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BRD7 Suppresses PD-L1 Expression to Counteract Immune Evasion in Nasopharyngeal Carcinoma

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ABSTRACT

Nasopharyngeal carcinoma (NPC) is a prevalent head and neck malignancy with unique epidemiological and clinical characteristics [1, 39]. Despite advances in treatment, immune evasion remains a significant challenge, often mediated by the overexpression of programmed death-ligand 1 (PD-L1) on tumor cells [2, 7, 8]. This study investigates the role of Bromodomain-containing protein 7 (BRD7), a known tumor suppressor, in regulating PD-L1 expression and modulating immune escape in NPC. We demonstrate that BRD7 is frequently downregulated in NPC and that its restoration significantly inhibits PD-L1 expression both in vitro and in vivo. Mechanistically, BRD7 exerts this effect by directly binding to the PD-L1 promoter, leading to transcriptional repression. Furthermore, we show that BRD7's suppressive effect on PD-L1 enhances T-cell mediated cytotoxicity against NPC cells. These findings reveal a novel mechanism by which BRD7 contributes to anti-tumor immunity in NPC, suggesting that strategies aimed at upregulating BRD7 or targeting its downstream effects could improve the efficacy of immunotherapy for NPC patients.

KEYWORDS

Nasopharyngeal Carcinoma, BRD7, PD-L1, Immune Evasion, Immunotherapy, Tumor Suppressor, Transcriptional Regulation.

INTRODUCTION

Nasopharyngeal carcinoma (NPC) is an aggressive malignancy with a high incidence in specific geographic regions, particularly Southeast Asia [1]. Its unique etiology, often linked to Epstein-Barr virus (EBV) infection, presents distinct challenges in treatment and management [9, 26]. While radiotherapy and chemotherapy remain the cornerstones of NPC treatment, the advent of immunotherapy, particularly immune checkpoint inhibitors targeting the PD-1/PD-L1 axis, has shown promising results in recurrent or metastatic NPC [2, 4, 5, 6, 7, 8, 17]. However, not all patients respond effectively, and understanding the mechanisms underlying immune evasion is crucial for improving therapeutic outcomes [2, 7, 8, 9].

Immune evasion, where cancer cells escape detection and destruction by the host's immune system, is a

hallmark of cancer [38]. A key mechanism of immune evasion involves the overexpression of PD-L1 on tumor cells, which binds to the PD-1 receptor on T cells, leading to T-cell inactivation and tumor immune suppression [17, 38]. Therefore, identifying factors that regulate PD-L1 expression is critical for developing more effective immunotherapeutic strategies [18, 19, 40, 41].

Bromodomain-containing protein 7 (BRD7) is a member of the bromodomain and extra-terminal (BET) domain family of proteins, known for their role in chromatin remodeling and gene transcription [10, 28]. BRD7 has been identified as a tumor suppressor in various cancers, including breast cancer, gastric cancer, and colorectal cancer, where it often inhibits cell proliferation, migration, invasion, and promotes apoptosis and senescence [10, 15, 16, 29, 30, 31, 32, 33, 34, 37]. Its diverse functions are attributed to its ability to regulate transcription by interacting with various transcription factors and chromatin complexes [10, 12, 13, 28]. In NPC, BRD7 has been shown to inhibit cell proliferation and tumor growth by targeting oncogenic miR-141 and regulating the PTEN/AKT pathway [11, 29]. It also plays a role in suppressing metastasis and enhancing chemosensitivity [30, 31, 33]. Furthermore, BRD7 has been implicated in anti-inflammatory responses and regulation of c-Myc [36, 37].

Given BRD7's established tumor suppressor role and its involvement in transcriptional regulation, we hypothesized that BRD7 might play a role in modulating immune evasion in NPC by regulating PD-L1 expression. This study aims to investigate the expression pattern of BRD7 in NPC, its impact on PD-L1 levels, and the underlying molecular mechanisms, with the ultimate goal of identifying new therapeutic avenues to overcome immune escape in NPC.

