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Exploring the Anti-cancer Potential of the New BRD4 Inhibitor, OPT-0139, in Human Hepatic Carcinoma

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ABSTRACT

Objective: Bromodomain-containing protein 4 (BRD4) is integral to the regulation of cell growth and differentiation. Inhibition of BRD4 can impede the expression of its associated genes, thereby suppressing cancer cell growth. This study investigates the potential anti-cancer properties of OPT-0139, a novel inhibitor targeting BRD4.

Method: Human hepatic carcinoma cell lines (SK-Hep1 and Huh-7) were employed to explore the impact of OPT-0139 treatment, both singly and in combination with Sorafenib. Cell viability, proliferation, cell cycle arrest, and apoptotic cell death were assessed using MTT and ATP assays, flow cytometry, Annexin V assay, and caspase-3 activity assay. The expression of BRD4 and apoptosis-related molecules was evaluated via RT-PCR and Western blot. An in vivo experiment utilizing a mouse xenograft model analyzed tumor growth, weight, and mRNA levels.

Results: OPT-0139 significantly reduces cell viability and proliferation, inducing cell cycle arrest and apoptotic cell death. The mouse xenograft model demonstrates significant alterations in tumor growth and the expression of BRD4 and apoptosis-related proteins. Combined treatment with Sorafenib synergistically enhances apoptotic cell death and suppresses tumor growth, both in vitro and in vivo.

Conclusion: This investigation validates OPT-0139's efficacy and mechanism in human hepatic carcinoma cell lines. The study underscores OPT-0139's potential as a promising therapeutic agent for human hepatocellular carcinoma.

Keywords: BRD4 inhibitor, hepatocellular carcinoma, OPT-0139, anti-cancer potential

INTRODUCTION

Hepatocellular carcinoma (HCC) remains a global health concern, the third leading cause of worldwide cancer-related mortality.[1] Chronic infections by hepatitis B or C viruses are the predominant risk factors driving the prevalence of HCC.[2] While surgical interventions offer a potential cure for select cases of HCC, the disease often presents at advanced stages, constraining treatment options to palliative care measures.[3,4] Regrettably, the challenges of late-stage diagnosis pose a significant barrier to the efficacy of conventional chemotherapy such as Sorafenib (Nexavar).[5] Sorafenib, an FDA-endorsed treatment for advanced HCC, directs its focus towards distinct enzymes and receptors, encompassing VEGF receptor tyrosine kinases, RAF family serine/threonine kinases, and platelet-derived growth factor receptor.[6-10]

At the forefront of molecular protagonists, the BET family proteins, comprising BRD2, BRD3, BRD4, and BRDT, have emerged as pivotal players in cellular dynamics.[11,12] Each BET protein unveils a conserved architecture marked by tandem bromodomains (BD1 and BD2), encompassing approximately 110 amino acids with a specific affinity for acetylated lysines. These proteins also feature an extra-terminal (ET) domain facilitating protein-protein interactions.[13] Within this BET constellation, BRD4 has garnered pronounced attention in gastrointestinal (GI) cancers due to its heightened expression in cancerous tissues and cell lines, rendering it a promising subject of inquiry and a potential therapeutic target.[14] Elevated BRD4 expression significantly fuels GI cancer cells' growth,

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differentiation, and metastasis, correlating with adverse patient clinical outcomes.[15,16] By directly binding to oncogenic promoters, BRD4 drives their overexpression, thus fueling cancer progression.[17-20]

Additionally, BRD4's interaction with acetylated lysines on transcriptional factors such as Twist or Snail activates the epithelial-to-mesenchymal transition (EMT) process, fostering the survival and differentiation of EMT cells and consequently facilitating metastatic propagation in GI cancers.[21,22]

Steering therapeutic paradigms towards innovation, Bromodomain and extra-terminal (BET) protein inhibitors emerge as a compelling avenue for various cancers, including those afflicting the gastrointestinal domain.[14] These inhibitors adeptly engage BET proteins, impeding their interaction with acetylated lysines and yielding potent anti-cancer effects. Dysregulated transcriptional control, a consequence of BET protein upregulation, assumes a central role in driving tumor initiation and progression.[23] The anti-cancer potential of BET inhibitors hinges upon their capacity to suppress BET protein expression and inactivate their function. A cadre of BET inhibitors targeting BET bromo-domains (BD) is undergoing clinical exploration, complemented by preclinical insights supporting their applicability in GI cancer treatment.[14]

This preclinical study aims to investigate the potential of BRD4 inhibition using a new molecule, OPT-0139, as a prospective therapeutic approach for liver cancer, either as a monotherapy or combined with Sorafenib. By meticulously evaluating BRD4 inhibition's impact on hepatic carcinoma cells, we aspire to unearth the underlying molecular mechanisms and ascertain the therapeutic potency of BRD4 targeting within the intricate landscape of hepatocellular carcinoma.

