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ORIGINAL PAPER

Targeting BRD4 mediates immune response against cervical cancer cells enhancing the efficacy of radiotherapy

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Abstract

Conventional therapy including chemo- and radiotherapy is the current standard treatment for cervical cancer. However, the cancer may become resistant to such modalities, necessitating the exploration of new therapeutic strategies. BRD4, an epigenetic reader that binds to acetylated histones and regulates transcription, plays a critical role in the development and progression of various cancers through its involvement in transcriptional regulation.

Purpose In this study, we examined the efficacy of BRD4 inhibition using different inhibitors in cervical cancer cells.

Methods The organoid cultures derived from SiHa and HeLa cells were treated with three different BRD4 inhibitors (JQ1, MZ1, and AZD5153) at the concentration of 1 μM. The organoids were subjected to RNA extraction and whole transcriptome sequencing, respectively.

Results The results reveal that targeting BRD4 in the organoid model altered the expression of genes involved in immune response against tumor cells including PDCD1LG2, TGFB1, and NECTIN2. These genes are associated with immune cells targeting of tumor cells. Specifically, the downregulation of PDCD1LG2 (PDL2) suggested enhanced immune checkpoint pathways, while TGFB1 is known for its immunosuppressive functions and its roles in the tumor microenvironment. NECTIN2 is involved in immune cell adhesion and activation.

Conclusions Targeting BRD4 has been shown to improve the immune response to tumors, suggesting its potential as a strategy for enhancing antitumor immunity in cancer treatment. These findings highlight the potential of BRD4 inhibitors to increase the efficacy of radiotherapy through enhancing immune response to tumor cells, which can also be activated by radiation, making BRD4 a promising therapeutic target for treating cervical cancer in combination with radiotherapy.

Keywords BRD4 · Cervical cancer · Organoid · Immune response

1 Introduction

Cervical cancer remains a significant global health challenge, with nearly 90% of cases attributed to persistent high-risk human papillomavirus (HPV) infections. The burden is particularly severe in low- and middle-income countries, where access to preventive measures and early detection is limited [1]. Although standard treatments—surgery, radiotherapy, and cisplatin-based chemotherapy—have improved outcomes, treatment resistance and recurrence remain significant hurdles. These issues are especially pronounced in advanced cases where radioresistance contributes to poor survival rates, underscoring the urgent need for novel therapies. BRD4 (Bromodomain-containing protein 4) is an essential regulator in the field of cancer epigenetics.

As a member of the BET (Bromodomain and Extra-Terminal domain) protein family, BRD4 plays a key role in transcriptional regulation and chromatin remodeling by interacting with acetylated histones [2]. It is crucial in maintaining oncogenic transcriptional programs and supporting cancer cell proliferation and survival. BRD4 inhibitors work by disrupting transcriptional super-enhancers, which reduces

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tumor growth and increases sensitivity to other therapies. Recent studies have revealed that BRD4 not only influences the intrinsic properties of cancer cells but also significantly impacts the tumor microenvironment (TME) [3]. The TME, which consists of various non-cancerous cells, including immune cells, fibroblasts, endothelial cells, and extracellular matrix components, is crucial for supporting tumor growth and progression [4]. BRD4 modulates the TME by regulating the expression of genes involved in immune evasion, angiogenesis, and inflammation, thereby shaping the landscape in which tumor cells thrive [5].

Our study investigated the role of BRD4 in tumor persistence by examining its potential effect on immune response by analyzing the transcriptomic data derived from cervical cancer organoids with BRD4 inhibition. The results showed that the inhibition downregulated immune-related gene expression, suppressing tumor immunity and promoting tumor survival. These findings suggest that targeting BRD4 could enhance cancer immunotherapy by boosting the immune response and sensitizing tumors to immune checkpoint blockade therapies, making BRD4 a critical therapeutic target within the tumor microenvironment (TME).

