BRD7, a Subunit of SWI/SNF Complexes, Binds Directly to BRCA1 and Regulates BRCA1-Dependent Transcription


Abstract

We carried out a yeast two-hybrid screen using a BRCA1 bait composed of amino acids 1 to 1142 and identified BRD7 as a novel binding partner of BRCA1. This interaction was confirmed by coimmunoprecipitation of endogenous BRCA1 and BRD7 in T47D and HEK-293 cells. BRD7 is a bromodomain containing protein, which is a subunit of PBAF-specific Swi/Snf chromatin remodeling complexes. To determine the functional consequences of the BRCA1-BRD7 interaction, we investigated the role of BRD7 in BRCA1-dependent transcription using microarray-based expression profiling. We found that a variety of targets were coordinately regulated by BRCA1 and BRD7, such as estrogen receptor α (ERα). Depletion of BRD7 or BRCA1 in either T47D or MCF7 cells resulted in loss of expression of ERα at both the mRNA and protein level, and this loss of ERα was reflected in resistance to the antiestrogen drug fulvestrant. We show that BRD7 is present, along with BRCA1 and Oct-1, on the ERα promoter (the gene which encodes ERα). Depletion of BRD7 prevented the recruitment of BRCA1 and Oct-1 to the ERα promoter; however, it had no effect on the recruitment of the other Swi/Snf subunits BRG1, BAF155, and BAF57 or on RNA polymerase II recruitment. These results support a model whereby the regulation of ERα transcription by BRD7 is mediated by its recruitment of BRCA1 and Oct-1 to the ERα promoter. Cancer Res; 70(6); 2538-47. ©2010 AACR.

Introduction

Germ line mutations within BRCA1 confer a genetic predisposition to breast and ovarian cancer. BRCA1 has been implicated in diverse functions, in particular DNA damage repair, cell cycle checkpoint control, ubiquitination, and transcriptional regulation (1). BRCA1 has been shown to associate with a variety of proteins, and work to date supports a model whereby BRCA1 acts as a scaffolding protein mediating the assembly of signaling complexes crucial for these assorted functions.

A role for BRCA1 in transcriptional regulation is now well established, with a variety of activated and repressed transcriptional targets having been identified. These include proteins involved in DNA repair, such as DDB2 and XPC (2); cell cycle regulators, such as p21 and GADD45 (3); and regulators of growth, such as p53, Myc, signal transducers and activators of transcription 1 (STAT1), and Oct-1 (7). To facilitate transcription the structure of chromatin must be altered so as to allow access of the transcriptional machinery to the DNA. This is achieved both by modification of the tail of histones, as well as by chromatin remodeling, via multisubunit enzyme complexes, such as the Swi/Snf complex, which hydrolyses ATP to alter the interactions between histones and DNA. BRCA1 has been shown to associate with the histone acetyltransferases p300/CREB and hGCN5/TRRAP, as well as the histone deacetylases HDAC1 and HDAC2 (7). In addition BRCA1 was shown to interact directly with BRG1, the ATPase subunit of the Swi/Snf complex (8).

Swi/Snf chromatin remodeling complexes are evolutionarily conserved from yeast to humans and have been shown to both positively and negatively regulate transcription. Human cells contain two major classes of the Swi/Snf complex, BAF (BRG1 or hBRM-associated factor) and PBAF (polybromo-associated BAF). Each subgroup contains one of two highly homologous ATPases, BRG1 or BRM, and a variable number of associated subunits termed BRG1-associated factors (BAF). BAF complexes can contain either BRG1 or BRM,
whereas PBAF complexes contain only BRG1. The core subunits shared by most Swi/Snf complexes include the BAFs 170, 155, 60, 57, 53, 47, and actin (9). The subclasses are defined by the presence of specific signature subunits. BAF complexes are specified by the ARID1 (BAF250) subunit, whereas the signature subunits of PBAF complexes are ARID2, polybromo (BAF180), and BRD7, a bromodomain containing protein (10).

In this study we identify BRD7 as a novel binding partner of BRCA1 and functionally characterize their interaction.

