Tumor Suppressive Effects of Bromodomain-Containing Protein 7 (BRD7) in Epithelial Ovarian Carcinoma

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Abstract

Purpose: Bromodomain-containing protein 7 (BRD7), which is a subunit of SWI/SNF complex, has been recently suggested as a novel tumor suppressor in several cancers. In this study, we investigated the tumor suppressive effect of BRD7 in epithelial ovarian cancer.

Experimental Design: We analyzed the expression of BRD7 in human ovarian tissues with real-time PCR. To investigate the functional role of BRD7, we transfected ovarian cancer cells (A2780 and SKOV3) with BRD7 plasmid and checked the cell viability, apoptosis, and invasion. The activities of BRD7 in the signaling pathways associated with carcinogenesis were also tested. In addition, we used the orthotopic mouse model for ovarian cancer to evaluate tumor growth-inhibiting effect by administration of BRD7 plasmid.

Results: The BRD7 expression was downregulated in the ovarian cancer tissues compared with normal tissue (P < 0.05). High-grade serous cancer exhibited significantly decreased expression of BRD7 compared with low-grade (P < 0.01) serous cancer. Transfection of BRD7 plasmid to A2780 (p53-wild) or SKOV3 (p53-null) ovarian cancer cells showed the tumor suppressive effects assessed by cell viability, apoptosis, and invasion assay and especially significantly decreased tumor weight in orthotopic mouse model (A2780). Moreover, we found that tumor suppressive effects of BRD7 are independent to the presence of p53 activity in ovarian cancer cells. BRD7 negatively regulated β-catenin pathway, resulting in decreased its accumulation in the nucleus.

Conclusions: These results suggested that BRD7 acts as a tumor suppressor in epithelial ovarian cancers independently of p53 activity, via negative regulation of β-catenin pathway. Clin Cancer Res; 20(3); 565–75.

Introduction

Ovarian carcinoma is the fifth leading cause of cancer-related death among females worldwide (1, 2). Functional inactivation of the p53 tumor suppressor protein, expression of the BRCA1/BRCA2 phenotype, and upregulation of several growth-regulatory genes that activate the ras-raf-MEK-ERK, β-catenin, or PI3K pathways have been suggested as potential mechanisms of ovarian carcinogenesis (3–5). Among these, mutation of p53 is most frequently thought to be the primary cellular event leading to the development of ovarian cancer (6–8).

Bromodomain-containing protein 7 (BRD7, chromosome 16q12) is a subunit of the SWI/SNF complex specific for polybromo BRG1-associated factor (PBAF; ref. 9). Several previous studies have described a tumor suppressor role for BRD7 (10–13). BRD7 expression was shown to be reduced in several subsets of cancer relative to that of individually matched normal cells (11, 12). In nasopharyngeal cancer cells, BRD7 reduced the activity of both ras-raf-MEK-ERK and Rb/E2F signaling, and also inhibited the β-catenin pathway (12). Our laboratory previously investigated the tumor suppressive role of BRD7 in endometrial cancer cells (14). However, the detailed mechanism of BRD7’s tumor suppressive role in ovarian cancer remains to be elucidated.

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suppressive effects remains unclear in the case of epithelial ovarian cancer.

In this study, we investigated the tumor suppressive effects of BRD7 in ovarian cancer. We found that transfection of the BRD7 plasmid into ovarian cancer cells induced tumor suppressive effects in vitro and significantly decreased tumor weights in the orthotopic mouse model (A2780). Moreover, we found that tumor suppressive effects of BRD7 are independent of the presence of p53 activity in ovarian cancer cells. BRD7 negatively regulated β-catenin pathway resulting in decreased its accumulation in the nucleus. These results suggested that BRD7 acts as a tumor suppressor in epithelial ovarian cancers independently of p53 activity, via negative regulation of β-catenin pathway.

