Bromine domain protein 4 is an important marker for prognosis of ovarian cancer and tumor immune infiltration

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Research Article

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Abstract

Background

Ovarian cancer (OC) is one of the most common malignant gynecological tumors, but its pathogenesis is unclear. Bromine domain protein 4 (BRD4) is involved in the malignant transformation of cells, as well as the invasion and metastasis of tumor cells. The biological role of BRD4 in ovarian cancer is yet to be determined.

Methods

The differential expression of BRD4 in OC and corresponding normal tissues was evaluated by exploring the Tumor Immune Assessment Resources (TIMER) and the Oncomine database. The correlation between the expression level of BRD4 and the prognosis of OC patients was evaluated using the Kaplan-Meier Plotter database. Using TIMER, we further studied the correlation between BRD4 and tumor immune cell infiltration.

Results

The expression of BRD4 was significantly higher in patients with OC, and high BRD4 expression was closely related to low overall survival rate. The BRD4 expression was associated with the levels of immune markers of macrophages, dendritic cells, neutrophils, and various effector T cells. Taken together, these findings show that BRD4 expression is significantly related to immune infiltration in OC and suggest that BRD4 might play an important role in the immune evasion of OC cells.

Conclusion

The expression level of BRD4 in OC tissues is significantly upregulated, and its high expression is significantly associated with poor prognosis of patients and is closely related to tumor immune infiltration. These results suggest that BRD4 can be used as a prognostic marker and a marker of immune infiltration in OC.

1 Introduction

Among the common gynecological malignancies, ovarian cancer (OC) has the highest mortality rate worldwide [1]. The high mortality rate can be attributed to the lack of effective biomarkers to detect the disease and predict the prognosis of patients from heterogeneous biological subgroups. More than 75% of patients are not diagnosed until the disease progresses to an advanced stage (III or IV), and the 5-year overall survival (OS) is less than 30% [2, 3]. Therefore, reliable early diagnostic and accurate prognostic biomarkers and new molecular targeted therapeutic strategies are urgently needed.

Bromine domain protein 4 (BRD4) is the most important functional protein in the bromodomain and superterminal family protein (BET) family, composed of two bromodomains and one superterminal domain [4]. Upregulation of BRD4 expression is closely related to the occurrence and development of lung cancer, breast cancer, acute myeloid lymphoma, liver cancer, and other tumors [5–7], but its relevance in OC and its relationship with patient prognosis have rarely been reported. Therefore, we searched the relevant gene expression databases to explore the differential expression of BRD4 in OC tissues and normal control tissues, and its relationship with patient survival and tumor immune infiltration.

2 Methods

2.1 Oncomine database analysis

The Oncomine database (https://www.oncomine.org/resource/main.html) was used to analyze the expression of BRD4 in OC.

2.2 Kaplan-Meier Plotter database

The Kaplan-Meier Plotter (http://kmplot.com/analysis/) database was used to assess the potential prognostic significance of BRD4 [8]. Patients were divided into two categories based on their median BRD4 expression level, high and low. The hazard ratios (HRs) at 95% confidence intervals (CIs) were calculated to determine the log-rank p value.

2.3 Tumor Immune Assessment Resources (TIMER) data analysis

TIMER analysis systematically evaluates the characteristics of tumor immune interactions (https://cistroes.shinyapps.io/timer/) [9]. This website has records of 10897 samples from 32 different cancer types from The Cancer Genome Atlas (TCGA) dataset. TIMER utilized the previously reported deconvolution analysis method to identify the excessive tumor-infiltrating immune cells through gene expression profiling.

2.4 c-BioPortal database

c-BioPortal (http://cbioportal.org) has a multidimensional cancer genomics dataset [10]. The c-BioPortal tool was used to analyze the mutation and copy number variation (CNV) of BRD4 in OC.

2.5 Statistical analysis

A student's t test was used to determine whether the expression levels of BRD4 were significantly different between OC and normal tissues. A p value < 0.05 was considered statistically significant. We compared the survival of OC patients based on the median expression levels of specific genes using a log-rank test, with $p < 0.05$ considered statistically significant.

3 Results

3.1 BRD4 gene expression analysis in OC tissue

We used the Oncomine database to analyze the expression of BRD4 in tumor tissues and corresponding normal tissues. BRD4 expression was significantly increased in tumor tissues in patients with OC (Figure 1A, B). This suggests that BRD4 may play a role in the occurrence and development of OC.