MATERIALS AND METHODS

Cell Lines and Drug Acquisition

Human liver cancer cell lines (HepG2, Huh-7, and SK-Hep1) were procured from the American Type Culture Collection (VA, USA). An immortalized normal human ovarian surface epithelial cell line (HOSEpic) was also obtained from ScienCell Research Laboratories, Inc. (Carlsbad, CA, USA). The novel BRD4 inhibitor, OPT-0139, was acquired from JBKLAB (Seongnam, Korea). At the same time, Sorafenib was purchased from Sigma-Aldrich (St. Louis, MO, USA). Sorafenib was solubilized in dimethyl sulfoxide (DMSO) for in vitro experiments and 0.9% saline for in vivo studies.

Cell Viability and Proliferation Assays

Cell viability was assessed using PrestoBlue Cell Viability Reagent (Invitrogen, Carlsbad, CA, USA). Cells were seeded in 96-well plates with 10% FBS and 1% penicillin-streptomycin-supplemented media and incubated overnight at 37°C with 5% CO2. Following overnight incubation, OPT-0139 at concentrations ranging from 0.1 to 10 μM was administered for 48 hours in a media volume of 100 μL. The final DMSO concentration did not exceed 0.1%. Subsequently, the cells were exposed to 10% PrestoBlue in darkness for one hour, and absorbance was measured at 540 nm.

The Cell Counting Kit-8 (CCK-8) Reagent (Dojindo Laboratories Co. Ltd., Tokyo, Japan) was utilized for cell proliferation assessment. Cells were seeded at 1×10^4 cells/well density in 96-well plates and treated with varying concentrations of OPT-0139 (0.1, 0.5, 1,5 and 10 μM) for 48 hours. The CCK-8 assay was conducted 48 hours post-treatment, replacing the medium with serum-free medium and adding 10 μL of CCK-8. After incubation at 37°C and 5% CO2 for one hour, absorbance was measured at 450 nm. All measurements were performed in triplicate.

Immunofluorescence Staining, Caspase-3 Activity, Annexin V-FITC, and Propidium Iodide Apoptosis Detection Assay

Immunofluorescence staining was performed by seeding cells at 5×10^4 cells/well on poly-L-lysine-coated cover glass in a 6-well plate. The fixed and permeabilized cells were blocked with 1% bovine serum albumin and incubated with an anti-BRD4 monoclonal antibody. Alexa Fluor Plus 488-conjugated Goat anti-rabbit immunoglobulin G. MitoTracker Orange was used to stain mitochondria. The samples were observed and photographed using a fluorescence microscope.

For Caspase-3 activity assessment, cells were seeded at 1×10^4 cells/well in white-walled 96-well plates and treated with varying concentrations of OPT-0139 (0.1, 0.5, 1, 5 and 10 μM) for 48 hours. Caspase-Glo 3/7 Reagent was added, and luminescence was measured after a 30-minute incubation.

Apoptosis detection was carried out using the Annexin V-FITC/PI apoptosis detection assay kit (Invitrogen). Cells were seeded in 6-well plates at 5×10^5 cells/well, treated with OPT-0139 (0.1, 0.5, 1, 5 and 10 μM) for 48 hours, harvested, stained, and analyzed by flow cytometry (FACSCalibur, BD Bioscience, CA, USA).

Cell Cycle Arrest Analysis

Cell cycle arrest was assessed by fixing treated cells with 70% ethanol at -20°C overnight, followed by staining with FxCycle™PI/RNase Staining Solution (Invitrogen). The samples were analyzed using a FACSCalibur flow cytometer.

Cell-Derived Mouse Tumor Xenograft Model

Forty BALB/c nude female mice (7 weeks old) were obtained from ORIENT BIO Inc. (Seongnam, Korea). The mice were housed under specific conditions, and Huh-7 cells were subcutaneously injected into the right flanks to establish tumors. Mice were divided into three groups (n = 10 each): tumor control, low-dose OPT-0139 (5 mg/kg), and high-dose OPT-0139 (20 mg/kg). Tumor areas were measured, and OPT-0139 was administered intravenously. Body weight was monitored, and the mice were euthanized after four weeks.

Immunohistochemical Staining

Tumor tissues from the xenograft model were subjected to immunohistochemical staining. Tissue sections were processed and incubated with primary antibodies, followed by appropriate secondary treatments. Visualization and analysis were performed.

Quantitative Real-Time PCR Analysis

Total RNA was extracted from tumor samples using TRIzol reagent (Invitrogen). cDNA synthesis and real-time PCR were conducted using the SYBR Green master mix (BioRad) and specific primers for target genes. Gene expression levels were normalized to GAPDH.

Protein Preparation and Western Blot

Cells treated with drugs were lysed, and total cell extracts were subjected to SDS-PAGE and transferred to PVDF membranes. Membranes were probed with primary antibodies, and signals were visualized using chemiluminescence. β-actin was used for normalization.

Data Analysis

Statistical analyses were performed using GraphPad Prism version 9.4.1 for Windows. Significant values were considered at $p < 0.05$, and more significant values were considered at $p < 0.01$, compared with the control.