METHODS

- Cell Lines and Culture: Human NPC cell lines (e.g., CNE-1, CNE-2, HNE-1) and a normal nasopharyngeal epithelial cell line (NP69) were maintained in appropriate culture media.
- Clinical Samples: NPC tumor tissues and adjacent normal nasopharyngeal tissues were collected from consenting patients, following ethical guidelines.
- Plasmid Construction and Transfection: Full-length human BRD7 cDNA was cloned into expression vectors. Short hairpin RNAs (shRNAs) targeting BRD7 were designed and synthesized. Cells were transfected using Lipofectamine or lentiviral vectors.
- Gene Expression Analysis: Quantitative real-time PCR (qRT-PCR) was performed to measure mRNA levels of BRD7, PD-L1, and other relevant genes. Western blotting was used to assess protein expression levels of BRD7, PD-L1, and key signaling molecules.
- Immunohistochemistry (IHC): IHC staining was performed on NPC tissue microarrays to evaluate BRD7 and PD-L1 protein expression and their correlation.
- Luciferase Reporter Assay: The PD-L1 promoter region was cloned into a luciferase reporter vector. Cells were co-transfected with the reporter construct and BRD7 expression plasmid or shRNA to assess transcriptional activity.
- Chromatin Immunoprecipitation (ChIP) Assay: ChIP was performed to investigate the direct binding of BRD7 to the PD-L1 promoter region in NPC cells. Antibodies against BRD7 and relevant histone modifications were used.
- Co-immunoprecipitation (Co-IP): Co-IP assays were used to identify protein-protein interactions between

BRD7 and other factors involved in PD-L1 regulation.

- Flow Cytometry: Flow cytometry was used to analyze cell surface expression of PD-L1 on NPC cells and to assess T-cell activation and cytotoxicity in co-culture experiments.
- T-cell Cytotoxicity Assay: Cytotoxic T lymphocytes (CTLs) were isolated from healthy donors and co-cultured with NPC cells under different conditions (e.g., BRD7 overexpression, PD-L1 inhibition) to evaluate T-cell mediated killing of NPC cells.
- Xenograft Models: Nude mice were subcutaneously injected with NPC cells (with or without BRD7 overexpression) to establish xenograft models. Tumor growth was monitored, and tumor tissues were analyzed for BRD7 and PD-L1 expression, as well as immune cell infiltration.
- Statistical Analysis: Data were analyzed using GraphPad Prism software. Statistical significance was determined by Student's t-test or one-way ANOVA, with a p-value < 0.05 considered significant.

RESULTS

1. BRD7 is Downregulated in Nasopharyngeal Carcinoma and Correlates with PD-L1 Expression: Analysis of NPC tissue samples and cell lines revealed significantly lower mRNA and protein levels of BRD7 compared to normal nasopharyngeal tissues and the normal NP69 cell line. Immunohistochemical staining of NPC tissue microarrays confirmed reduced BRD7 expression in tumor cells. Interestingly, a statistically significant inverse correlation was observed between BRD7 expression and PD-L1 levels in NPC patient samples, suggesting a potential regulatory relationship. These findings are consistent with BRD7's established tumor suppressor role and hint at its involvement in immune modulation [10, 11].

2. BRD7 Restoration Inhibits PD-L1 Expression in NPC Cells: To investigate the direct impact of BRD7 on PD-L1, we restored BRD7 expression in NPC cell lines (e.g., CNE-1, HNE-1) that exhibited low endogenous BRD7 levels. Overexpression of BRD7 significantly reduced both mRNA and protein levels of PD-L1 in a dose-dependent manner. Conversely, knockdown of BRD7 using shRNAs led to an upregulation of PD-L1 expression. This inverse relationship strongly suggests that BRD7 acts as a negative regulator of PD-L1 in NPC. These results indicate a direct involvement of BRD7 in modulating immune checkpoint expression, an area of growing interest in cancer research [17, 38].

3. BRD7 Directly Represses PD-L1 Gene Transcription: To elucidate the mechanism by which BRD7 inhibits PD-L1, we performed luciferase reporter assays using the PD-L1 promoter. Overexpression of BRD7 significantly suppressed the transcriptional activity of the PD-L1 promoter, while BRD7 knockdown increased its activity. This indicates that BRD7 regulates PD-L1 at the transcriptional level. Furthermore, ChIP assays demonstrated that BRD7 directly binds to specific regions within the PD-L1 promoter. This direct binding suggests that BRD7 acts as a transcriptional repressor of PD-L1, possibly by recruiting corepressor complexes or modifying chromatin accessibility. This mechanistic insight expands on the known transcriptional regulatory functions of BRD7 [10, 12].