3.2 Prognostic value of BRD4 in OC

The PrognoScan database was used to analyze the correlation between BRD4 (probes, ID: 202102_at and ID:12779) and the survival rate in OC. The cumulative survival rate of patients with high BRD4 expression was significantly lower than that of the low-expression group (Figure 2A, ID: 202102_at, P = 0.000538; Figure 2B, ID: 12779, P = 0.029596). Further, using the Kaplan-Meier Plotter database, we performed multivariate Cox regression analysis on the data related to BRD4 expression and OC patients. The cumulative overall survival (OS) of patients in the BRD4 high expression group was significantly lower than that of patients in the low expression group (Figure 2C, ID: 202102_at, P = 0.027; Figure 2D, 226054_at, P = 0.012). Patients in the BRD4 high expression group had significantly shorter progression-free survival (PFS) than those in the low expression group (Figure 2E, ID: 202102_at, P = 0.000247; Figure 2F, 226054_at, P = 0.0029). These results indicate that BRD4 is an important factor affecting the prognosis of patients with OC.

3.3 Subgroup analysis of the correlation between BRD4 expression and patient survival

To further understand the correlation between BRD4 expression and OC and the potential mechanism, we explored the relationship between BRD4 expression and clinical characteristics of OC patients using the Kaplan-Meier Plotter database (Table 1). High BRD4 expression was associated with poor OS of serous ovarian cancer ($P < 0.05$). In patients with early stage OC (stage 1-2), high BRD4 expression was significantly associated with poor prognosis (Table 1). Pathological registry analysis also showed that high expression of BRD4 was significantly associated with poor OS (Table 1), independent of the presence of TP53 mutation ($P < 0.05$). Most importantly, we found that after chemotherapy with various drugs, high expression of BRD4 was significantly associated with poor OS ($P < 0.05$). These results suggest that BRD4 may be an important factor that hinders chemotherapeutic treatment of OC.

3.4 Genomic changes of BRD4 in OC

Next, we used c-BioPortal to determine the type and frequency of BRD4 genome changes in OC based on sequencing data from OC patients in TCGA. Twenty-two percent of OC patients had BRD4 genome changes (Figure 3A). In addition, BRD4 CNV was significantly correlated with the OS and disease-specific survival (DSS) of OC patients (Figure 3B-C).

3.5 Analysis of BRD4 expression and immune infiltration level in OC

Lymphocyte infiltration is an independent predictor of cancer patient survival and lymph node metastasis [11]. We evaluated the relationship between BRD4 expression and the degree of immune invasion in

patients with OC tumors. First, we found that BRD4 expression was significantly positively correlated with tumor purity (r = 0.196, P = $1.47´10^{-5}$), indicating that BRD4 is related to tumor purity and is closely related to prognosis. We also found that BRD4 expression was positively correlated with the degree of infiltration by immune cells, including CD8+ T cells (P = $6.28´10^{-8}$), macrophages (P = $1.1´10^{-12}$), centrioles (P = $1.85´10^{-13}$), and dendritic cells (P = $1.30´10^{-9}$) (Figure 4). To further study the correlation between BRD4 and several types of infiltrating immune cells, we used the TIMER and GEPIA datasets to determine the correlation between BRD4 and different immune cell markers in OC. TIMER database analysis showed that BRD4 was significantly correlated with immune markers of macrophages, dendritic cells, and various effector T cells in OC (Table 2). GEPIA analysis results confirmed that BRD4 was significantly correlated with immune markers of macrophages, dendritic cells, and various effector T cells, as well as centrioles, in OC (Figure 5). Based on the above results, we believe that BRD4 expression is related to immune cell infiltration in OC and that BRD4 may play an important role in the immune evasion of OC cells.

4. Discussion

BRD4 acts as a transcriptional co-factor that regulates the expression of a variety of genes. Because of its extensive cellular biological functions, BRD4 is also closely associated with a variety of tumors [5]. Miguel et al. found that BRD4 expression was significantly upregulated in primary and metastatic melanoma tissues. BET inhibitors can inhibit the proliferation of melanoma cells *in vitro* and the growth and metastasis of tumors *in vivo*, and the same effect can be obtained by silencing the BRD4 gene [12]. RNA sequencing of cells treated with BET inhibitors showed that it had a significant impact on cell growth, proliferation, cell cycle regulation, and differentiation. Tan et al. found that BRD4 expression was significantly increased in prostate cancer tissues and cells. Inhibition of BRD4 through short hairpin RNA or JQ1 can reduce prostate cancer cell proliferation, induce G0/G1 phase arrest and apoptosis, reduce cell invasion and migration *in vitro*, and inhibit tumor growth in the body. Thus, abnormal expression of BRD4 may induce prostate cancer [13]. In summary, BRD4 as an epigenetic regulator and transcription co-factor is closely linked with gene transcription, cell cycle, apoptosis, invasion, and metastasis. Abnormal expression of BRD4 can cause a variety of gene expression disorders, thereby affecting the function of related genes, and is closely related to the occurrence and development of lung cancer, breast cancer, acute myeloid lymphoma, and other tumors. Therefore, the mechanism of action of BRD4 and its inhibitors in various tumors requires comprehensive and in-depth multi-omics investigation.