Ethics Statement

All animal experiments were conducted under the guidelines of the Seoul National University Bundang Hospital Institutional Animal Care and Use Committee (IACUC) (Approval No. BA-2111-332-010-02, approval date: Nov 26, 2021).

RESULTS

1. BRD4 Inhibitor (OPT-0139) as a Therapeutic Target in Liver Cancer

The study unveiled higher BRD4 expression levels in liver cancer cell lines (Huh-7, SK-Hep1 and HepG2) compared to the non-tumorigenic HOSEpic cell line. Remarkably, SK-Hep1 and Huh-7 exhibited elevated BRD4 expression compared to HepG2 (Fig. 1), implying the potential of BRD4 as a therapeutic target in liver cancer.

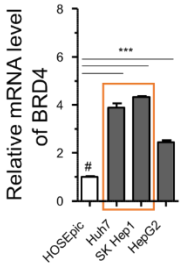

Fig. 1. BRD4 Expression Levels in Liver Cancer Cell Lines. Relative expression levels of BRD4 in liver cancer cell lines (Huh-7, SK-Hep1 and HepG2) and the immortalized normal human ovarian surface epithelial cell line (HOSEpic) were assessed by Western blot analysis. The results emphasize a significant overexpression of BRD4 in liver cancer cell lines in comparison to HOSEpic.

2. BRD4 Inhibitor (OPT-0139) Suppress Cancer Survival and Induce Apoptotic Cell Death in Liver Cancer Cells

Cell viability and proliferation evaluation demonstrated a dose-dependent reduction in both parameters upon treatment with BRD4 inhibitor (OPT-0139) in SK-Hep1 and Huh-7 cell lines. The half-maximal inhibitory concentration (IC50) values of OPT-0139 were established using PrestoBlue assay (Fig. 2A and 2B). Subsequent analyses using Presto-Blue and CellTiter-

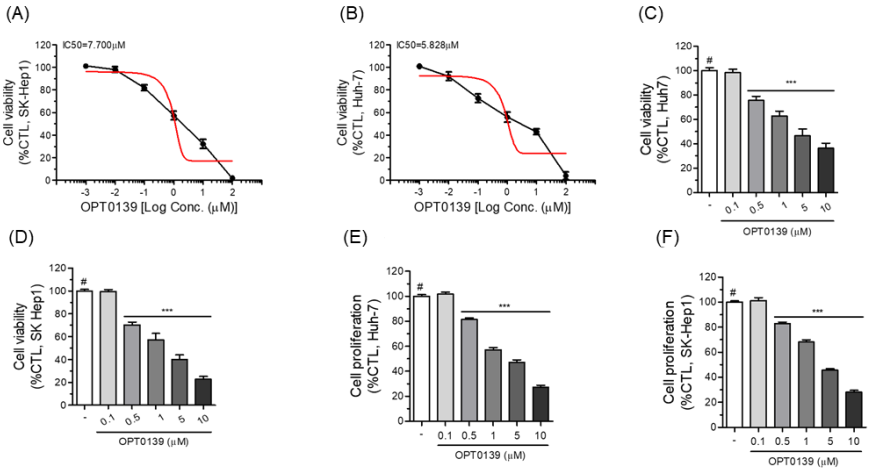

Fig. 2. Inhibition of Cell Survival by OPT-0139 in SK Hep1 and Huh-7 cells. Figurs 2A and 2B present the half-maximal inhibitory concentration (IC50) values of OPT-0139 in SK-Hep1 and Huh-7 cells, measuring 7.700 μM and 5.828 μM, respectively. Figures 2C and 2D show the inhibition of cell viability in Huh-7 and SK-Hep1 cells treated with varying concentrations of OPT-0139 (0.1–10 μM) for 48 hours. Cell viability was assessed using the PrestoBlue assay, revealing a dose-dependent decrease in proliferation. Figures 2E and 2F further illustrate the suppression of cell proliferation through analysis of ATP content in OPT-0139-treated Huh-7 and SK-Hep1 cells using the CellTiter-Glo assay.

Glo assays confirmed dose-dependent inhibition of cell viability (Fig. 2C and 2D) and cell proliferation (Fig. 2E and 2F). Annexin V and Caspase-3 assays revealed increased apoptotic activity and caspase-3 activation upon OPT-0139 treatment (Fig. 3A-D). Additionally, OPT-0139 caused a dose-dependent decreased in BRD4 and BCL-2 and a concomitant increase in C-C3 and C-P expression (Fig. 3E and 3F), underscoring its potential to inhibit cell survival and induce apoptosis in liver cancer cells.

3. BRD4 Inhibitor (OPT-0139) Induces Cell Cycle Arrest and Apoptosis in Liver Cancer Cells

Flow cytometry analysis demonstrated that OPT-0139 treatment led to cell cycle arrest in the G1 phase and an increase in the G2/M phase in SK-Hep1 and Huh-7 cells, indicating its ability to impede cell cycle progression (Fig. 4A and 4B). This finding underscores the role of OPT-0139 in inhibiting liver cancer cell growth and survival through cell cycle arrest and apoptosis induction.