**Materials and Methods**

**Patients and tissue specimens**

Study subjects were recruited from the Department of Obstetrics and Gynecology at the Samsung Medical Center, Sungkyunkwan University School of Medicine in Seoul, Korea, between 2003 and 2010. A total of 41 tissue specimens were collected including 11 samples of normal ovarian epithelium, 7 low-grade (grade 1/3), and 34 high-grade (grade 3/3) serous ovarian cancer tissue samples. Tumor tissue specimens were obtained intraoperatively from women with epithelial ovarian carcinoma. Normal ovarian epithelium specimens were collected from women who underwent hysterectomy for benign disease. All specimens were immediately snap frozen at −80°C. The specimens were stained with hematoxylin and eosin and evaluated by a single gynecologic pathologist. If the specimen had greater than 90% tumor cells, it was used in the analysis. Histo-sing followed the International Federation of Gynecology World Health Organization criteria, whereas surgical staging followed the International Federation of Gynecology and Obstetrics standards. The Institutional Review Board at our hospital approved this study (IRB #2011-04-008-002), and informed consent was obtained from all patients between 2003 and 2010.

**RNA extraction, real-time PCR, and semiquantitative PCR**

Total RNA including mRNA was extracted from the tissue using mirVana miRNA Isolation Kit (Ambion). cDNA was synthesized from total RNA using oligo dT primer (Invitrogen) according to the TaqMan RNA Assay protocol (PE Applied Biosystems). Real-time PCR was performed using an Applied Biosystems 7900HT Sequence Detection system (Applied Biosystems) according to the manufacturer’s protocol. Data normalizations were performed using GAPDH. To avoid amplification of genomic DNA, the primers and probes for amplifying BRD7 and GAPDH were chosen to hybridize at the junction between the 2 exons as follows: BRD7 (#4351732; Applied Biosystems) and GAPDH (#4310884E; Applied Biosystems). Relative quantification of BRD7 expression was calculated using the 2−ΔΔCt method. For semiquantitative PCR, we designed primers specific to the individual genes. The sequences of the primers were as follows: BRD7, sense 5′-TGAAGCCTTCTCAACAGCT-3′; antisense 5′-CCCGGGCCCATCCTCACCA-3′; p53, sense 5′-CCGGAGGCCCATCCTCACCA-3′; antisense 5′-TGG CTG AGATGACCC CTG CT-3′; MMP2 sense 5′-CACCCTACCA AAAG GCCCTC-3′; antisense 5′-AAC ACCGCTTCCTCT- CCAG-3′; b-actin sense 5′-GATGCAGAAGGAGATCAG- TG-3′; antisense 5′-AGTCATAGTCCGCTAGAG-3′.

**Cell culture**

Ovarian carcinoma cell lines 2774, PA-1, OVCAR3, SKOV3, SW626 (colonic origin of ovarian cancer cell line), and Caov4 were obtained from the American Type Culture Collection (ATCC), and A2780 was obtained from the European Collection of Cell Cultures (ECACC). HeyA8 was a gift from Dr. Anil K. Sood (Department of Cancer Biology, University of Texas M.D. Anderson Cancer Center, TX). We were informed about the authentications of whole cell lines when obtained them by the described providers. We checked the certificates of cell lines that include the results of the tests (species verification and short tandem repeat DNA profiling assay) and procedures for authentication. More detailed information of the cell lines were stated in Supplementary Table S1.

OVCAR3, 2774, and SKOV3 cells were maintained in RPMI and PA-1 cells were maintained in MEM.SW626 and Caov4 cells were maintained in Leibovitz’s L-15 Medium. Cells were grown at 37°C in 5% CO2 and supplemented with 10% FBS, penicillin (100 units/ml), and streptomycin (100 μg/ml).

