The Activity of p53 Is Differentially Regulated by Brm- and Brg1-containing SWI/SNF Chromatin Remodeling Complexes*

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Brahma (Brm) and Brahma-related gene-1 (Brg1) ATPases share similarities in structure and function, but their presence in human SWI/SNF chromatin remodeling complexes is mutually exclusive. Although Brm and Brg1 can compensate for each other, it is possible that Brm and Brg1 have their unique properties to differentially regulate gene expression in vivo. To explore this, we examined the requirement of Brm and Brg1 for p53-dependent transcription, especially p53-mediated induction of p21 and MDM2, using cell lines in which Brm or Brg1 could be inducibly knocked down. We found that Brg1, but not Brm, is required for p21 induction in MCF7 cells. However, in Brg1-deficient H1299 cells, Brm is also required for p21 induction. Likewise, Brm is necessary for induction of p21 in MCF7 cells in which Brg1 is stably knocked down. In contrast, Brg1 has little, if any, effect on p53-mediated induction of MDM2 in cells that have Brm and vice versa. In addition, we demonstrated that the impaired induction of p21 upon Brg1 knockdown is at least in part due to decreased p53 binding to the p21 promoter. Taken together, we provided evidence that Brg1 is preferentially recruited by p53 for inducing a subset of target genes through chromatin remodeling. Thus, we hypothesize that the potential tumor suppressor function for Brg1 is mediated in part through the p53 pathway.

The highly condensed chromatin packaged with genomic DNA forms a repressive structure that tends to restrict the access of transcription factors and co-factors to DNA, and relief from this repression by chromatin remodeling is critical for gene transcription (1–3). Generally, chromatin remodeling is achieved through two mechanisms: covalent modifications such as acetylation of histone tails and disruption or alteration of the local histone-DNA association by ATP-dependent chromatin remodeling complexes (3, 4). Currently, four distinct classes of chromatin remodeling complexes, SWI/SNF, ISWI, Mi-2, and Ino80, have been identified (5–7).

All chromatin remodeling complexes contain a core ATPase that has a homology to the yeast SWI2/SNF2 (8). Human SWI/SNF chromatin remodeling complexes contain either Brahma (Brm)3 or Brahma-related gene 1 (Brg1) as an ATPase subunit (7). Brm and Brg1 are evolutionarily conserved with a considerable degree of amino acid sequence identity, and their functions appear to be partially redundant. It has been well established that Brg1 and Brm are involved in transcriptional activation as well as transcriptional repression for a subset of genes (9). For example, both Brm and Brg1 are required for activation of genes such as CD44 (10) and EPO (11) and interact with prohibitin (12) and TopBP (13) for transcriptional repression. Moreover, Brg1 can functionally replace Brm and vice versa (14). Nevertheless, the presence of Brm and Brg1 in an SWI/SNF chromatin remodeling complex is mutually exclusive, and functional differences between Brm and Brg1 have been implicated from in vitro and in vivo studies. First, knock-out mice studies revealed that Brg1 is embryonically lethal, but heterozygous Brg1+−/− is viable and cancer-prone (15). In contrast, Brm−−/− develops normally with increased body weight and is not prone to cancer (16). Second, Brm and Brg1 are differentially expressed in various normal tissues. For instance, Brg1 is predominantly expressed in cells that constantly undergo proliferation or self-renewal (17). Third, Brm and Brg1 display variable binding affinities to certain transcription factors in vitro. For example, Brg1 specifically associates with zinc finger proteins, whereas Brm preferentially interacts with ankyrin repeat family proteins (18). These differences between Brm and Brg1 suggest that distinct target genes may be regulated by Brg1 and Brm.

The tumor suppressor p53 functions as a potent transcription factor that activates or represses a plethora of target genes to regulate multiple cellular pathways, such as the cell cycle, apoptosis, DNA repair, and metabolism (19). In response to DNA damage and other stress signals, p53 is stabilized and activated. Upon binding to consensus p53-responsive elements present in the promoters, p53 trans activates many downstream targets including p21 and MDM2 (19). Because the accessibility of p53 to its target gene promoters is the first step for activating gene expression, it has been subject to extensive investigations (20–22). Indeed, recent studies have demonstrated that p53-dependent recruitment of histone acetyltransferases and targeted acetylation of histones at the promoters of p53 target genes is mediated in part through the p53 pathway.

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3 The abbreviations used are: Brm, Brahma; Brg1, Brahma-related gene 1; siRNA, small interfering RNA; ChIP, chromatin immunoprecipitation; HA, hemagglutinin.
genes correlate well with the ability of p53 to activate gene expression (23–25). However, when compared with histone acetylation, the role of the SWI/SNF chromatin remodeling complex in p53-dependent transcription is less understood. Although the exogenous Brg1 was shown to interact with, and enhance, p53 to activate a reporter under the control of the p21 promoter (26), whether endogenous Brg1 and Brm play a role in the p53 pathway remains unknown.