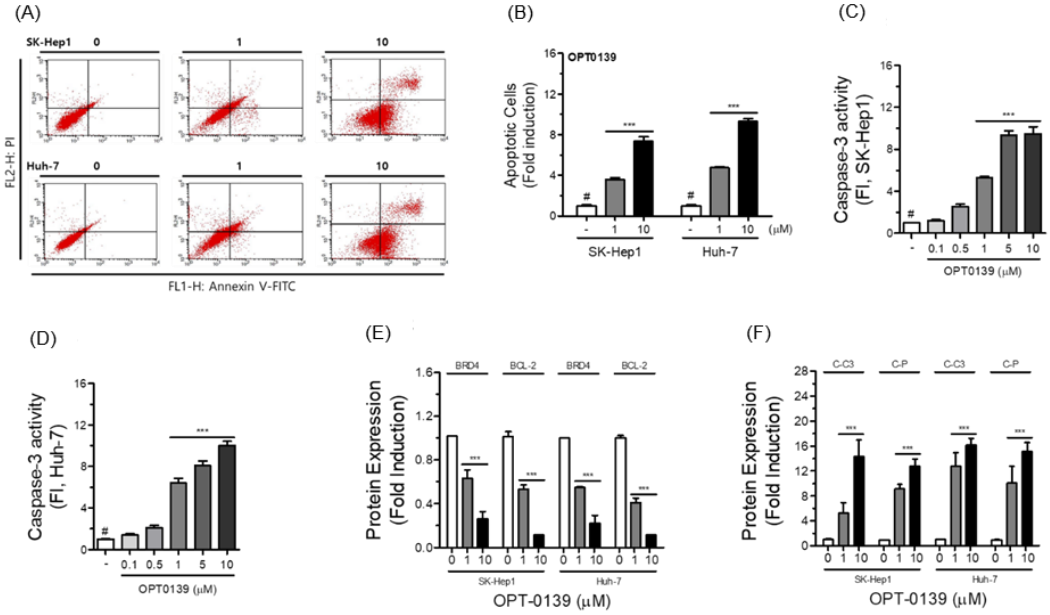

Fig. 3. Inhibition of Cell Survival and Induction of Apoptotic Cell Death by OPT-0139 in SK-Hep1 and Huh-7 Cells. Figure 3A presents representative FACS analysis demonstrating apoptosis induction in SK-Hep1 and Huh-7 cells treated with OPT-0139, along with positive controls. Annexin V and PI double-staining was performed, followed by flow cytometry for quantitative assessment. Figure 3B, flow cytometric analysis of Annexin V-FITC/PI-stained cells determines apoptotic rates in SK-Hep1 and Huh-7 cells under varying OPT-0139 concentrations. Figures 3C and 3D illustrate caspase-3 activity in OPT-0139-treated SK-Hep1 and Huh-7 cells, measured using the Caspase-Glo 3/7 assay. There is a notable dose-dependent increase in apoptosis and caspase-3 activity. Figures 3E and 3F exhibit Western blot analysis of BRD4, BCL-2, cleaved caspase-3 (C-C3), and cleaved PARP (C-P) expression in OPT-0139-treated SK-Hep1 and Huh-7 cells, providing insights into its anti-cancer effects.

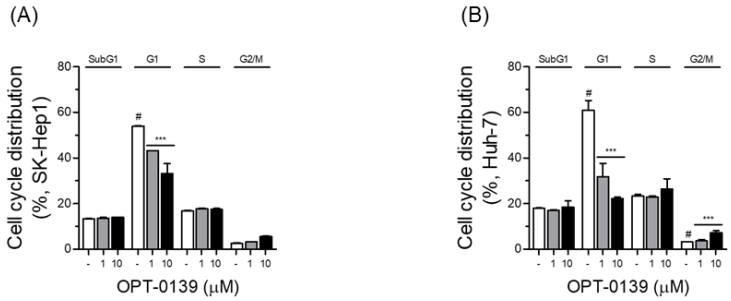

Fig. 4. Cell Cycle Arrest of SK-Hep1 and Huh-7 Cells by OPT-0139. Figures 4A and 4B present DNA content percentages in each cell cycle phase for SK-Hep1 and Huh-7 cells treated with OPT-0139. The data, represented as means ± S.E.M compared to vehicle control, reveal a decrease in G1 phase and an increase in G2/M phase populations. This indicates cell cycle arrest induced by OPT-0139, as highlighted by the statistical significance (***P < 0.001). No significant changes in the S phase percentage are observed. These findings elucidate OPT-0139's capacity to induce apoptosis and cell cycle arrest.

4. Combination of OPT-0139 and Sorafenib Therapy Reduces Cancer Cell Survival and Promotes Apoptotic Cell Death in Liver Cancer Cells

Co-treatment of SK-Hep1 and Huh-7 cells with OPT-0139 and Sorafenib exhibited an enhanced reduction in cell viability and increased apoptosis compared to individual treatments (Fig. 5A-E). This combination demonstrated synergistic effects in decreasing cancer cell survival and inducing apoptotic cell death.