**Plasmid construct and siRNA**

We tested 2 plasmid constructs, #1 and #2, that includes full length of human BRD7. #1 and its parent vector,
pcDNA3.0 were kindly gifted from Fr. Giannino Del Sal (Laboratorio Nazionale CIB, Trieste, Italy) and #2 was obtained from ATCC (#10436864), and its parental vector that was used as mock control was made by recirculating the BRD7 construct. p53 siRNA 5’-CACUACAACUAUACUGUUGUGUA-3’, β-catenin siRNA 5’-CGUUCUCCAGAUGACUGUUG-3’, and AccuTarget Negative control siRNA were obtained from Bioneer (Bioneer). Cells were transfected with the described combination of the corresponding expression plasmid and/or siRNA in each experiment. After 36 hours, cell lysates were subjected to Western blot analysis to determine the protein levels for each gene. The Western blot results demonstrated that the corresponding plasmid and/or siRNA efficiently regulated the expression of each gene.

Transfection
Cells were transfected using the described combination of the corresponding expression plasmid and/or siRNA using lipofectamine (Invitrogen) according to the manufacturer’s protocol. For upregulation of BRD7 in vivo, we injected the BRD7 plasmid or mock vector into the xenografts using GenJet Plus transfection reagent (SignaGen Laboratories) according to the manufacturer’s protocol. We used high-quality plasmid DNA (A260/A280 ratio of 1.8 or higher). We transfected 10 μg of plasmid via intraperitoneal injection once weekly for 1 month beginning 7 days after cancer cell injection.

MTT assay
Cells were seeded in 96-well plates at a density of 5,000 cells/well for 24 hours. Cells were then transfected with the corresponding expression plasmid and/or siRNA as indicated for 36 hours. MTT assays were performed as previously described (15). Values are expressed as means of three independent experiments ± SD.

Apoptosis assay
Cells were transfected with the corresponding expression plasmid and siRNA as indicated for 12 hours and then treated with etoposide (Sigma-Aldrich) for 24 hours for the induction of apoptosis. Because SKOV3 is p53 null cell line, we estimated apoptosis by measuring activated caspase-3 on an ELISA assay (Invitrogen), which is a common final mediator of apoptosis in both p53-dependent and independent pathways. The apoptosis for each subset were determined as previously described (16). Values are expressed as means of 3 independent experiments ± SD.

Flow cytometry (FACS) and sorting
For the FACS assay, cells were seeded in 175 T flasks for 24 hours and then starved in a serum-free medium for 24 hours. Cells were trypsinized for dissociation into a single cell in potassium-buffered saline (PBS), pH 7.5. Cells suspensions were rinsed twice with ice-cold PBS and added dropwise ice-cold 70% ethanol for 12 hours at 4°C. After removing ethanol, cells were treated with RNase A (1 μg/mL) followed by addition of propidium iodide (Sigma; 0.5 mg/mL) for 30 minutes. Cells were analyzed with a FACSort flow cytometer (BD Bioscience). Data were processed using CellQuest (BD Bioscience) and ModFit (www.Vsh.com) and presented by using FCSPress 1.4 (http://www.fcspress.com/).

Preparation of cellular extracts and Western blot
Cells were lysed using a lysis buffer (INTRON BIO Technology). To avoid degradation and dephosphorylation of proteins, a protease and phosphatase inhibitor cocktail (Sigma-Aldrich) was added. Extraction of cytoplasm and nuclear lysates from whole cells was performed using NE-PER Nuclear and Cytoplasm Extraction Reagent Kit (Pierce) according to the manufacturer’s protocol. The following antibodies were subsequently used: BRD7 (Lifespan Biotechnology), β-catenin (R&D), MMP-2, α-catenin, lamin β receptor (LBR), cyclin B1 and MEK1 (Epitomics), MMP-9 (Thermo), β-actin, cyclin D1, and p53 (Santa Cruz Biotechnology), and cyclin E2, ERK, and p-ERK(1/2) (Thr202/Tyr204; Cell Signaling). Protein bands were then labeled with horseradish peroxidase-conjugated anti-mouse or anti-rabbit antibody (Amersham).

