships between BRD4 expression and the following genes: WIZ (Widely Interspaced Zinc Finger), CHERP (Calcium homeostasis endoplasmic reticulum protein), SETD1A (SET domain protein 1 A), MAML1 (mastermind-like 1), CREBBP (CREB-binding protein).
protein), SRCAP (SNF2-related CREBBP activator protein), POM121C, ZNF142 (zinc finger protein 142), UBAP2L (ubiquitin-associated protein 2-like), and GATAD2A (GATA zinc finger domain-containing protein 2A) obtained from the GEPIA2. (C) Detailed cancer types and the corresponding heat map data. (D) GO annotation results obtained using the Metascape platform.

**Figure 7**

The flow chart of analysis