5. BRD4 Inhibitor (OPT-0139) Inhibit Tumor Growth in Liver Cancer Cell Mouse Models

In vivo, experimentation using xenograft models with Huh-7 cells demonstrated that OPT-0139 treatment resulted in smaller tumor volumes compared to the control group (Fig. 6A). Two-way ANOVA analysis revealed significant inhibition of liver cancer (LC) xenograft growth by OPT-0139 (Fig. 6B) without observed signs of toxicity throughout the treatment (Fig. 6C and 6D).

6. Down-regulation of Expression of Target Genes by BRD4 Inhibitor (OPT-0139) in LC-Derived Mouse Xenografts

OPT-0139 treatment exhibited dose-dependent downregulation of target genes such as BRD4, HIF-1α, VEGF-A, Nanog, Oct-4, and Bcl-2 in LC-derived mouse xenografts (Fig. 7), indicating its potential to modulate key molecules associated with cancer progression.

7. BRD4 Inhibitor OPT-0139 in Combination with Sorafenib Inhibits Tumor Growth in LC Cell Mouse Models

Combination treatment of OPT-0139 with Sorafenib led to reduced tumor volumes and weights compared to control and single-agent-treated groups (Fig. 8A-C). No signs of toxicity were observed during treatment (Fig. 8D and 8E), highlighting the potential of combining OPT-0139 with Sorafenib to inhibit tumor growth in liver cancer.

These results collectively underscore the promising therapeutic potential of BRD4 inhibitor (OPT-0139) in liver cancer treatment, offering insights into their ability to suppress cancer survival, induce apoptotic cell death, impede cell cycle progression, and synergize with Sorafenib for enhanced anti-tumor effects. These findings contribute to the growing understanding of BRD4 inhibition as a novel approach in liver cancer therapy.

DISCUSSION

The results presented in this study provide significant insights into the potential therapeutic role of BRD4 inhibitor OPT-0139 in treating liver cancer. The comprehensive experimental approach encompassing both in vitro and in vivo analyses sheds light on the mechanisms underlying the inhibitory effects of OPT-0139 on liver cancer cell survival, proliferation, and tumor growth, as a single agent and in combination with Sorafenib. The discussion below elaborates on the implications and significance of each set of results.

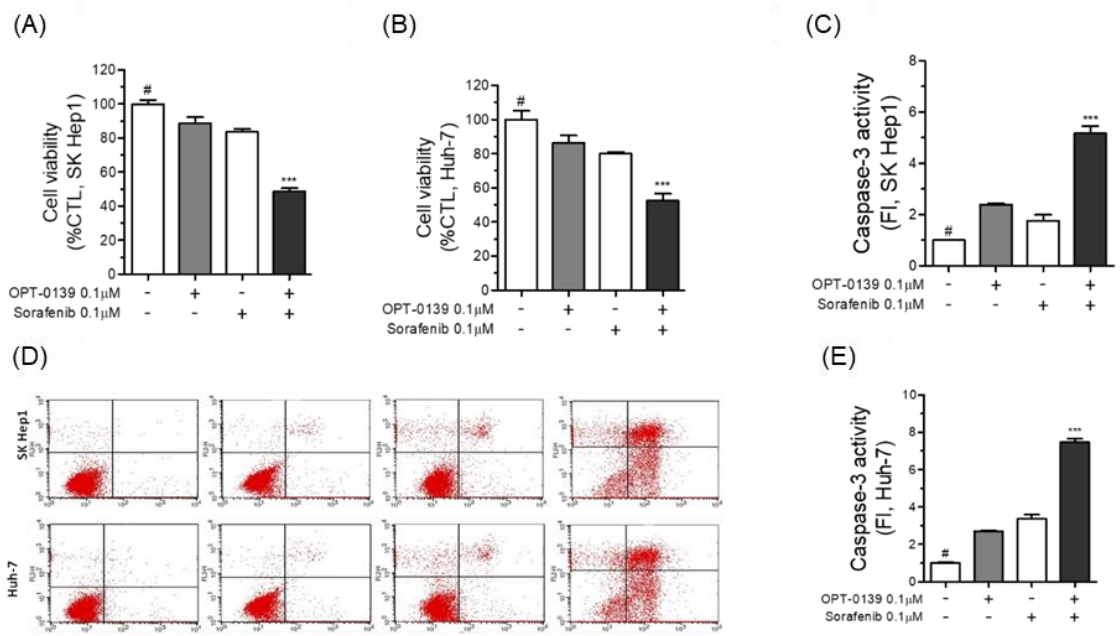

Fig. 5. Effect of Combination Therapy using OPT-0139 and Sorafenib on SK-Hep1 and Huh-7 Cell Survival and Apoptotic Cell Death. Figures 5A and 5B underscore decreased cell viability in SK-Hep1 and Huh-7 cells treated with Sorafenib and OPT-0139 in combination, evaluated through the PrestoBlue and CellTiter-Glo assays. Figure 5C, representative FACS analysis illustrates apoptosis induction by OPT-0139 and Sorafenib combination therapy. Figures 5D and 5E highlight increased caspase-3 activity in combination treatment compared to individual treatments with OPT-0139 or Sorafenib alone.