Wound healing assay
For the wound healing assay, confluent and quiescent monolayers of cells that were transfected with corresponding expression plasmid and siRNA as indicated were wounded using the end of a sterilized tip. After 24 hours, to observe changes involved with healing, photographs were taken 9 and 12 hours after wounding of each subset of SKOV3 and A2780 cells, respectively, based on the migratory ability of each cell line. All photographs were taken at 100× magnification.

Cell invasion assay
For measuring the ability of cells to invade, we used a transwell system (8-μm pore size; Corning). Cells were transfected with the corresponding expression plasmid and/or siRNA for 12 hours and trypsinized for dissociation into single cells in a serum-free medium. A total of 100 μL of the cell suspension (1 × 10^5 cells) was then added to the upper chamber of the insert that was coated with matrigel (50 μg/mL; BD Biosciences). Cells were allowed to invade for 12 hours in response to FBS as a chemoattractant in the lower compartment. After incubation, cells on the upper surface of the inner membrane were removed with the cotton swab, and the final number of invaded cells was determined using crystal violet staining.

ELISA assay
For quantitative measurement of MMP2 and MMP9 secretion, we used ELISA assays (R&D and Abnova, Taoyuan Country, Taiwan, respectively). Cells were transfected with the corresponding expression plasmid and siRNA as indicated for 36 hours. Protein from suppurates of each subset were then purified and concentrated using Amicon Ultra.
Centrifugal Filter (Millipore). To measure the quantity of MMP-2 in tumor tissues from the xenografts, tumor samples were placed into 200 μL of protein lysis buffer (INTRON BIO Technology) and homogenized. The quantities of MMP2 or MMP9 were assessed using ELISA assay according to the manufacturer’s protocol.

Animal care and orthotopic implantation of tumor cells
Female BALB/c nude mice were purchased from ORIENT BIO. This study was approved by the Institutional Animal Care and Use Committee (IACUC) of the Samsung Biomedical Research Institute (SBRI). SBRI is an Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International, protocol No. H-A9-003) accredited facility and abides by the Institute of Laboratory Animal Resources (ILAR) guide. To establish the orthotopic model, A2780 cells (1 × 10⁶ cells/0.2 mL HBSS) were injected into the peritoneal cavity (17). The mice were 6 to 8 weeks of age. Mice (n = 6 per group) were monitored daily for tumor development and were sacrificed on day 35 after the cancer cell injection. Body weight, tumor weight, and the number of tumor nodules were recorded. Tumors were fixed in formalin and embedded in paraffin or snap frozen in OCT compound (Sakura Finetek Japan).

Immunohistochemistry
Immunohistochemical procedures for Ki67 and BRD7 were performed as previously described (14, 16). To quantify Ki67 expression, the number of Ki67-positive cells and the total number of tumor cells were counted in 5 random fields at 100× magnification, followed by calculation of the percentage of positive cells.

Luciferase assay
For the luciferase assay, cells were transiently transfected with Firefly luciferase reporter (100 ng) containing wild-type T-cell factor (Tcf) (OT) with the corresponding expression plasmid (5 μg). Renilla luciferase reporter (100 ng) was cotransfected in individual cases to normalize transfection efficiency. All reporter plasmids were kindly gifted from Dr. Sunjoo Jeong (Dankook University, Yongin Si, Korea). After 24 hours, cells were harvested and lysed in passive lysis buffer from the Luciferase Assay Kit (Promega). All readings were made using a Wallac Victor2 1420 Microplate Reader (PerkinElmer Life Sciences Inc.). Relative firefly luciferase activities were calculated by normalization with Renilla luciferase activity.

Data analysis
Mann–Whitney U tests were used to evaluate the significance between gene expression in tumors and normal ovarian tissues as assessed by real-time PCR, and to compare differences among the groups for both in vitro and in vivo assays. All statistical tests were two-sided, and P-values < 0.05 were considered to be statistically significant. All statistical analyses were performed using statistical SPSS software package (SPSS Inc.).