Our results showed that BRD4 expression was significantly increased in patients with OC. As expected, patients with BRD4 overexpression had poor survival rates, while overexpression of BRD4 in OC patients was significantly associated with multiple case characteristics. BRD4 showed genomic changes in 22% of OC patients, suggesting that BRD4 mutations also lead to a significant decrease in survival. These results suggest that BRD4 gene expression and genome mutations are significantly correlated with the survival rate of OC patients. We also found that BRD4 is closely related to tumor immune infiltration. These findings suggest that small-molecule therapy targeting BRD4 may significantly improve survival by

inhibiting tumor-infiltrating immune cells. Therefore, the development of BRD4 inhibitors is expected to provide new research targets for applications in OC.

Declarations

Acknowledgements

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Funding

Not applicable.

Availability of data and materials

Direct web links of datasets about; Oncomine: http://oncom ine.org; UALCAN:http://ualca n.path.uab.edu; The Human Protein Atlas: https ://www.prote inatlas.org; cBioPortal: http://cbiop ortal .org; LinkedOmics: http://linke domic s.org; TIMER: https ://cistr ome.shiny apps.io/timer /; HCCDB: http://lifeo me.net/database/hccdb /home.html. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare that they have no conflicts of interest.

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Tables

Due to technical limitations, the tables are only available as downloads in the supplementary files.

Figures

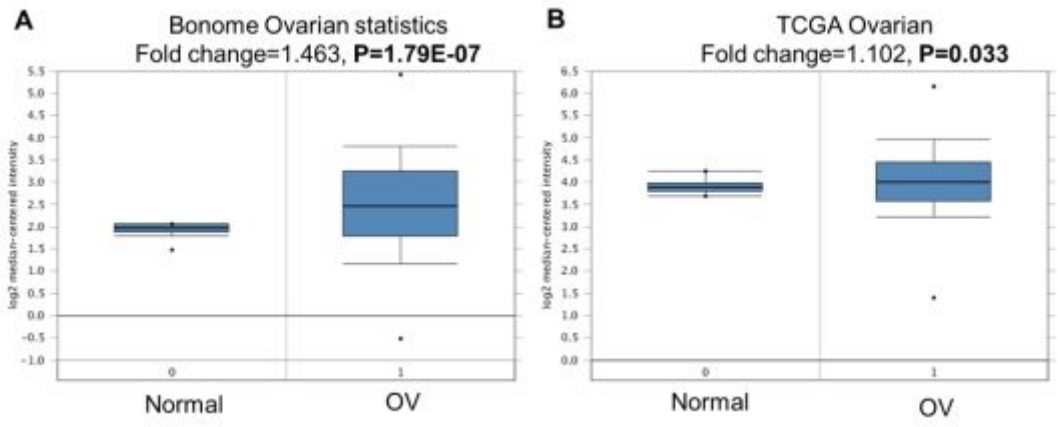

Figure 1

Transcriptional expression analysis of BRD4 in OC. A. Box diagram showing the level of BRD4 mRNA in OC tissues. B. Block diagram showing BRD4 copy numbers in TCGA.