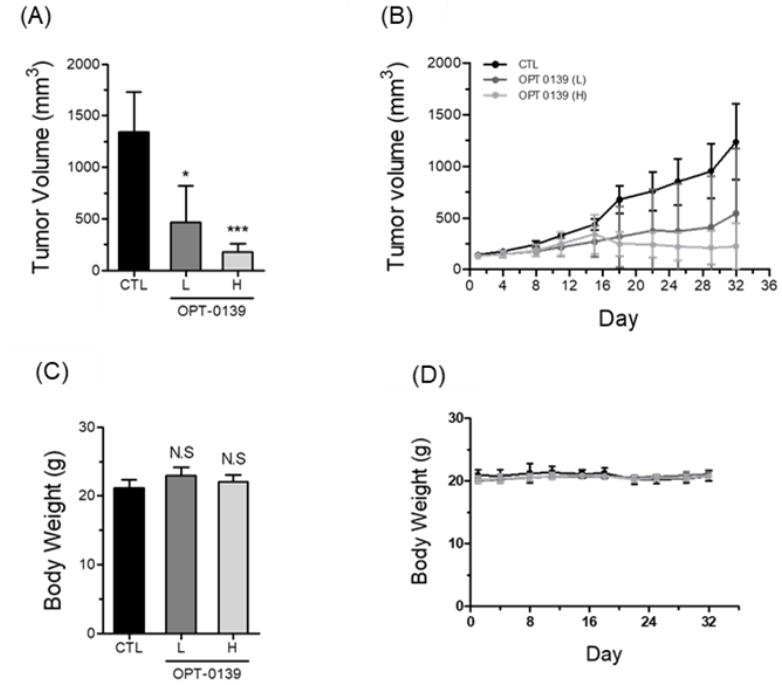

Fig. 6. OPT-0139 Low and High Doses Inhibit Tumor Growth in Liver Cancer Xenograft Mouse Models. Figure 6A displays individual tumor volumes with statistical analysis using a t-test (*P < 0.05, ***P < 0.001), revealing smaller tumor volumes in OPT-0139-treated groups. Figure 6B shows changes in tumor volume over time, indicating smaller average tumor volumes in the high and low OPT-0139 doses on day 14. Figures 6C and 6D depict body weight changes during treatment, indicating no noticeable toxicity. These results demonstrate the inhibition of tumor growth by both low and high doses of OPT-0139 in liver cancer xenograft mouse models.

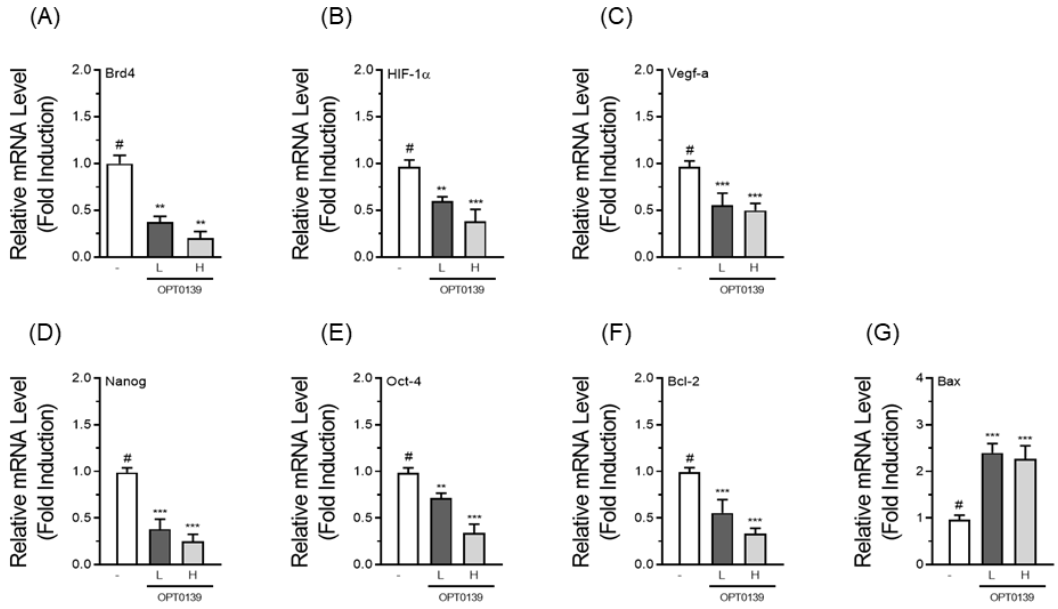

Fig. 7. Down-Regulation of Target Gene Expression by BRD4 Inhibitor OPT-0139. Figure 7 presents mRNA expression of target genes (BRD4, HIF-1α, VEGF-A, Nanog, Oct-4, Bcl-2, Bax) treated with low and high doses of BRD4 inhibitor OPT-0139. The relative mRNA expression, normalized with GAPDH, displays significant down-regulation of target genes by OPT-0139 treatment (**P < 0.01, ***P < 0.01). These findings indicate the potential therapeutic role of BRD4 inhibitor in modulating gene expression in liver cancer treatment.