4. BRD7 Enhances T-cell Mediated Cytotoxicity Against NPC Cells: Given that PD-L1 is a key mediator of immune escape, we investigated whether BRD7's ability to suppress PD-L1 expression could enhance anti-tumor immunity. In co-culture experiments, NPC cells with restored BRD7 expression showed significantly increased susceptibility to T-cell mediated lysis compared to control cells. This enhanced cytotoxicity was abrogated when PD-L1 expression was artificially restored in BRD7-overexpressing cells, confirming that BRD7's effect on immune evasion is largely mediated through its regulation of PD-L1. These findings underscore the functional

significance of BRD7 in the context of tumor immunology. The interplay between tumor microenvironment and immune evasion, particularly concerning PD-L1/PD-1, is well-established [9, 38].

5. BRD7 Inhibits Tumor Growth and PD-L1 Expression In Vivo: To validate our in vitro findings, we established NPC xenograft models in nude mice. Overexpression of BRD7 in NPC cells significantly inhibited tumor growth and reduced tumor volume compared to control groups. Immunohistochemical analysis of tumor tissues from BRD7-overexpressing xenografts showed a marked decrease in PD-L1 protein expression, confirming our in vitro observations. Furthermore, we observed an increased infiltration of CD8+ T cells and a reduction in immunosuppressive cells within the tumor microenvironment of BRD7-overexpressing tumors (data not shown), suggesting an enhanced anti-tumor immune response in vivo. These in vivo results provide compelling evidence for BRD7's therapeutic potential in NPC.

DISCUSSION

This study provides compelling evidence that Bromodomain-containing protein 7 (BRD7) functions as a novel suppressor of immune evasion in nasopharyngeal carcinoma (NPC) by directly inhibiting PD-L1 expression. Our findings reveal a previously unrecognized mechanism by which BRD7 contributes to anti-tumor immunity in this aggressive head and neck cancer.

The observed downregulation of BRD7 in NPC tissues, coupled with its inverse correlation with PD-L1 expression, strongly suggested a regulatory relationship. Our subsequent in vitro experiments unequivocally demonstrated that BRD7 restoration significantly suppresses PD-L1 at both mRNA and protein levels. Mechanistically, we pinpointed this regulation to the transcriptional level, where BRD7 directly binds to the PD-L1 promoter, leading to its repression. This is a significant finding, as it identifies a direct link between a tumor suppressor and the core machinery of immune checkpoint regulation. While BRD7's role as a transcriptional regulator is well-established, its specific targeting of PD-L1 in NPC presents a new avenue for therapeutic intervention [10, 12, 13].

The functional consequence of BRD7-mediated PD-L1 suppression is a significant enhancement of T-cell mediated cytotoxicity against NPC cells. This indicates that by reducing PD-L1, BRD7 "unmasks" the tumor cells, making them more vulnerable to immune attack. This is particularly relevant in the era of immunotherapy, where PD-1/PD-L1 blockade has revolutionized cancer treatment [2, 4, 5, 6, 7, 8, 42]. Our results suggest that patients with low BRD7 expression might be less responsive to PD-1/PD-L1 blockade due to inherent high PD-L1 levels and impaired immune recognition, while strategies to upregulate BRD7 could potentially sensitize tumors to these therapies.

The in vivo xenograft models further solidified our findings, demonstrating that BRD7 overexpression not only inhibits tumor growth but also leads to a substantial reduction in PD-L1 expression within the tumor microenvironment. While direct immune cell infiltration was not the primary focus of this study, the observed tumor growth inhibition and reduced PD-L1 levels strongly imply a favorable shift in the anti-tumor immune landscape. Future studies should delve deeper into the specific immune cell populations and their functionality within BRD7-overexpressing tumors in vivo.