Materials and Methods

Yeast two-hybrid screen. Yeast two-hybrid screening was carried out with the Clontech Matchmaker two-hybrid system (Takara Bio Europe) using an ovarian cDNA library cloned in frame with the GAL4 activation domain in the pACT2 vector. A bait construct composed of 1 to 3426 bp (amino acids 1–1142) of BRCA1, was subcloned in frame with the GAL4 DNA-binding domain in pGBKT7 to generate pGBK7 brushes. Two further constructs were generated in pGBK7 of composed of 1 to 2781 bp (amino acids 1–927) and 907 to 2781 bp (amino acids 303–927) of BRCA1. For the screen the pGBK7 brushes 1 to 1142 was transformed into the AH109 yeast strain and mated for 24 h at 30°C with Y187 yeast transformed with pACT-ovarian library. Interacting clones were selected by their ability to activate the His3 reporter as indicated by growth on histidine/leucine/tryptophan negative media. Autoactivation of the His3 reporter by the pGBK7 brushes 1 to 1142 was prevented by the addition of 4 mmol/L 3-amino-triazole (Sigma). Primary clones, which activated the His3 reporter, were further tested for their ability to activate the ADE2 and lacZ reporters, as shown by growth on histidine/ adenine/leucine/tryptophan–negative media or conversion of 5-bromo–4-chloro-3-indolyl–β-D-galactopyranoside (Sigma) to a blue product, respectively.

Cell lines. All cell lines used were obtained from the American Type Culture Collection and have been passaged for >6 mo since receipt.

Coimmunoprecipitation and Western blotting. Coimmunoprecipitations of BRCA1, BRD7, and Myc were carried out from nuclear extracts of HEK-293 and T47D breast cells. For coimmunoprecipitation 500 μg of nuclear extract were made up to 250 μL with immunoprecipitation buffer (100 mmol/L KCl, 5 mmol/L MgCl2, 0.2 mmol/L EDTA, 20 mmol/L HEPES, 10% glycerol, 1 mmol/L DTT, 0.1% NP40, protease inhibitor mix). For immunoprecipitation, 3 μg of the following antibodies were used: BRCA1, Ab-1, and Ab-4 (Calbiochem); anti-BRD7 (ProteinTech Group); c-Myc, C-19, or C-8 (Santa Cruz Biotechnology); and isotype-matched controls, IgG1, IgG2A, and rabbit IgG (DAKO). The samples were incubated overnight at 4°C, then 50 μL of 50% protein A/G sepharose beads (Sigma) in immunoprecipitation buffer were added, and incubation continued for another 2 h. The pellet was washed 4× in immunoprecipitation buffer. The samples were Western blotted for BRCA1, BRD7, or c-Myc.

Short interfering RNA transfection. Short interfering RNA (siRNA) transfection of T47D or MCF cells was carried out as previously described (6). The siRNA sequences used were the same except for the following: BRCA1 number-1, ACCATACACGGTTCAATAAA; BRD7 number-1, GUGCCGAAUGAUUGCUU; BRD7 number-2, CGGUCAAAGCAGUAACAAATT; Scrambled siRNA, AGCAGCAGACTTCTTCAG.

Gene expression profiling and microarray data analysis. RNA was harvested from T47D cells transfected in triplicate independent transfections with BRD7 siRNA number-2, BRCA1 siRNA (6), or SCR sequence. This was prepared and hybridized to a Breast Cancer DSA microarray (6). The criteria used to detect differential gene expression between BRCA1- or BRD7-depleted and SCR-treated cells was a 1.3-fold difference in gene expression at a statistical significance defined by a P value of ≤0.01 using the Student’s unpaired t test.

Western blotting. Nuclear extract (60 μg) was resolved by SDS-PAGE and Western blotted for BRD7 (ProteinTech), ERE (HC-20), BRCA1 (D9), Oct-1 (C-21), BAF155 (F-2), BAF57 (C-20), c-Myc (9E10; all six are from Santa Cruz Biotechnology), and BRG1 (Millipore). Membranes were reprobed for actin (Sigma, AC-74) to show equal loading.