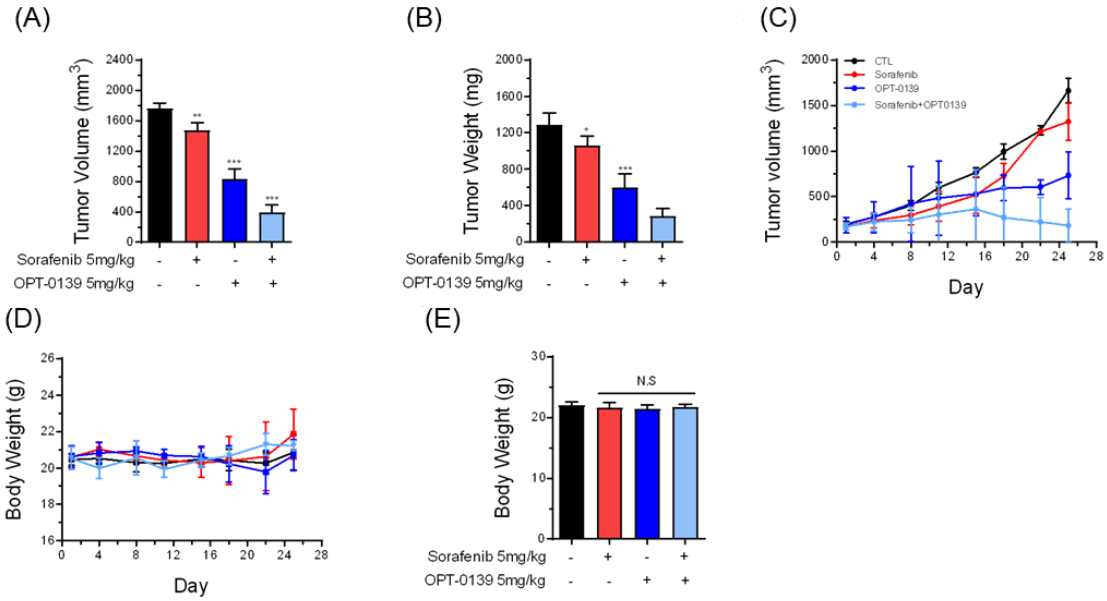

Fig. 8. Inhibition of Tumor Growth by OPT-0139 in Combination with Sorafenib. Figures 8A and 8B exhibit individual tumor volumes and weights with statistical analysis using a t-test (*p < 0.05, **p < 0.01, ***p < 0.001), revealing smaller tumor volume and weight in the OPT-0139 and Sorafenib combination-treated group. Figure 8C presents changes in tumor volume over time), indicating smaller average tumor volumes in the combination-treated group on day 14. Figures 8D and 8E illustrate body weight changes during treatment, suggesting no noticeable toxicity. These findings underscore the potential therapeutic value of combining OPT-0139 with Sorafenib in inhibiting tumor growth.

Therapeutic Target Potential of BRD4 Inhibitor in Liver Cancer

The elevated BRD4 expression in liver cancer cell lines compared to non-tumorigenic cells underscores the potential significance of BRD4 as a therapeutic target in liver cancer. This finding aligns with existing literature that highlights the pivotal role of BRD4 in promoting cancer cell growth and survival through the regulation of gene transcription and cell cycle progression.[15,24] The increased expression of BRD4 in liver cancer cells suggests its involvement in driving tumorigenesis. It provides a rationale for targeting this molecule to curtail cancer progression.

The dose-dependent inhibition of cell viability and proliferation by OPT-0139 in liver cancer cell lines corroborates the potential cytotoxic effects of BRD4 inhibition. The substantial increase in apoptotic cell death, as indicated by Annexin V binding and Caspase-3 activation, further substantiates the proapoptotic role of OPT-0139; these findings are consistent with previous studies on other BRD4 inhibitors in HCC cells,[18,19,24,25] which supports the validity and consistency of our study, and suggest that OPT-0139 may function as an inducer of apoptotic cell death in liver cancer cells, which is a critical therapeutic outcome in cancer treatment.