In this study, we analyzed p53-mediated induction of p21 and MDM2 using cell lines in which Brm or Brg1 can be inducibly knocked down. We found that p21 induction is impaired by Brg1 but not Brm knockdown in MCF7 cells. However, in Brg1-deficient H1299 cells, p21 induction is also decreased by Brm knockdown. Likewise, Brm is necessary for induction of p21 in MCF7 cell in which Brg1 is stably knocked down. In contrast, Brm and Brg1 have little, if any, role in p53-mediated induction of MDM2. We also demonstrated that the impaired induction of p21 upon Brg1 knockdown is at least in part due to decreased p53 binding to the p21 promoter. Taken together, we provided evidence that Brm and Brg1 differentially regulate a subset of p53 target genes through chromatin remodeling.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—To generate an inducible small interfering RNA (siRNA) against Brg1 or Brm, an siRNA was cloned into pBabe-H1 vector as described previously (28), and the resulting construct was designated as pBabe-H1-siBrg1 or pBabe-H1-siBrm. The sense oligonucleotide in pBabe-H1-siBrg1 (targeting region shown in upper case) is 5′-gatccccAACATGCACCAATGCACAGTtgcaagaCTTGTGACATCTGGTGACAACGGTTCAGAGTAACAGGCTAAGG-3′, and the antisense oligonucleotide is 5′-agcttttccccaaaaAACATGCACCAATGCACAGTtgcaagaCTTGTGACATCTGGTGACAACGGTTCAGAGTAACAGGCTAAGG-3′, and the antisense oligonucleotide is 5′-agcttttccccaaaaAACATGCACCAATGCACAGTtgcaagaCTTGTGACATCTGGTGACAACGGTTCAGAGTAACAGGCTAAGG-3′. The sense oligonucleotide in pBabe-H1-siBrm is 5′-gatccccAACATGCACCAATGCACAGTtgcaagaCTTGTGACATCTGGTGACACGGTTCAGAGTAACAGGCTAAGG-3′, and the antisense oligonucleotide is 5′-agcttttccccaaaaAACATGCACCAATGCACAGTtgcaagaCTTGTGACATCTGGTGACACGGTTCAGAGTAACAGGCTAAGG-3′. To generate a construct stably expressing Brg1 siRNA, an siRNA was cloned into pBabe-H1 vector, and the resulting construct was designated as pBabe-U6-siBrg1 or pBabe-U6-siBrm. The sense oligonucleotide in pBabe-H1-siBrg1 is 5′-agcttttccccaaaaAACATGCACCAATGCACAGTtgcaagaCTTGTGACATCTGGTGACACGGTTCAGAGTAACAGGCTAAGG-3′, and the antisense oligonucleotide is 5′-agcttttccccaaaaAACATGCACCAATGCACAGTtgcaagaCTTGTGACATCTGGTGACACGGTTCAGAGTAACAGGCTAAGG-3′. To generate a construct stably expressing Brg1 siRNA, an siRNA was cloned into pBabe-H1 vector, and the resulting construct was designated as pBabe-U6-siBrg1 or pBabe-U6-siBrm. The sense oligonucleotide in pBabe-U6-siBrg1 is 5′-tcgaggttcAACATGCACCAATGCACAGTtgcaagaCTTGTGACATCTGGTGACACGGTTCAGAGTAACAGGCTAAGG-3′, and the antisense oligonucleotide is 5′-tcgaggttcAACATGCACCAATGCACAGTtgcaagaCTTGTGACATCTGGTGACACGGTTCAGAGTAACAGGCTAAGG-3′. To generate cell lines in which Brg1 or Brm can be inducibly knocked down under the control of the tetracycline-inducible promoter, pBabe-H1-siBrg1 or pBabe-H1-siBrm was transfected into MCF7 cells in which a tetracycline repressor is expressed by pcDNA6 (28). Brg1 and Brm knockdown cell lines were selected with puromycin and confirmed by Western blot analysis. To generate cell lines in which p53 is stably knocked down and is inducibly knocked down, pBabe-U6-sip53 was co-transfected with pcDNA4, which confers zeocin resistance, into inducible Brg1 knockdown MCF7 cells and then selected with zeocin. To generate cell lines in which Brg1 is stably knocked down and Brm is inducibly knocked down, pBabe-U6-siBrm was co-transfected with pcDNA4 into Brm-inducible knockdown MCF7 cells.

**Reverse Transcription-PCR**—Total RNAs were isolated from MCF7 cells, which were uninduced or induced to express Brg1 siRNA for 3 days along with or without treatment of 0.5 μM doxorubicin for 8 h. First-strand cDNA was synthesized by using iScript according to the instructions of the manufacturer (Bio-Rad). A 160-bp cDNA fragment of p21 was amplified with forward primer 5′-CATGTTGACCTGTCACTGTGCTGCT-AACGGTGTCGACACAGTGCTTGG-3′ and the reverse primer 5′-CTCGTGGAGAAGATCCGGACC-3′. A 225-bp cDNA fragment of actin was measured as a loading control, which was amplified with forward primer 5′-CTGAGGATACCCATCGAAGCAGGCA-3′ and the reverse primer 5′-GGATACGACACGCTGTGGAACAGC-3′.