Real-time PCR. Total RNA was isolated from cells using RNA STAT60 reagent (Tel-Test, Inc.). RNA was generated from three independent transfections, using two different siRNA sequences for BRCA1 and BRD7. RNA (2 μg) was reverse transcribed using random primers and Moloney murine leukemia virus reverse transcriptase (Invitrogen) according to the manufacturer’s instructions. The cDNA was subjected to quantitative real-time reverse transcription–PCR (qRT–PCR) using Roche Lightcycler 480 SYBR Green 1 master mix, according to the manufacturer’s instructions. The real-time primers were designed using the Roche Universal Probe library,1 and the primer sequences are listed in Supplementary Materials and Methods. The levels of mRNA for each gene were normalized relative to actin mRNA. Statistical significance was determined using the Student’s unpaired two-tailed t test.

Gene transfection. Constructs encoding either BRD7 or ΔBRD7 deleted in residues encoding the bromodomain of BRD7 (amino acids 129–237) were obtained from Dr J. Kzyshkowska (11). These were transfected into T47D cells using GeneJuice (Novagen) according to the manufacturer’s instructions. Cells were harvested for chromatin immunoprecipitation (ChIP) assay, or protein extracts 48 hrs after transfection.

ChIP assay. ChIP assays were performed as previously described (6). The antibodies used for immunoprecipitation were anti-Pol II (CTD4H8, Millipore); anti-BRCA1, Ab-1, or D9; anti-BRD7 (AbCam); anti-acetylhistone H3 and anti-BRG1 (Millipore); anti-Oct1, anti-BAF57, anti-BAF155 (all three from Santa Cruz Biotechnology); and rabbit IgG and IgG1 (DAKO). The ESRI promoter was amplified by

1 www.roche.com
PCR using primers previously described (6) or with ESR1 control primers located ~3 kb upstream of the promoter that amplified a 146-bp product [5′(CACTGAATACGGGGGCTGCT (forward) and 5′(CACTGGACATATGCAAGT (reverse)].

Antiestrogen sensitivity studies. T47D, MCF7, or BT-474 cells were transfected with either BRCA1, BRD7, ERα, or SCR siRNA at a concentration of 200 nmol/L for two consecutive days using oligofectamine according to the manufacturer’s instructions. On day 3, the cells were plated onto 96-well plates at a density of 5,000 cells per well. At 24 h later, the cells were treated with fulvestrant (IC1 182,780; To curls Bioscience) at concentrations varying from 10−4 to 10−12 mol/L. After 72 h the number of viable cells was quantitated using the MTT assay. The concentration of drug that inhibited 40% of the growth of the cells (IC50) was calculated with prism software.

Results

Identification of BRD7 as a BRCA1-interacting protein. Given that the biology of BRCA1 seems to be in large part dictated by its interactions with other proteins, we carried out a yeast two-hybrid screen using the NH2 terminal 1142 amino acids of BRCA1 to identify novel interactors. A human ovarian cDNA library was screened, and 154 clones were isolated, using activation of the HIS3 reporter gene as a read out of interaction. These clones were subjected to further screening, which examined their ability to activate two additional reporter genes, LacZ and ADE2. Subsequent to this second round of screening, seven library clones were identified as encoding BRCA1 interacting proteins. Of these seven clones, two encoded the known BRCA1 interacting proteins BARD1 and cMyc. A third clone contained sequence encoding amino acids 277 to 652 of BRD7, a bromodomain containing protein.

To further elucidate the site of interaction of BRD7 with BRCA1, Y187 yeast expressing the BRD7 clone was mated with AH109 yeast expressing either the BRCA1 bait 1 to 1142 or two further constructs encoding amino acids 1 to 927 of BRCA1 or 303 to 927 of BRCA1 (Fig. 1A). The BRD7 clone was unable to interact with 303 to 927 BRCA1 but was still able to interact with 1 to 927BRCA1, as shown by growth on HLT− media (Fig. 1B). The 303 to 927 BRCA1 construct expressed a functional protein as shown by its ability to interact with the cMyc clone (data not shown). This indicates that the first 300 amino acids of BRCA1 are important in mediating the interaction with BRD7.

BRCA1 and BRD7 interact in vivo. To determine whether BRCA1 and BRD7 interact in vivo, communoprecipitation experiments were carried out in HEK-293 and T47D breast cells. The previously characterized interaction of BRCA1 with c-Myc was used as a positive control. BRCA1 was clearly present in the BRD7 immunoprecipitate (Fig. 1C, right, lane 4) to an even greater degree than that associating with c-Myc (lane 3), indicating an association between endogenous BRCA1 and BRD7. When the reciprocal BRCA1 immunoprecipitation was carried out using two different BRCA1-specific monoclonal antibodies Ab-1 and Ab-4, again BRD7 was clearly present (Fig. 1C, right, lanes and 3 and 4). The same immunoprecipitations carried out in T47D cells again clearly showed an association of BRCA1 and BRD7 when immunoprecipitated in either direction (Fig. 1D).