2 Method

2.1 Chemical reagent

According to product data sheets, the BRD4 inhibitors JQ1 and AZD5153 were purchased from Sigma-Aldrich (Merck, Darmstadt, Germany) and MZ1 was purchased from MedChemExpress (Monmouth Junction, NJ, USA). They were dissolved in dimethyl sulfoxide (DMSO) and stored at − 80 degrees Celsius. The stock solutions were diluted in complete medium to achieve the final DMSO concentrations.

2.2 Gene expression profiling and data analysis

After establishing cervical cancer organoids from SiHa and HeLa cell lines obtained from ATCC, cells were cultured in organoid medium with advanced DMEM/F12 at 37 °C and 5% CO_2 in a humidified incubator. The BRD4 inhibitors JQ1, MZ1, and AZD5153 (1 µM each) were initiated. RNA (Ribonucleic acid) was extracted using the RNeasy Plus Universal Mini Kit (Qiagen, #73404), and sequencing was performed by Macrogen (South Korea) on the Illumina NovaSeq6000 platform, generating 100 bp paired-end reads. Quality control was conducted using FastQC, and low-quality reads were trimmed with cutadapt. Clean reads were aligned to the GRCh38 genome using STAR. Differential gene expression analysis, performed with DESeq2 on six biological replicates per condition, identified differentially expressed genes using a P-value $\leq 10^{-9}$ and a log2 fold change ≥ 2.

3 Final Considerations

To elucidate the molecular mechanisms of BRD4 inhibitors in cervical cancer, RNA sequencing was performed on HeLa and SiHa organoids treated with JQ1, MZ1, and AZD5153 (1 µM) or DMSO as a control for 24 h. This approach enabled a comprehensive comparison of gene expression profiles, with Differentially expressed genes (DEGs) identified using a stringent significance threshold (adjusted p-value $< 10^{-9}$) to ensure statistical and biological relevance.

The analysis generated a heatmap of gene expression changes, with upregulated genes shown in red and downregulated genes in blue. Emphasis was placed on the downregulated genes following BRD4 inhibition (Fig. 1), which identified PDCD1LG2, TGFB1, and NECTIN2 as key targets, indicating BRD4's role in promoting immune evasion. The overlap in differentially expressed genes (DEGs) across the three BRD4 inhibitors in both cell lines was also assessed. In HeLa cells, JQ1 treatment resulted in 12 DEGs, MZ1 in 16 DEGs, and AZD5153 in 12 DEGs. Of these, 10 genes were commonly affected: HSPD1, CASC2, and DLEC1 were upregulated, while MR1, NRG1, RBMS3, KLF4, TGFB1, NECTIN2, and PDCD1LG2 were downregulated. Notably, DAPK1 was modulated by both JQ1 and MZ1, and FBXO38 was regulated by both MZ1 and AZD5153 (Fig. 2). In SiHa cells, JQ1 treatment produced 15 DEGs, MZ1 led to 13 DEGs, and AZD5153 resulted in 13 DEGs. Seven genes were commonly modulated, with FBXO38, TNFRSF10B, and KIF4 upregulated, and IL11, PDCD1LG2, TGFB1, and NECTIN2 downregulated. JQ1 also specifically impacted genes like HSPD1, RELA, PRDX2, RBMS3, and CASC2, while MZ1 uniquely regulated DLEC1. MR1 was shared by both MZ1 and AZD5153 (Fig. 3). Importantly, three genes—PDCD1LG2, TGFB1, and NECTIN2—were consistently downregulated across all three treatments in both organoid models. Notably, KLF4 expression was differentially regulated between the two cell lines: it was downregulated in HeLa cells but upregulated in SiHa cells following BRD4 inhibition.