Induction of Cell Cycle Arrest and Apoptosis by BRD4 Inhibitor

The perturbation of the cell cycle and the induction of apoptosis by OPT-0139 provide mechanistic insights into its anti-cancer effects. The altered cell cycle distribution, characterized by decreased G1 phase and increased G2/M phase populations, suggests that OPT-0139 could impede cell cycle progression, possibly interfering with key cell cycle regulators. The increased Caspase-3 activity and Annexin V binding further supports the induction of apoptosis by OPT-0139, signifying its potential to trigger programmed cell death pathways. These results suggest that the anti-proliferative effects of OPT-0139 on liver cancer cell lines are mediated through G1 arrest and apoptosis. The apoptotic cell death observed was attributed to the decreased expression of BRD4 and BCL-2, along with increased expression of C-C3 and C-P in SK-Hep1 and Huh-7 cells, which indicates that OPT-0139 exerts its effects on the apoptosis pathway in liver cancer cells.

Enhanced Effects of OPT-0139 and Sorafenib Combination Therapy

The synergistic reduction in cell viability and enhancement of apoptotic cell death observed with the combination of OPT-0139 and Sorafenib underscore the potential of multi-modal therapeutic strategies in liver cancer treatment. Sorafenib, a standard chemotherapeutic drug, is known for its anti-angiogenic and anti-proliferative effects; its mode of action involves inhibition of crucial enzymes and receptors, including vascular endothelial growth factor (VEGF) receptor tyrosine kinases (KDR and FLT4), RAF family serine/threonine kinases (c-Raf and B-Raf), and platelet-derived growth factor receptor (PDGFR).[6-9] Based on the distinct action pathways for OPT-0139 and Sorafenib as anti-tumor agents, the conceptual framework for investigating their combination in HCC treatment emerged from the different pathways.[8,9,19,25]

Inhibition of Tumor Growth by BRD4 Inhibitor in Liver Cancer Cell Mouse Models

The in vivo mouse model experiments demonstrate the potential of OPT-0139 to inhibit tumor growth in a living organism. The significant reduction in tumor volume and delayed growth kinetics in response to OPT-0139 treatment reinforces its therapeutic potential. Notably, the absence of apparent treatment-related toxicity supports the safety profile of OPT-0139, a crucial consideration for clinical translation.

Down-Regulation of Target Genes Expression by BRD4 Inhibitor

The down-regulation of target gene expression, including BRD4 itself, VEGF-A, HIF-1α, Oct-4, Nanog and Bcl-2 in response to OPT-0139 treatment further elucidates its mechanism of action. This observation suggests that OPT-0139 may exert its anti-cancer effects by modulating key proteins involved in cell survival, angiogenesis, and pluripotency. These molecular insights enhance our understanding of the cellular pathways impacted by BRD4 inhibition.

Combination of BRD4 Inhibitor and Sorafenib Inhibits Tumor Growth in xenograft mouse models

The promising outcome of combining OPT-0139 with Sorafenib in inhibiting tumor growth in the xenograft mouse models underscores the translational potential of this research. Combination therapies targeting multiple pathways have shown increased efficacy and are of great interest in cancer treatment. The synergy between OPT-0139 and Sorafenib suggests a novel approach for improving therapeutic outcomes in liver cancer patients.[26,27]

The findings of this study are consistent with previous research that has shown the potential of BRD4 inhibitors as a therapeutic target for various cancers, including liver cancer.[4,12-14] The study's results support the notion that targeting BRD4 could be a viable treatment strategy for liver cancer, and further research could be conducted to validate these findings. Additionally, combining BRD4 inhibitors with Sorafenib could be explored as a potential therapy for liver cancer.

The study demonstrates notable strengths through its comprehensive assessment of the anti-cancer effects of the inhibitor across various assays, including MTT and ATP assays, flow cytometry, Annexin V assay, Caspase-3 activity assay, RT-PCR, and Western blot analysis. Moreover, the utilization of a mouse xenograft model in an in vivo experiment substantiates the inhibitor's anti-tumor potential, substantiated by reductions in tumor growth, tumor weight, and mRNA levels.

Nevertheless, certain limitations warrant consideration. Firstly, the investigation solely addresses the anti-cancer potential of the inhibitor within two human liver cancer cell lines, necessitating further exploration within diverse cell lines and primary tumor specimens. Additionally, the study overlooks exploring the potential adverse effects of the inhibitor, mandating further dedicated inquiry.

Overall, the study's findings underscore the promise of BRD4 inhibitors, both as standalone agents and in conjunction with Sorafenib, for effectively restraining the proliferation of liver cancer cells. These outcomes offer valuable insights into the efficacy of BRD4 inhibition in liver cancer, potentially heralding forthcoming clinical trials. Ultimately, this research is a stepping stone toward developing more efficacious treatment avenues for individuals afflicted with hepatocellular carcinoma.

In conclusion, this study contributes significantly to our understanding of the potential of BRD4 inhibitors, particularly OPT-0139, as a promising therapeutic avenue for liver cancer treatment. The findings provide compelling evidence of its ability to inhibit cancer cell survival, induce apoptosis, and suppress tumor growth. The mechanistic insights from this research may pave the way for developing novel and more effective therapeutic strategies for liver cancer patients, offering hope for improved clinical outcomes. Interpret results, compare with previous studies, address limitations, and discuss academic implications.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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