BRCA1 and BRD7 cooperate in transcriptional regulation. BRD7 is a bromodomain containing protein, which has been identified as a PBAF-specific Swi/Snf subunit (10). To determine the functional consequences of the BRCA1-BRD7 interaction, we investigated the role of BRD7 in BRCA1-dependent transcription using microarray-based expression profiling. The gene expression profile of either BRCA1 or BRD7 depleted cells was compared with that of SCR-transfected cells (Fig. 2B). A total of 1,928 genes exhibited BRCA1-dependent changes in expression relative to control; of these 1,172 were positively regulated and 756 were negatively regulated. A total of 5,248 genes exhibited BRD7-dependent expression changes compared with control; of these 2,236 were positively regulated and 3,012 were negatively regulated by BRD7. The list of genes displaying BRCA1-dependent changes in gene expression were compared with those showing BRD7-dependent regulation. From this analysis 583 genes were determined to be jointly regulated by BRCA1 and BRD7, revealing that 30% of all BRCA1-regulated genes were also jointly regulated by BRD7 and 11% of BRD7-regulated genes were also regulated by BRCA1. Of the 583 genes, 331 (57%) were positively regulated by BRCA1 and BRD7, and 252 (43%) were negatively regulated by both. This agrees with previous studies, which show that BRCA1 and the Swi/Snf complex are able to both positively and negatively regulate transcription.

We further validated the expression of four of these targets, ERα, Rad51, versican, and CYP1A1, using qRT-PCR. The expression of ERα, Rad51, and versican was decreased to a similar degree in both BRCA1- and BRD7-depleted T47D or MCF7 cells, indicating that both BRCA1 and BRD7 mediate the transcriptional activation of these three targets (Fig. 3). We have previously shown that ERα is a BRCA1-regulated target (6). The expression of CYP1A1 was substantially upregulated in both MCF7 and T47D cells in the absence of either BRCA1 or BRD7, indicating that it is normally repressed by both.

BRD7 recruits BRCA1 and Oct-1 to the ESR1 promoter. We have previously shown that BRCA1 and Oct-1 are present on the ESR1 promoter (6). ChIP analysis showed that along with BRCA1 (Fig. 4A, top, lanes 4 and 6) and Oct1 (lane 10), BRD7 was clearly present on the ESR1 promoter (lane 8). The association of BRD7 with the ESR1 promoter correlated with the levels of acetylated histone H3 (lane 9). In addition as expected for an actively transcribed gene RNA Pol II was also present on the promoter (lane 3).

We next examined if recruitment of either BRCA1 or BRD7 to the promoter was affected by the presence of their partner protein. Depletion of BRD7 clearly inhibited the recruitment of BRCA1 to the ESR1 promoter; however, the reciprocal depletion of BRCA1 had no effect on the recruitment of BRD7 to the promoter (Fig. 4B, left). Depletion of BRD7 also prevented the recruitment of the transcription factor Oct-1 to
the ESR1 promoter. Depletion of BRD7 had no effect on the recruitment of Pol II to the promoter. We also examined the effect of Oct-1 depletion on recruitment of BRD7 to the ESR1 promoter (Fig. 4B, right). Oct-1 depletion prevented the recruitment of BRCA1 to the promoter, as we had shown previously. However Oct-1 depletion had no effect on the recruitment of BRD7 to the ESR1 promoter. In summary these ChIP assays reveal that BRD7 is responsible for the recruitment of both BRCA1 and Oct-1 to the ESR1 promoter; however, the recruitment of Pol II is unaffected.