Results
The expression of BRD7 is decreased in high-grade serous ovarian cancer relative to normal tissue or low-grade serous ovarian cancer
We estimated the relative expression pattern of BRD7 using human ovarian tissue from 11 normal ovarian epithelium and 41 serous cancer tissue specimens, which included 7 low-grade and 34 high-grade cancers. Actually, we did tried estimation of protein level of BRD7 in the ovarian cancer tissues through immunohistochemistry (IHC), but the qualities of antibodies were not sufficient for sensitive analysis for the expression pattern of BRD7 (data not shown). We found that BRD7 mRNA expression was significantly lower in the serous ovarian cancer tissues relative to the normal tissues (P = 0.02; Fig. 1A).

Figure 1. Real-time PCR analysis of BRD7 expression in human ovarian tissue. A, the expression levels of BRD7 mRNA were significantly reduced in serous ovarian cancers relative to normal ovarian tissue (*, P = 0.02). B, expression was significantly reduced in high-grade serous ovarian cancer relative to low-grade serous cancer (*, P = 0.01) or normal tissue (†, P < 0.01).
Interestingly, although there was no statistical difference in BRD7 expression between normal tissues and low-grade carcinomas ($P = 0.49$), high-grade carcinomas showed significantly decreased expression of BRD7 compared with normal ($P = 0.01$) or low-grade cancer tissues ($P = 0.01$; Fig. 1B).

**Upregulation of BRD7 affects cell survival and apoptosis of ovarian cancer cells**

We evaluated endogenous expression of BRD7 in epithelial ovarian cancer cell lines using Western blot analysis. BRD7 was variably expressed among the cell lines (Fig. 2A), so we chose to study A2780 and SKOV3 cells because of their relatively regular and minor expression, respectively. Transfection of BRD7 plasmid construct either #1 or #2 increased expression of BRD7 in a dose-dependent manner in both cells with similar transfection efficiency (approximately up to 3-fold upregulation of BRD7 in both 2 cell lines; Fig. 2B). Cells were transiently transfected with BRD7 plasmid for 36 hours, and cell survival was estimated using MTT assay. The cell survival was significantly decreased by BRD7 plasmid construct #1 or #2 in a dose-dependent manner in both cell lines compared with controls as assessed by MTT assays after 36 hours of transfection with corresponding BRD7 plasmid construct (*, $P < 0.05$ and **, $P < 0.01$). Apoptosis was estimated by detection of activated caspase-3 using ELISA assay after 36 hours of transfection with BRD7 plasmid (*, $P < 0.05$ and **, $P < 0.01$). Values are expressed as means of 3 independent experiments ± SD.

Figure 2. BRD7 overexpression affects cell survival and apoptosis in ovarian cancer cells. A, Western blot analysis exhibited variable expression of BRD7 in ovarian cancer cell lines. B, A2780 and SKOV3 cells were transfected with BRD7 plasmid construct #1 or #2 and Western blot analysis subsequently showed upregulation of BRD7 in a dose-dependent manner in both cell lines. C, upregulation of BRD7 decreased cell survival in both cell lines as assessed by MTT assays after 36 hours of transfection with BRD7 plasmid construct. D, apoptosis was estimated by detection of activated caspase-3 using ELISA assay after 36 hours of transfection with BRD7 plasmid. Values are expressed as means of 3 independent experiments ± SD.
manner in both cells (Fig. 2C; $P < 0.05$). Comparison showed that plasmid constructs induced upregulation of BRD7 expression was similar, but the reduction of cell viability by construct #1 was relatively more effective than by #2 in both 2 cell lines. So we selected construct #1 for the rest tests for the estimation of the cellular effects by BRD7 (marked as BRD7 plasmid).

BRD7 also significantly increased apoptosis in both cells compared with mock control (Fig. 2D; $P < 0.05$).