The identification of differentially expressed genes (DEGs) following treatment with JQ1, MZ1, and AZD5153 suggests that these BRD4 inhibitors influence gene expression, particularly in immune response pathways within the tumor microenvironment [6]. DAPK1, a serine/threonine kinase involved in apoptosis and autophagy, may be reactivated by BRD4 inhibition, restoring pro-apoptotic signaling. Studies indicate that BRD4 regulates both intrinsic and extrinsic apoptosis pathways, and inhibiting it could enhance DAPK1 expression or activity [7]. FBXO38, an

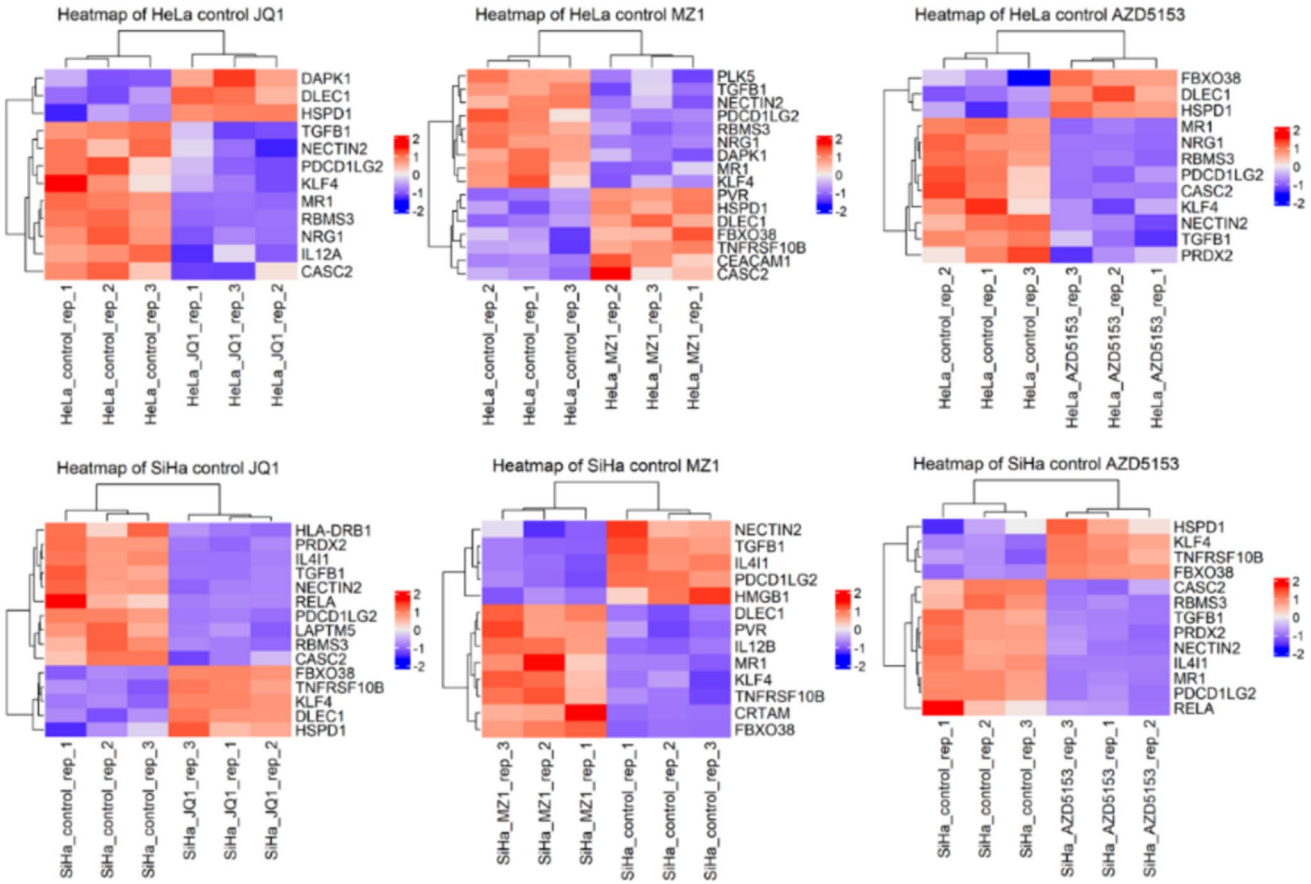
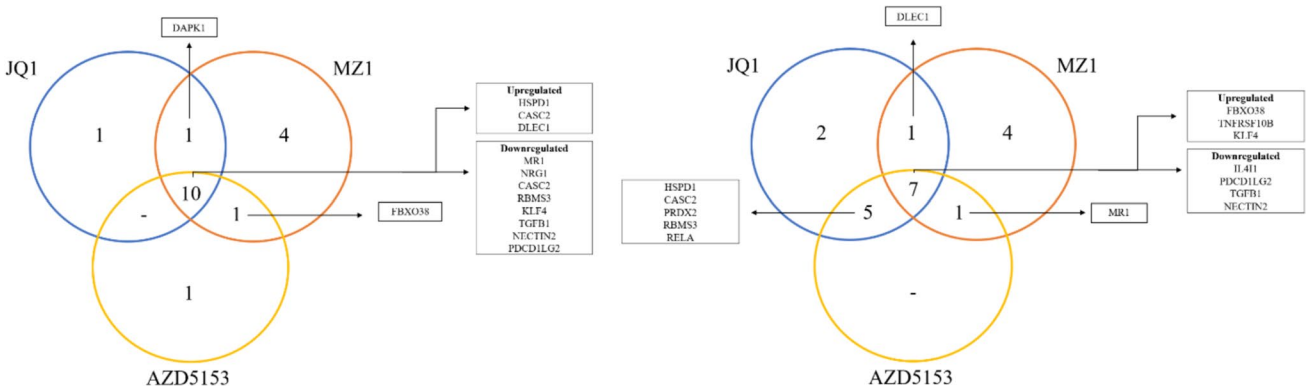