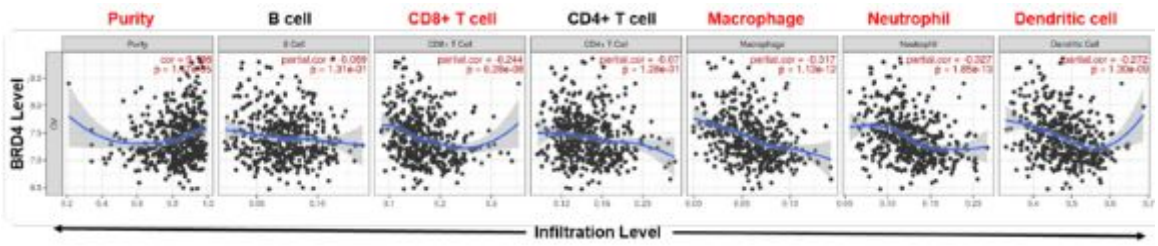

Figure 2

Survival analysis of BRD4 in OC patients. A. Analysis of OS in two groups of OC patients (DUKE-OC and ID: 202102_at). B. Analysis of OS in two groups of OC patients (GSE8841 and ID: 12779). C. Kaplan-Meier survival analysis for determining the impact of BRD4 (ID: 202102_at) on the OS of OC patients. D. Kaplan-Meier survival analysis for determining the impact of BRD4 (ID: 226054_at) on the OS of OC patients. E. Kaplan-Meier survival analysis for determining the impact of BRD4 (ID: 202102_at) on the PFS of OC patients. F. Kaplan-Meier survival analysis for determining the impact of BRD4 (ID: 226054_at) on the PFS of OC patients.

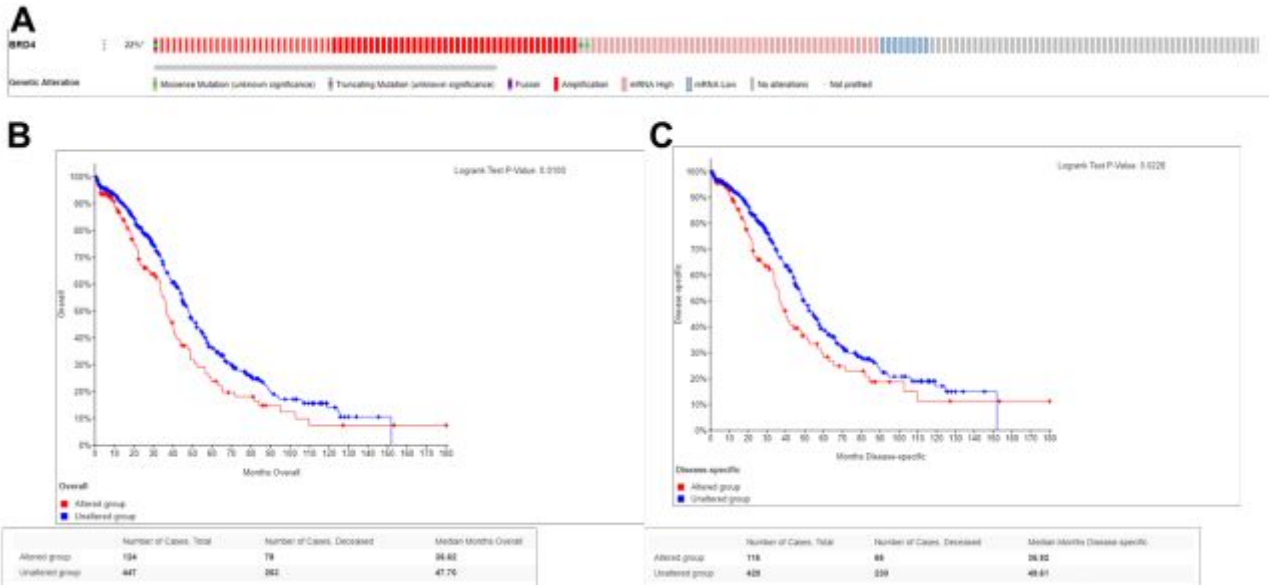

Figure 3

Analysis of BRD4 genome changes in OC. A. Analysis of BRD4 genome changes in the OC cohort. Various genomic changes are highlighted in different colors. B. Kaplan-Meier analysis comparing the total OS with and without BRD4 gene expression changes. C. Kaplan-Meier analysis comparing DSS with and without BRD4 gene expression changes.