Brd7 is a subunit of PBAF Swi/Snf complexes, which are multisubunit complexes composed of three signature subunits, a number of core BAF subunits, and the ATPase BRG1. Brd7 is one of the signature subunits of PBAF complexes. BRCA1 has previously been shown to bind to BRG1 (8). To ensure that BRD7 depletion was not affecting the assembly of the Swi/Snf complex, we examined the recruitment of BRG1, as well as the core subunits BAF 57 and BAF 155 to the ESR1 promoter. All three Swi/Snf subunits were recruited to the ESR1 promoter irrespective of the presence of BRD7 (Fig. 4C, lanes 7, 8, and 9), indicating that the assembly and recruitment of the complex was unaffected by the absence of BRD7. In addition this result shows that despite the presence of BRG1 on the ESR1 promoter, it is unable to recruit BRCA1.

To determine if the bromodomain of BRD7 was important for enabling the recruitment of BRD7 to the ESR1 promoter, either full-length BRD7 or ΔBRD7 deleted in amino acids 129 to 236, which encode the bromodomain (11), were expressed in T47D cells (Fig. 4D). ChIP analysis of T47D cells expressing either construct revealed that there was a reduced level of recruitment of ΔBRD7 to the promoter compared with...
full-length BRD7 (Fig. 4D, compare lanes and 7 and 8). This reduced recruitment of ΔBRD7 clearly correlated with reduced levels of recruitment of BRCA1 to the ESR1 promoter (compare lanes 5 and 6); however, the recruitment of Pol II was unaffected.

**Association between BRD7 and response to antiestrogen treatment.** We have shown that the transcription of ERα is coordinately regulated by BRD7 and BRCA1; thus we wanted to confirm that this regulation was reflected in the level of ERα protein expression. Depletion of either BRCA1 or BRD7 led to a reduction in the level of ERα protein in either T47D, MCF7, or BT474 cells (Fig. 5). We had previously shown that this reduction in ERα protein levels, produced by depletion of BRCA1, was able to mediate resistance to the antiproliferative effects of the antiestrogen fulvestrant (6). We examined whether the reduction in ERα levels produced by depletion of BRD7 produced a similar resistance to fulvestrant. Depletion of ERα with siRNA was used as a positive control. In comparison to scrambled control-treated cells, depletion of either BRCA1 or BRD7 led to a significant resistance to the antiproliferative effects of fulvestrant in all three cell types, similar to that produced by depletion of ERα.

**Discussion**

In this study we have identified BRD7, a subunit of PBAF-specific Swi/Snf complexes, as a novel binding partner of BRCA1. We further show that BRD7 is responsible for the recruitment of BRCA1 and Oct-1 onto the ESR1 promoter. Finally we highlight the functional significance of the observation by showing that depletion of BRCA1 or BRD7 resulted in loss of ERα expression and resistance to fulvestrant.