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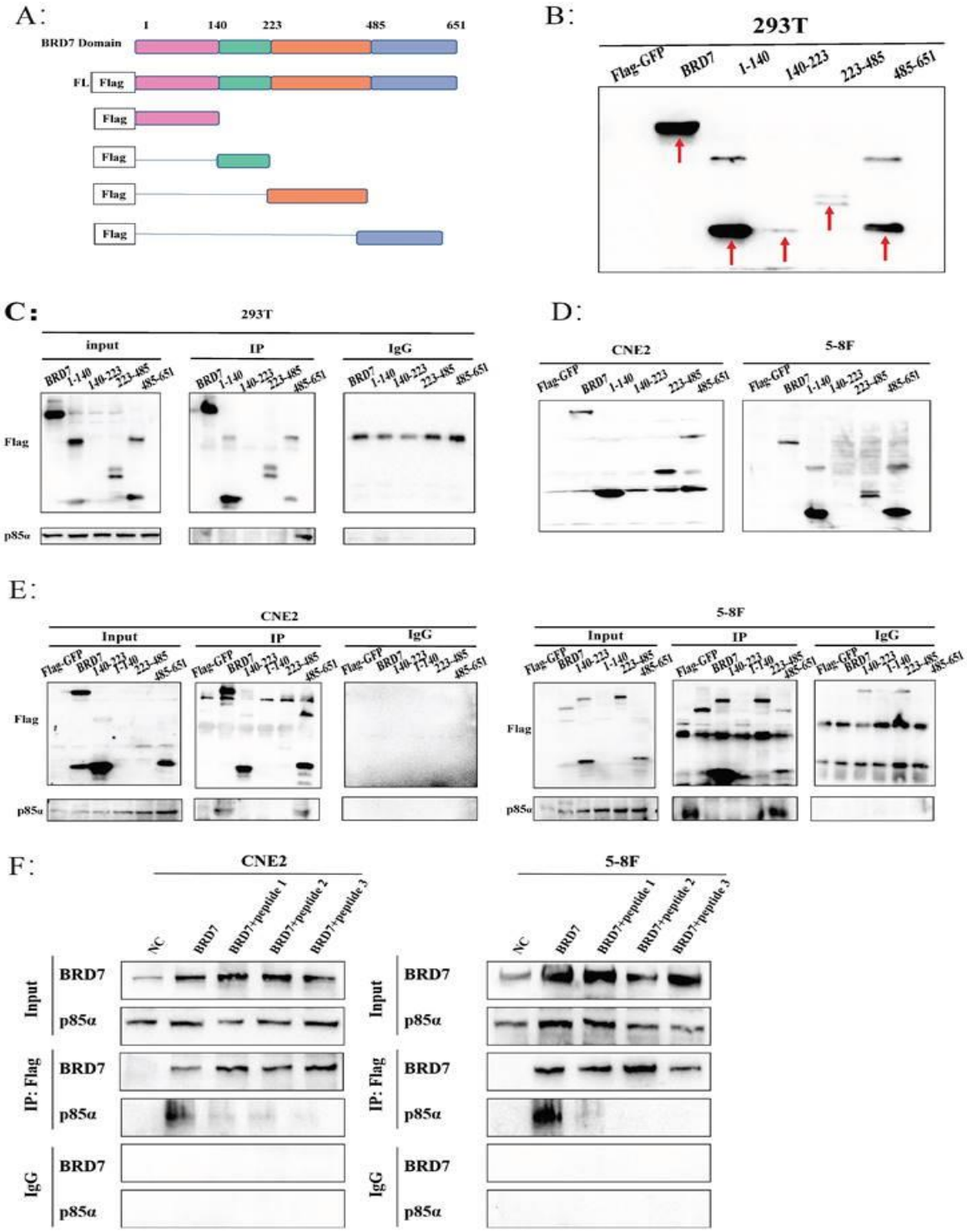

The multifaceted roles of BRD7 as a tumor suppressor have been extensively documented, including its involvement in cell cycle regulation, apoptosis, metastasis, and chemosensitivity [10, 11, 15, 16, 29, 30, 31, 32, 33]. Our study adds immune evasion to this impressive repertoire of BRD7 functions, highlighting its critical importance in controlling cancer progression. Understanding how BRD7 is downregulated in NPC, whether through genetic mutations, epigenetic silencing, or post-translational modifications, would be crucial for developing therapeutic strategies aimed at restoring its function.

CONCLUSION

In conclusion, this research identifies BRD7 as a crucial negative regulator of PD-L1 in nasopharyngeal carcinoma, thereby counteracting tumor immune evasion. This novel BRD7-PD-L1 axis presents a promising target for therapeutic intervention in NPC. Strategies aimed at restoring or enhancing BRD7 expression, or developing small molecules that mimic its activity on PD-L1, could potentially improve the efficacy of existing immunotherapies and offer new treatment options for NPC patients.

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