We also assessed the effect of BRD7 to the progress of cell cycle in ovarian cancer cells. First, we sorted the cells in the individual phase of cell cycle, G0–G1, S, and G2–M by using FACS assay and then isolated whole mRNA and analyzed the expression of BRD7 mRNA. Compared with the G0–G1 phase, the expression of BRD7 in either S or G2–M phase did not show significant differences in both the cell lines (Supplementary Fig. 1A). Subsequently, we estimated the regulation of cyclin D1 and E2 expression followed by upregulation of BRD7. And we found that both cyclins were not regulated by BRD7 in either cell line (Supplementary Fig. 1B). These results imply that BRD7 might not be associated with the process of cell cycle in ovarian cancer cells.

Upregulation of BRD7 decreases cell migration/invasion and MMP2 activity in ovarian cancer cells

BRD7 inhibited cell migration in both cells as assessed by the wound healing assay (Fig. 3A). Using transwell-invasion assay, we identified that cell invasion was more obviously inhibited by BRD7 than cell viability in both cells (Fig. 3B). In addition, MMP2 expression was significantly decreased by BRD7 both cells (Fig. 3C), although there were no changes in MMP9 expression in either cell (data not shown). Using ELISA assay, we subsequently found that secretion of MMP2 was significantly reduced by BRD7 (Fig. 3D; $P < 0.01$). The secretion of MMP9 was not changed by BRD7 in either cell (data not shown).

**BRD7 overexpression inhibits tumor growth and metastatic capability in orthotopic model**

Orthotopic model of ovarian cancer was established in nude mice by intraperitoneal injection of A2780 cells. We injected the BRD7 plasmid or the mock vector into the mice once weekly starting 7 days after cancer cell injection. Plasmid DNA injections resulted in significantly decreased tumor weight and the number of tumor nodules (Fig. 4A and B; $P < 0.05$). In addition, when we examined tumor cell

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Figure 3. BRD7 overexpression reduces cell migration/invasion and MMP2 activity. A, the wound healing assay showed that upregulation of BRD7 inhibited the migratory ability of ovarian cancer cells. All photographs were taken at 100× magnification. B, the transwell-invasion assay used to determine cell invasion showed that upregulation of BRD7 inhibited invasion of ovarian cancer cells (†, $P < 0.05$ and ‡, $P < 0.01$). C, upregulation of BRD7 reduced expression of MMP2 as measured on Western blot analysis. D, upregulation of BRD7 reduced secretion of MMP2 as measured on an ELISA assay (**, $P < 0.01$). Values are expressed as means of 3 independent experiments ± SD.
proliferation using Ki67 IHC in harvested tumor tissues, positive staining was significantly decreased in the BRD7 plasmid-treated group (Fig. 4C; \( P < 0.05 \)). When we measured the MMP2 levels using ELISA assay, we found that secretion of MMP2 was also significantly decreased by BRD7 (Fig. 4D; \( P < 0.05 \)). These results were consistent with the in vitro effects. Upregulation of BRD7 in tumor tissues was estimated by Western blot analysis (Supplementary Fig. S2).

BRD7 regulates cell survival, apoptosis, and cell migration/invasion independent of the p53 tumor suppressor protein

We confirmed that BRD7 mediates antitumor effects in both A2780 (p53 wild) and SKOV3 (p53 null) cells, suggesting that BRD7 may act as a tumor suppressor gene in a p53-independent manner. To more precisely test this hypothesis, we evaluated whether BRD7 could mediate antitumor effects when p53 is downregulated in A2780 cells. Cells were cotransfected with p53 siRNA and BRD7 plasmid. We then examined the protein expression levels using Western blot analysis (Fig. 5A) and found that BRD7-mediated changes in cell survival were not affected by downregulation of p53 (Fig. 5B, top; \( P < 0.01 \)). BRD7 also induced a 1.2-fold increase in apoptosis when cells were cotransfected with p53 siRNA (\( P < 0.05 \); Fig. 5B, bottom).