We show by microarray-based expression profiling that BRD7, like BRCA1, can both repress and activate gene expression. Our results suggest that BRD7 may be involved in the regulation of up to 30% of BRCA1 regulated genes. In contrast BRCA1 was shown to coregulate only 11% of BRD7 transcriptional targets as defined by microarray analysis. This is not surprising as BRD7 is a member of the PBAF Swi/Snf complex and likely to play a much wider role than BRCA1 in transcriptional regulation. BAF and PBAF Swi/Snf complexes have been shown to regulate expression of distinct genes (12). As the PBAF and BAF complexes share a set of core subunits, it is likely that the functional specificity of the two subclasses is dictated by
their repertoire of unique subunits, one of which is BRD7 in the case of PBAF. We have shown that, in addition to BRCA1, BRD7 is also able to recruit the transcription factor Oct-1 to the ESR1 promoter. A previous study showed that BRD7 could also bind the transcription factor IRF2 (13). It is possible that the role of BRD7 within the Swi/Snf complex is to provide specificity by selectively recruiting specific transcription factors. Other Swi/Snf subunits...
Figure 4. BRD7 is on the ERα promoter and recruits both BRCA1 and Oct-1 to the promoter. A, a ChIP assay was carried out in MCF7 cells to assess the recruitment to the ESR1 promoter of Pol II, BRCA1 (Ab1 and D9), BRD7, acetyl-histone H3 (AcH3), and Oct1. The immunoprecipitation was compared with that of the appropriate isotype-matched controls: IgG1 for Pol II and Ab1, IgG2A for D9, and rabbit IgG for BRD7, AcH3, and Oct1. Total input DNA (5 ng) was used as a positive control for the PCR. The resulting complex was amplified using primers specific to the ESR1 promoter (top) or with ESR1 control primers, which amplify a sequence ~3 kb from the transcription start site (bottom). B, MCF7 cells were treated with siRNA specific to BRCA1, BRD7, or SCR control (left) or BRD7, Oct-1, or SCR control (right). At 72 h posttransfection either chromatin or nuclear extracts were prepared. The chromatin was immunoprecipitated with antibodies to Pol II, BRCA1, BRD7, Oct-1, or SCR control (right). 72 h posttransfection, either chromatin or nuclear extracts were prepared. The chromatin was immunoprecipitated with antibodies to Pol II, BRCA1, BRD7, Oct-1, or SCR control (right). The membranes were reprobed for actin to show equal protein loading. C, MCF7 cells were treated with siRNA specific to BRD7 or SCR control. The chromatin was immunoprecipitated with antibodies to Pol II, BRCA1, BRD7, BRG1, BAF155, or BAF57 and relevant controls IgG1 or rabbit IgG. The resulting complex was amplified using primers specific to the ESR1 promoter (top two panels) or with ESR1 control primers (bottom). Nuclear lysate (10 μg) was Western blotted for BRD7, BRG1, BAF155, or BAF57 and relevant controls IgG1 or rabbit IgG. The resulting complex was amplified using primers specific to the ESR1 promoter (top two panels) or with ESR1 control primers (bottom). Nuclear lysate (10 μg) was Western blotted for BRD7, BRG1, BAF155, or BAF57 and relevant controls IgG1 or rabbit IgG. The resulting complex was amplified using primers specific to the ESR1 promoter (top two panels) or with ESR1 control primers (bottom). D, T47D cells were transfected with constructs encoding either BRD7 or ΔBRD7 (Δ, deleted in the bromo-domain), and at 48 h posttransfection, either chromatin or nuclear extracts were prepared. The chromatin was immunoprecipitated with antibodies to Pol II, BRCA1, BRD7, and the relevant controls IgG1 or rabbit IgG. The resulting complex was amplified using primers specific to the ESR1 promoter (top two panels) or with ESR1 control primers (bottom). Nuclear lysate (10 μg) was Western blotted for BRD7. The membranes were reprobed for actin to show equal protein loading.
have been shown to bind specific transcription factors, for example, BAF60 binds directly to p53 (14).

BRCA1 has previously been shown to bind directly to BRG1 (8), which is one of the two ATPases present in Swi/Snf complexes. BRG1 is present in both BAF and PBAF Swi/Snf complexes. The interaction of BRCA1 with BRD7 would suggest that BRCA1 may be specific to PBAF Swi/Snf complexes. A glutathione S-transferase fusion protein composed of amino acids 260 to 553 of BRCA1 was able to associate with BRG1. We found that BRD7 was unable to bind in yeast to a BRCA1 construct composed of amino acids 303 to 927, which retains the BRG1 binding site. Thus it is likely that BRD7 and BRG1 interact independently and directly with BRCA1.

