

Amplification of the bromodomain-containing protein 4 gene in ovarian high-grade serous carcinoma is associated with worse prognosis and survival

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Received June 30, 2015; Accepted July 22, 2015

DOI: 10.3892/mco.2015.622

Abstract. High-grade serous carcinoma (HGSC) of the ovary is an aggressive and devastating neoplasm and the identification of novel therapeutic targets may result in a significant decrease in patient morbidity and mortality. Over the last few years, chromatin regulators have become attractive targets for cancer therapy. More specifically, bromodomain-containing protein 4 (BRD4), a protein that is associated with acetylated chromatin and transcriptional activation, has been shown to selectively regulate the transcription of key oncogenic drivers, such as *CMYC*, in several tumor types. The Cancer Genome Atlas (TCGA) project has molecularly characterized the genome of ovarian serous carcinomas, which enabled us to study the association of genomic alterations of *BRD4* with patient survival and clinicopathological characteristics. Our analysis using clinical and genomic data from the TCGA ovarian carcinoma samples revealed that somatic amplification of *BRD4* (observed in 12% of the cases) was correlated with increased *BRD4* mRNA levels and is significantly associated with worse overall and progression-free survival compared to wild-type cases. These findings support the hypothesis that future studies and trials investigating newly developed BRD4 inhibitors are required in a subset of patients with ovarian HGSC.

Introduction

Ovarian high-grade serous carcinoma (HGSC) is the most common type of epithelial ovarian carcinoma, with the highest mortality rate among gynecological malignancies. Following debulking surgery and adjuvant carboplatin and paclitaxel

chemotherapy, a significantly high percentage of cases eventually recur, with resistance to systemic therapy. The availability of targeted therapies is currently limited and death typically occurs within 5 years after tumor recurrence and development of chemoresistance (1).

Recently, the Cancer Genome Atlas (TCGA) project has characterized the genomic landscape of HGSC and highlighted a prevalence of somatic tumor protein p53 gene mutations and numerous DNA amplifications and deletions (2). Similar to previous studies, the TCGA study additionally confirmed that a significant number of HGSCs exhibited genetic alterations in the breast cancer 1, early onset (*BRCA1*) and *BRCA2*, cyclin E and *MYC* pathways, with extensive gene copy number alterations. However, the absence of high-frequency oncogenic point mutations in drug target genes has precluded the development of targeted therapeutics for HGSC (2-5).

The goal of this study was to investigate whether novel therapeutic target pathways with available inhibitors may be associated with specific survival outcomes in patients with HGSC. In recent years, chromatin regulators, such as bromodomain-containing protein 4 (BRD4), a member of the bromodomain and extra terminal domain family, which is associated with acetylated chromatin and transcriptional activation, have become attractive targets for cancer therapy. BRD4 has been shown to selectively regulate the transcription of key oncogenic drivers, such as *CMYC*, in several tumor types (6-9), whereas more recent analyses using epigenetic datasets have demonstrated that BRD4 specifically targets cell-type specific enhancer sequences (i.e., super enhancers) (10). The aim of the present study, was to determine whether the *BRD4* gene, which is a druggable gene product, is amplified in a subset of HGSCs, and whether its amplification is associated with worse prognosis and survival.

Patients and methods

Survival analysis. Clinical data from newly diagnosed patients with ovarian carcinoma from the TCGA study (2), specifically 489 cases with copy number alterations data, were integrated with *BRD4* amplification data from the TCGA data portal. Kaplan-Meier overall and disease-free survival analysis was performed with the cBio Cancer Genomics portal

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Key words: bromodomain-containing protein 4, ovarian cancer, serous carcinoma, MYC, The Cancer Genome Atlas project

(<http://cbioportal.org>; Memorial Sloan Kettering Cancer Center, New York, NY, USA); significance was estimated using the log-rank test (11,12).

Review of clinical and pathological data. Pathology reports were downloaded from the Cancer Genome Atlas data portal control-access directory (<https://tcga-data.nci.nih.gov/tcga>) or via the cBio portal. Quality control hematoxylin and eosin images from frozen and permanent sections were analyzed via the TCGA BioSig website hosted at Lawrence Berkeley National Laboratory (<http://tcga.lbl.gov:8080/biosig/tcgadownload.do>) and via the Cancer Digital Slide Archive (<http://cancer.digitalslidearchive.net>; Emory University, Atlanta, GA, USA).