Fig. 1 Heat maps in HeLa and SiHa organoids treated with 1 μM of BRD4 inhibitors for 24 h



Figs. 2–3 Venn diagram showing immune responses in HeLa and SiHa organoids treated with 1 μM of BRD4 inhibitors

F-box protein involved in ubiquitin-mediated degradation, is regulated by MZ1 and AZD5153, indicating that these inhibitors promote proteasomal degradation. MZ1, a PROTAC, specifically targets BRD4 for degradation, and FBXO38, as part of the SCF complex, may support similar ubiquitination processes, further enhancing the degradation of BRD4 or related proteins [7–9]. DLEC1, a tumor suppressor gene frequently silenced in various cancers, is found in SiHa cells treated with MZ1 and JQ1. While there is no direct link between BRD4 inhibitors and DLEC1, BRD4 inhibition typically reactivates tumor suppressor genes [9, 10], suggesting the possibility of DLEC1 reactivation. Genes like HSPD1 and CASC2, consistently upregulated across multiple BRD4 inhibitors, point to the influence of these inhibitors on common pathways involved in cellular stress response and tumor suppression [11–13]. Notably,

the down-regulation of PDCD1LG2, TGFB1, and NECTIN2 across all inhibitors in HeLa and SiHa organoids is significant. The reduction of PDCD1LG2, a key player in tumor immune evasion, suggests that BRD4 inhibitors may enhance immune surveillance [14]. Similarly, the decreased expression of the immunosuppressive cytokine TGFB1 and NECTIN2, involved in immune evasion [14–16], indicates that BRD4 inhibitors disrupt multiple immune resistance pathways.

These findings align with previous studies showing BRD4's role in regulating immune checkpoint molecules like PD-L1 in various cancers [17]. The consistent down-regulation of PDCD1LG2, TGFB1, and NECTIN2 in cervical cancer highlights BRD4 inhibitors' broader impact on immune regulation. Additionally, the divergent regulation of KLF4—up-regulated in SiHa and down-regulated in HeLa—may reflect cell line-specific responses to BRD4 inhibition, potentially involving distinct tumor progression mechanisms. Overall, these results underscore the therapeutic potential of BRD4 inhibitors in modulating immune responses in cancer treatment.

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Code availability The code for publication is available upon reasonable request to the corresponding author.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent to publish All authors have provided consent to publish this work.

Conflicts of interest The authors declare no conflicts of interests.

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