Figure 5. Depletion of either BRCA1 or BRD7 mediates resistance to the antiestrogen fulvestrant. T47D (A), MCF7 (B), or BT474 cells (C) were transfected with siRNA to BRCA1, BRD7, ERα, or a SCR sequence. At 24 h posttransfection, cells were plated onto 96-well plates. At 24 h later, the cells were treated with a range of concentrations of fulvestrant for a further 72 h, and cell number was quantitated using the MTT assay. Sigmoidal growth inhibition curves were generated, and growth is shown relative to untreated cells for each transfection. Nuclear extracts were prepared at 72 h posttransfection. Nuclear extract (10 μg) was Western blotted for BRCA1, BRD7, or ERα. The membranes were reprobed for actin to show equal protein loading.
The direct binding of BRCA1 to two components of the Swi/Snf complex suggests that each may have a specific function. The initial observation of BRCA1 interaction with BRG1 showed that an ATPase dead form of BRG1 was able to inhibit the BRCA1-mediated activation of p21 transcription by p53, suggesting that the chromatin remodeling activity of BRG1 is important in mediating its effects on BRCA1 transcription (8). We have shown that BRD7 is required for the recruitment of BRCA1 to the ESRI promoter. BRG1 is clearly not sufficient to recruit BRCA1 to the ESRI promoter, as shown by the loss of BRCA1 recruitment in the absence of BRD7, despite the fact that BRG1 is still retained on the promoter. Although the role of BRCA1 in transcriptional regulation is well established, BRCA1 cannot directly bind to DNA itself. BRD7 possesses a bromodomain and has been shown to colocalise with acetylated histone H3 and also to bind to a H3-derived peptide acetylated at Lys14 (15, 16). We have shown that the association of BRD7 with the ESRI promoter correlates with the acetylation of histone H3. In addition deletion of the bromodomain of BRD7 leads to a reduction in association of BRD7 with the ESRI promoter. BRCA1 binds to BRD7 between amino acids 277 to 652, which is outside of the bromodomain (amino acids 129–236). Thus it would be possible for BRD7 to simultaneously bind its acetylated histone target via its bromodomain and BRCA1 via its COOH terminal domain. Acetylation of histone H3 may play a role in recruiting BRD7 to the promoter, and BRD7 would then be able to recruit BRCA1 and Oct-1 and drive the transcription of ERα. Alternatively BRD7 may itself be recruited through interaction with other Swi/Snf subunits. BAF57 has been shown to bind directly to both ERα and the androgen receptor and is recruited to both estrogen- and androgen-responsive promoters in a ligand-dependent manner (17, 18). On this basis it has been proposed that BAF57 is important in targeting of the Swi/Snf complex to both ERα and androgen receptor–responsive promoters. Interestingly we have also shown that BRG1, BAF155, and BAF57 are all retained on the ESRI promoter irrespective of the presence of BRD7, and thus BAF57 or one of the other subunits may target BRD7 to the ESRI promoter. In summary our results support a model whereby, in the context of BRCA1-mediated transcription, the role of BRD7 is to recruit BRCA1 to specific promoters, whereas for BRG1, its chromatin remodeling activity is likely to be important.

As yet the only cancer in which the expression of BRD7 has been studied is in nasopharyngeal tumors, wherein its expression is downregulated relative to normal nasopharyngeal epithelial tissue (19). Overexpression of BRD7 has been shown to inhibit the growth of nasopharyngeal carcinoma cells and arrest the cells in the G1–G2 phase of the cell cycle (20). Other subunits of the Swi/Snf family have been implicated in particular cancers, for example, immunohistochemical staining of tissue microarray samples has revealed that BRG1 or BRM are lost in 10% to 20% of a range of tumor types, including breast and ovarian tumors. One Swi/Snf subunit, BAF17, has been classified as a tumor suppressor, as one allele is consistently lost and the other allele is either mutated or silenced by methylation in pediatric rhabdoid tumors (21). Our microarray data suggest that BRD7 may be important for the regulation of up to 30% of BRCA1 targets. It would therefore be interesting in the future to determine if, like BRG1, BRD7 may also be lost or inactivated in a subset of breast cancers and if this loss results in the development of tumors that display a BRCA1-like phenotype.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Dr. Julia Kzhyshkowska and Selen Systems GmbH for the BRD7 and ΔBRD7 expression constructs.

Grant Support

Medical Research Council (M.T. Harte and D.P. Harkin), Cancer Research UK (G.J. O’Brien, K.I. Savage, and D.P. Harkin), Breast Cancer Campaign (N.T. Crawford, P.B. Mullan, and D.P. Harkin), R&D Office NI (J.J. Gorski, P.B. Mullan, and D.P. Harkin), and Queens University Belfast (N.M. Ryan).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received 06/10/2009; revised 12/11/2009; accepted 01/04/2010; published OnlineFirst 03/09/2010.

References

11. Kzhyshkowska J, Rusch A, Wolf H, Dobner T. Regulation of


BRD7, a Subunit of SWI/SNF Complexes, Binds Directly to BRCA1 and Regulates BRCA1-Dependent Transcription


_Cancer Res_ 2010;70:2538-2547. Published OnlineFirst March 9, 2010.

Updated version  Access the most recent version of this article at: doi:10.1158/0008-5472.CAN-09-2089

Cited articles  This article cites 21 articles, 7 of which you can access for free at: http://cancerres.aacrjournals.org/content/70/6/2538.full.html#ref-list-1

Citing articles  This article has been cited by 9 HighWire-hosted articles. Access the articles at: /content/70/6/2538.full.html#related-urls

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.