This study was conducted in accordance with the TCGA publication policy for ovarian carcinomas (<http://cancer-genome.nih.gov>) and was approved by the Beth Israel Deaconess Medical Center HCC Institutional Review Board.

Results

Cases and survival. Clinical data from newly diagnosed patients with ovarian carcinoma from the TCGA study, specifically 489 cases with copy number alterations data, were integrated with *BRD4* amplification data from the TCGA data portal via the cBio Cancer Genomics Portal (2,11,12). Somatic amplification of *BRD4* was identified in 12% (57/489) of the cases. Survival analysis of all TCGA tumors with ovarian HGSC (n=489), revealed that patients with somatic *BRD4* amplification exhibited significantly worse overall and progression-free survival compared with those with non-amplified *BRD4* (log-rank test $P=0.0042$ and $P=0.013$, respectively; Fig. 1). The median number of months until relapse was 13 for cases with amplification, compared with 17 for cases without amplification. Patients with *BRD4* amplification exhibited a median survival of 36 months, compared with 44 months for patients without amplification.

mRNA data. We next examined the mRNA data for these cases. As shown in Fig. 2A, *BRD4* mRNA levels tended to increase with copy number. Using a stringent Z score threshold of +3.0 to define significant upregulation, *BRD4* mRNA was increased in 9% (43/489) of the cases, and increased *BRD4* mRNA levels were associated with worse overall survival (log-rank test $P=0.01$; Fig. 2B). By contrast, there were no significant differences in progression-free survival in cases with *BRD4* mRNA upregulation (log-rank test $P=0.59$). Again using a Z score threshold of +3.0, in 45% of the amplified cases (26/57), *BRD4* amplification resulted in mRNA upregulation. Conversely, 60% (26/43) of tumors with high mRNA levels harbored an underlying amplification.

Genes differentially expressed in *BRD4*-amplified tumors. To elucidate the mechanism through which *BRD4* amplification may affect patient survival, we assessed which genes may be differentially expressed in *BRD4*-amplified tumors, including *CMYC* or *NMYC*, which are known downstream targets of *BRD4* (7,13,14). In the TCGA cohort, a subset of *BRD4*-amplified tumors exhibited high *CMYC* and *NMYC* mRNA levels; however, there was only a weak positive association between *BRD4* copy number and *CMYC* mRNA