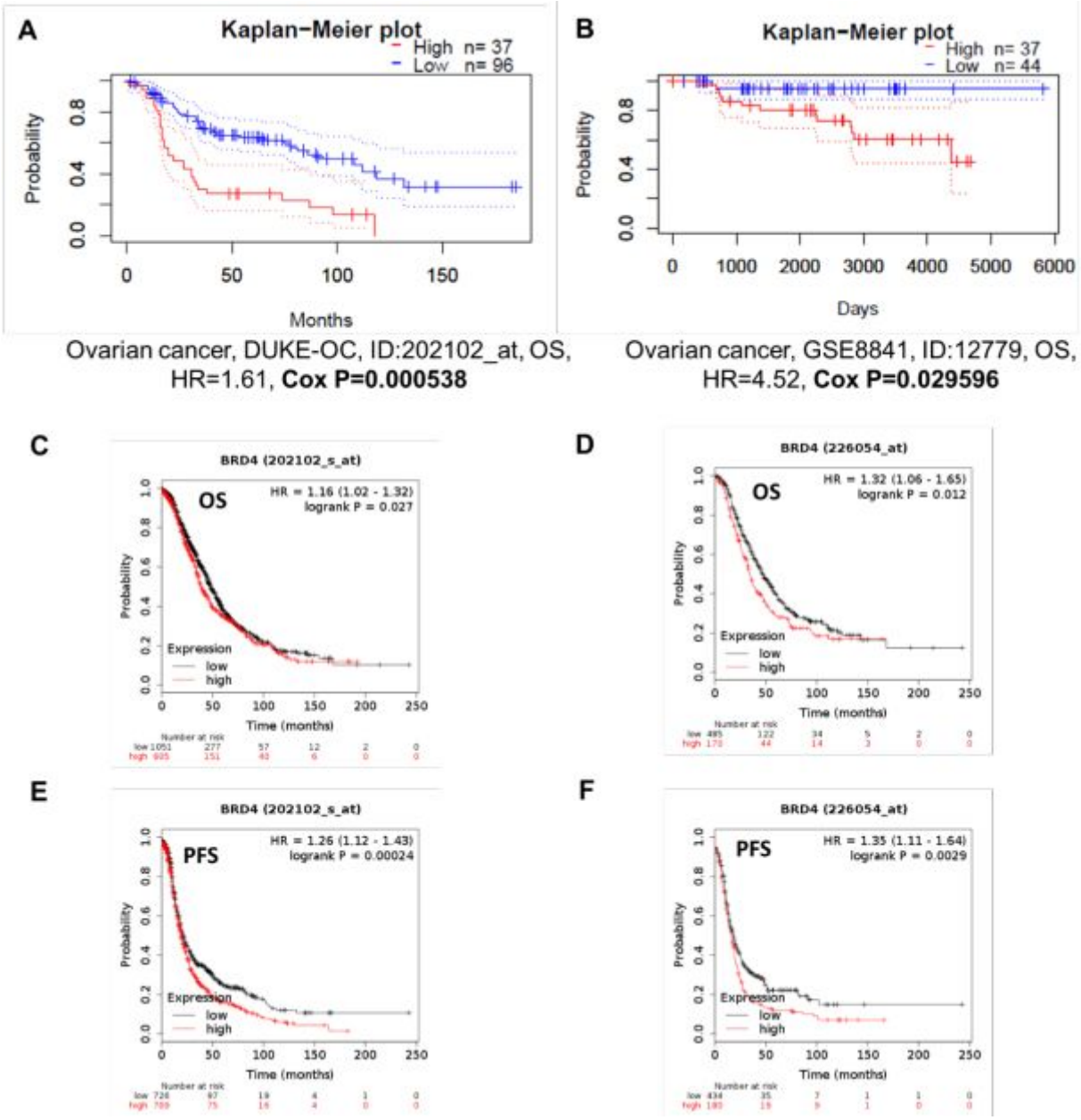

Figure 4

Correlation analysis between BRD4 expression in OC and the extent of immune infiltration.

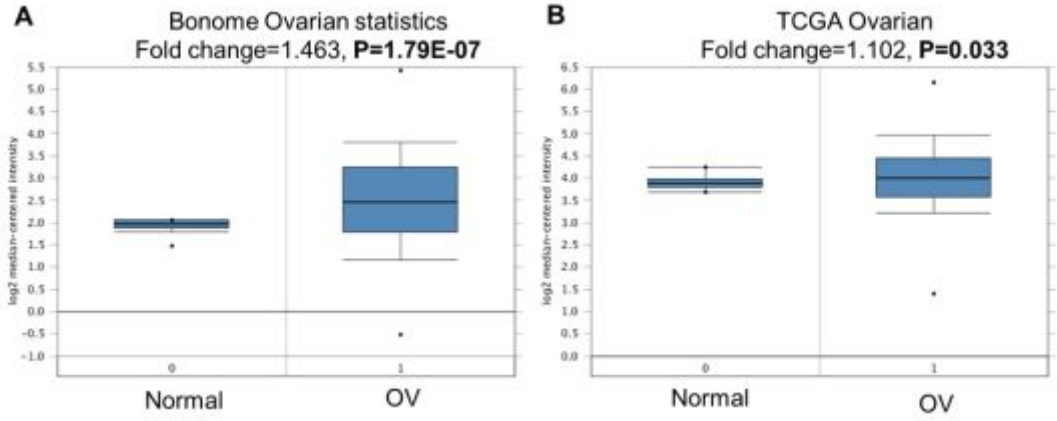

Figure 5

Correlation between BRD4 expression and immune markers in OC

Supplementary Files

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