Downregulation of p53 increased cell invasion in A2780 cells compared with negative control, and BRD7 continued to induce decreases in cell migration/invasion in the absence of p53 activity (Fig. 5C; \( P < 0.05 \)). In addition, we found that BRD7-mediated downregulation of MMP2 activity did not require p53 activity in ovarian cancer cells (Fig. 5D).

BRD7 negatively regulates \( \beta \)-catenin pathways

Based on our previous study of endometrial cancer and the negative regulation of the \( \beta \)-catenin pathway by BRD7 (14), we sought to determine whether BRD7 could upregulate \( \alpha \)-catenin and the accumulation of \( \beta \)-catenin in the cytoplasm of ovarian cancer cells. BRD7 did not upregulate \( \alpha \)-catenin in either cell (Supplementary Fig. S3), although it increased the accumulation of \( \beta \)-catenin in the cytoplasm in both cells (Fig. 6A). The expression of \( \beta \)-catenin was not modified by BRD7 in either cell (data not shown), suggesting that BRD7 may inhibit translocation of \( \beta \)-catenin to the nucleus in ovarian cancer cells. In addition, reporter gene silencing assay using a luciferase plasmid vector showed that BRD7 decreased \( \beta \)-catenin–mediated Tcf-responsive gene transcription in both cells (Fig. 6B). Transcriptional target genes of \( \beta \)-catenin have been known to play roles in various cellular events and MMP2 is also known as one of them. So we next tested whether the mechanism by which BRD7 downregulated MMP2 expression in ovarian cancer cells could be the negative regulation of \( \beta \)-catenin pathway. When \( \beta \)-catenin was downregulated by siRNA, BRD7 plasmid did not downregulate MMP2 expression (Supplementary Fig. S4) via representing BRD7-mediated downregulation of MMP2 is requiring \( \beta \)-catenin pathway. We also assessed MEK1 expression and phosphorylation of ERK1/2,
which is the final activating event of the ras-raf-MEK-ERK pathway, using Western blot analysis, but there was no influence on the transduction of the pathway by BRD7 (Supplementary Fig. S5).

Discussion

The key finding of this study is that BRD7 is a novel tumor suppressor in serous ovarian cancer independent of p53 activity. The expression of BRD7 was significantly lower in serous cancer tissues than in normal tissues, with high-grade serous cancers exhibiting markedly decreased expression of BRD7 compared with low-grade cancers. Although BRD7 has modest effects on cell viability and apoptosis, it could more effectively inhibit cell migration/invasion and the reduction of MMP2 expression and secretion in ovarian cancer cells. Moreover, we confirmed these BRD7-induced tumor suppressor effects in orthotopic mouse model of ovarian cancer, and found that not only cell proliferation but also secretion of MMP2 were significantly decreased by BRD7. These results are consistent with the observed in vitro results.

Recent studies have attempted to develop a more precise model of carcinogenesis with an emphasis on differences in cellular origins and molecular aberrations in serous ovarian cancer (18–20). These studies have suggested that tumor grade, a pathological index of an individual cellular aberration, could help to stratify serous ovarian carcinomas. Low-grade carcinomas tend to be slow growing, indolent, and typically arise from invasive foci of tumors with low malignant potential, whereas high-grade carcinomas are rapidly growing, aggressive, and usually lack a definitive precursor region. In this study, we found that the expression of BRD7 mRNA in high-grade tumors was significantly lower than that of low-grade tumors. These findings suggest that a decrease in BRD7 could play an important role in the aggressive behavior of high-grade tumors and may potentially be used as a prognostic marker for this disease.
shown to contribute specifications for assembly and targeting (21, 24, 25). Research studies have demonstrated that BRD7 regulates signaling pathways that regulate cellular events involving growth, apoptosis, cell cycle, and mobility (10, 12, 26, 27). In particular, BRD7 has been known to interact with p53 and p300 by affecting acetylation of histones and recruiting them to specific promoters of target genes (11, 27). Those studies have revealed that the interaction of BRD7 with p53-p300 is required for a subset of p53-regulated gene expressions through direction of promoter-binding and/or bridging of p53 to a favorable chromatin environment. In another study, the interaction between BRD7 and BRCA1 was shown to regulate transcriptional activity of downstream target genes of BRCA1 in breast cancer cells (9). In addition, other studies have found that BRD7 may act as a regulator of signaling pathways including ras-raf-MEK-ERK and RB/E2F (12, 26).