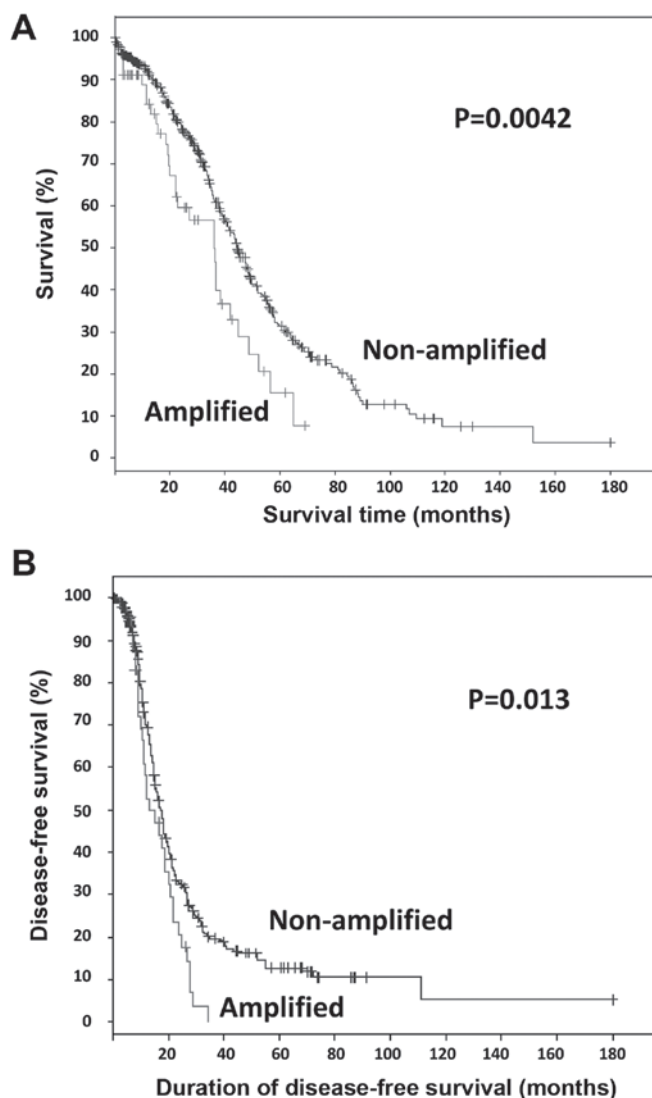


Figure 1. Survival Kaplan-Meier curves for bromodomain-containing protein 4 gene (*BRD4*)-amplified tumors. (A) Overall and (B) disease-free survival of The Cancer Genome Atlas cases with copy number alteration data (n=489), with or without *BRD4* amplification.

(Pearson, 0.130; and Spearman, 0.072), or *NMYC* mRNA levels (Pearson, 0.119; and Spearman, 0.063) (data not shown). By contrast, there was a strong association between *BRD4* amplification and increased mRNA levels of other genes located in close proximity to *BRD4*, such as *WIZ*, *PRKACA*, *NOTCH3* and *SMARCA4* (Table I), suggesting a potential collaborative regional amplification effect. Future studies with independent cohorts, cell culture and/or animal model systems are required to address the potential mechanisms through which *BRD4* amplification contributes to the pathogenesis and prognosis of ovarian HGSCs.

Discussion

BRD4 is a bromodomain protein and part of a chromatin remodeling complex, which regulates gene expression by binding acetylated chromatin at specific promoters and enhancers. Via its bromodomains, *BRD4* recruits positive transcription elongation factor b and triggers the release of

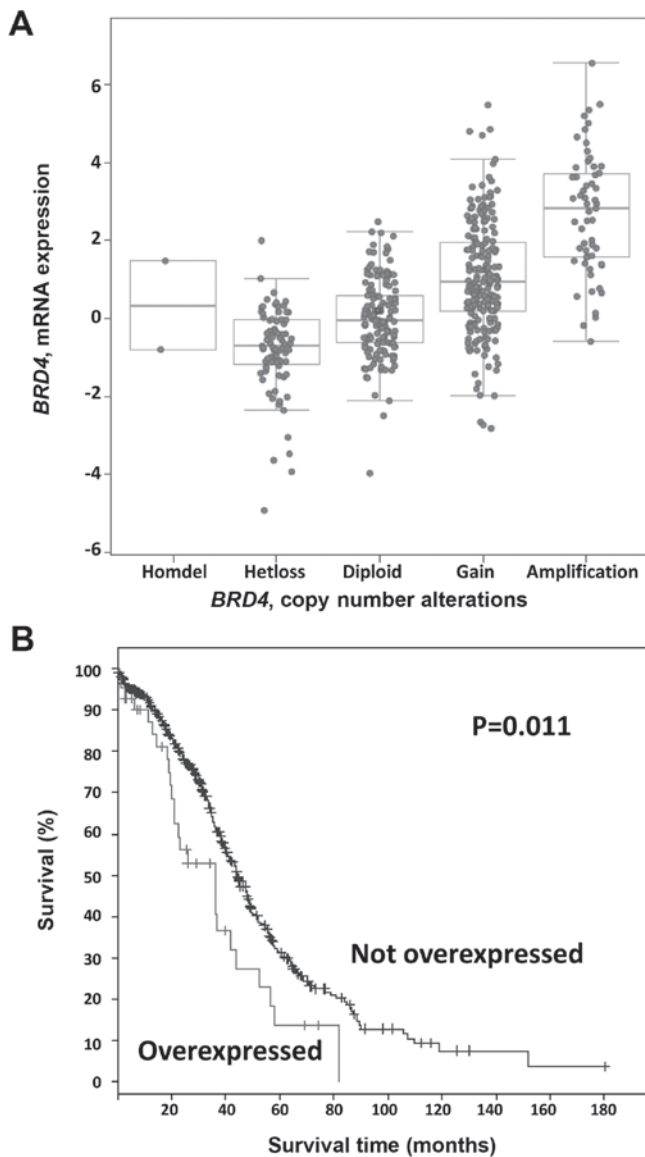


Figure 2. Bromodomain-containing protein 4 (*BRD4*) mRNA data. (A) *BRD4* mRNA levels tended to increase with increased copy number. (B) Kaplan-Meier curve depicting overall survival with *BRD4* mRNA upregulation, Z score threshold of +3.0 of *BRD4* mRNA levels.

RNA polymerase II from promoters, thereby resulting in productive transcriptional elongation and active gene expression of genes such as *CMYC* (13,15).

We exploited the TCGA data portal on ovarian carcinomas to interrogate specific genetic events of druggable genes associated with survival and clinicopathological characteristics, as well as to generate hypotheses for future testing. We herein propose that *BRD4* amplification in ovarian HGSCs may be associated with unfavorable prognosis and survival. However, due to the limitations of the TCGA study (i.e., relatively short follow-up time and lack of information on other comorbidities), these findings are exploratory and hypothesis-generating in nature rather than definitive. Future studies with independent patient cohorts are required to validate the TCGA findings.

Furthermore, the mechanism through which *BRD4* amplification may affect patient prognosis and survival remains unclear. Amplification of *BRD4* appears to segregate

Table I. Differentially expressed genes (mRNA) in bromodomain-containing protein 4 gene-amplified tumors.

Genes	Pearson	Spearman
<i>WIZ</i>	0.79	0.80
<i>AKAP8</i>	0.74	0.72
<i>CHERP</i>	0.69	0.70
<i>AKAP8L</i>	0.67	0.64
<i>SIN3B</i>	0.66	0.66
<i>DCAF15</i>	0.66	0.58
<i>MYO9B</i>	0.65	0.65
<i>MED26</i>	0.65	0.66
<i>PRKACA</i>	0.64	0.60
<i>CCDC130</i>	0.62	0.58
<i>NACCI</i>	0.61	0.60
<i>PKN1</i>	0.60	0.55
<i>CC2D1A</i>	0.60	0.58
<i>AP1M1</i>	0.59	0.58
<i>C19ORF44</i>	0.59	0.57
<i>EPS15L1</i>	0.59	0.59
<i>TECR</i>	0.58	0.56
<i>MAP1S</i>	0.57	0.56
<i>ZNF333</i>	0.57	0.53
<i>NOTCH3</i>	0.56	0.55
<i>SLC35E1</i>	0.56	0.56
<i>TRMT1</i>	0.55	0.50
<i>GTPBP3</i>	0.55	0.53
<i>KRI1</i>	0.55	0.53
<i>TNPO2</i>	0.54	0.50
<i>JUND</i>	0.53	0.57
<i>RAD23A</i>	0.53	0.51
<i>SMARCA4</i>	0.53	0.52
<i>TYK2</i>	0.53	0.49
<i>ILF3</i>	0.52	0.47

preferentially to the non-BRCAness molecular subgroup of ovarian HGSCs (16). A recently published study suggested that *BRD4* inhibitors may be effective in a subset of ovarian HGSCs that exhibit high *CMYC* or *MYCN* levels (17). In the TCGA cohort, there was a weak positive association between *BRD4* copy number alterations and *CMYC* or *MYCN* mRNA levels, suggesting that survival effects may be *MYC*-independent. By contrast, there was a strong association between *BRD4* amplification and increased mRNA levels of other genes located in close proximity to *BRD4*, such as *NOTCH3* and *SMARCA4*, suggesting a potential collaborative regional amplification effect. Supporting this hypothesis, *SMARCA4* (a member of the SWI/SNF chromatin remodeling complex, also known as Brg1) has been found to occupy the same *MYC* enhancer sites as *BRD4*, thereby enhancing *MYC* expression (18). In addition, *NOTCH1* has been shown to enhance *MYC* expression in T-cell leukemia independently of *BRD4* (19). In ovarian HGSC, our finding that worse overall survival is also observed with increased *BRD4* mRNA levels

in addition to *BRD4* amplification suggests that this survival effect may be specific to *BRD4* function.

Finally, we hypothesized that *BRD4*-amplified tumors may exhibit increased sensitivity to newly developed *BRD4* small-molecule inhibitors. Future studies, including animal xenografts or cell culture models, are required to further elucidate the mechanisms through which *BRD4* amplification and overexpression may affect tumor progression and patient survival. In summary, we herein presented evidence that the *BRD4* gene is amplified and overexpressed in a subset of HGSCs, and that its amplification and overexpression are associated with worse prognosis and survival. Our findings suggest that targeted inhibition of *BRD4* with specific inhibitors should be tested in a subset of ovarian carcinoma patients.

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