As the p53 mutation is a definitive feature in high-grade ovarian cancer, we initially considered BRD7 as a coactivator of p53. It is well established that p53 regulates cellular behavior of cancer cells by transcriptional regulation of specific genes or signaling pathways implicated in those activities (28–34). However, our results showed that BRD7 could induce tumor suppressive effects in either SKOV3 (p53 null) or A2780 (p53 wild type) cells. In addition, we found that downregulation of p53 activity did not alter BRD7-mediated tumor suppressive effects in A2780 cells. These results indicate that BRD7-mediated effects may involve a p53-independent signaling pathway in ovarian cancer cells.

As a key mediator of the Wnt signaling pathway, β-catenin accumulates in the nucleus where it complexes with the Tcf/LEF family of transcription regulators in response to Wnt, and directly binds the promoter of Tcf-responsive genes thereby activating transcription (35–37). Moreover, β-catenin is a part of an E-cadherin/catenin adhesion complex, acting as a cell-to-cell adhesion molecule by regulating the epithelial entities of cancer cells with characteristics of catenin proteins (35, 36). It is known that BRD7 upregulates α-catenin, which holds β-catenin in the membrane and cytoplasm in nasopharyngeal carcinoma cells (12). In addition, our previous study, we confirmed that BRD7 accumulates β-catenin in the cytoplasm in endometrial cancer cells (14). Interestingly, in this study, BRD7 did not alter the regulation of α-catenin but did decrease the accumulation of β-catenin in the nuclei of ovarian cancer cells. Accordingly, BRD7 negatively regulated β-catenin-mediated Tcf-responsive gene transcription. In addition, we found that the mechanism by which BRD7 reduces MMP2 expression could be the negative regulation of β-catenin pathway. Taken together, our results show that BRD7 induced the accumulation of β-catenin in the cytoplasm and inhibited transcription of Tcf-responsive genes, which may be the key mechanism by which BRD7 exhibits tumor suppressive effects in ovarian cancer cells.

This study demonstrated an association between BRD7 and high-grade serous ovarian carcinoma that is independent of p53. These findings are significant with respect to targeted therapy, as most patients with serous ovarian cancer exhibit poor responses to chemotherapeutic agents that target the p53 pathway. Thus, there is a new demand for the development of novel treatments targeting p53 independent pathways.

In conclusion, this study showed that BRD7 acts as a tumor suppressor in epithelial ovarian cancers independent of p53 activity via negative regulation of the β-catenin pathway. Establishment of the mechanism by which BRD7 negatively regulates the β-catenin pathway could result in promising diagnostic and therapeutic strategies for the treatment of ovarian cancer. Future study about
LOH and the correlation of the BRD7 protein expression and the clinicopathological factor could be helpful for the understanding the role of BRD7 in epithelial ovarian carcinoma.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: D.-S. Bae, Y.-A. Park, J.-W. Lee, T.-J. Kim
Acquisition of data (provided patients, acquired and managed patients, provided facilities, etc.): J.-W. Lee
Analysis and interpretation of data (e.g., statistical analysis, bioinformatics, clinical): J.-W. Lee, H.-S. Kim, C. Choi
Writing, review, and/or revision of the manuscript: J.-W. Lee, Y.Y. Lee

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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J.-W. Lee, Y.Y. Lee, J.-J. Choi, H.-K. Jeon, Y.-J. Cho

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