**Luciferase Assay**—Dual luciferase assay was performed in triplicate according to the instructions of the manufacturer (Promega). The pGL2-p21A luciferase reporter under the control of the two p53-responsive elements in the p21 promoter was used (29). Briefly, 100 ng of pGL2-p21A luciferase reporter, 100 ng of pcDNA3 or pcDNA3-HA-p53, and 5 ng of Renilla luciferase assay vector pRL-CMV (Promega) were co-transfected into H1299 or MCF7 cells. The -fold increase in relative luciferase activity is a product of the luciferase activity induced by p53 divided by that induced by an empty pcDNA3 vector.

**Chromatin Immunoprecipitation (ChIP) Assay**—ChIP assay was performed as described previously (30). After induction (+) or no induction (−) of Brg1 or Brm siRNA for 72 h, cells were treated with doxorubicin for the indicated time, cross-linked with 1% formaldehyde for 10 min at room temperature, and then sonicated to generate 500–1000-bp DNA fragments and immunoprecipitated with anti-p53 (DO-1) and control mouse IgG. After reverse cross-linking and phenol-chloroform extraction, the bound DNA fragments were purified by a Qia-gen column. PCR was performed to visualize the enriched DNA fragments. Primers designed to amplify the region from nucleotide −2312 to −2131 in the p21 promoter were forward primer 5′-CAGGCTGGGTCTCTGATTTGG-3′ and the reverse primer 5′-TTCAGAGTAAACAGGCTAAGG-3′. Primers designed to amplify the region from nucleotide +3779 to +3969 in the MDM2 intron 1 were forward primer 5′-GGATTGGGCGGTTTCACTGTTGG-3′ and the reverse primer 5′-GGTCTACCCTCAATC-GCCAC-3′.

**Colony Formation Assay**—MCF7 and H1299 cells were seeded at 500 per well in a 6-well plate were cultured in the absence or presence of tetracycline (1 μg/ml) for 12–14 days and then stained with crystal violet.

**Western Blot Analysis**—Whole cell extracts were prepared by lysing cells with 2× SDS sample buffer. Proteins were separated on 7–10% SDS-PAGE, transferred to a nitrocellulose membrane, and probed with the indicated antibodies followed by ECL detection. Antibodies against Brg1 (G7), Brm (E1), p21 (C19), cyclin B, p130, and MDM2 (SMP14) were purchased from Santa Cruz Biotechnology. Antibodies against p53, HA epitope, and actin were described previously (31).
Knockdown of Brg1 Impairs p53-Dependent Induction of p21 in MCF7 Cells—Overexpression of Brg1 can promote p53 to activate the p21 promoter by luciferase assay (26). However, it is not clear whether endogenous Brg1 is required for endogenous p53 to induce its target genes. To explore this, we examined induction of p21 and MDM2 in MCF7 cells in which Brg1 is inducibly knocked down. We found that upon knockdown of Brg1 and Brm had no effect on the level of Brg1 (Fig. 1C, compare lanes 1 and 2). Similarly, knockdown of Brg1 had no effect on the level of Brm (Fig. 1C, compare lanes 3 and 4). Furthermore, we found that knockdown of Brg1 or Brm had no significant effect on the level of SNF5, a core subunit of the SWI/SNF complex (Fig. 1C, compare lanes 1 and 3 with lanes 2 and 4, respectively). Together, we confirmed that Brg1 and Brm can be inducibly knocked down, and Brg1 and Brm do not compensate for each other at the level of expression.

Knockdown of Brg1 Impairs p53-Mediated Induction of p21 in MCF Cells—Overexpression of Brg1 can promote p53 to activate the p21 promoter by luciferase assay (26). However, it is not clear whether endogenous Brg1 is required for endogenous p53 to induce its target genes. To explore this, we examined induction of p21 and MDM2 in MCF7 cells in which Brg1 is inducibly knocked down. We found that upon treatment with Nutlin-3, an inhibitor of p53-MDM2 interaction (32), or doxorubicin, p53 was stabilized and subsequently p21 was induced (Fig. 2A). However, the extent of p21 expression was markedly decreased when Brg1 was inducibly knocked down (Fig. 2A, compare lanes 3, 5, 9, and 11 with lanes 4, 6, 10, and 12, respectively). Interestingly, the level of p53 stabilized by treatment with Nutlin-3 or doxorubicin were not changed significantly.
p53 Activity Is Differentially Regulated by Brm and Brg1

Although p21 is a bona fide target of p53, it can be regulated by other p53-independent mechanisms (33). To demonstrate that the effect of Brm knockdown on p21 induction is p53-dependent, we generated cell lines in which p53 is stably knocked down and Brm1 can be inducibly knocked down. One representative cell line, MCF7-Brm-KD-p53-KD-13, was chosen for further studies. As shown in Fig. 2D, upon induction of Brm1 siRNA, the level of Brm1 was decreased significantly in both MCF7-Brm-KD-p53-KD-13 (Fig. 2D, Brm1 panel, lanes 1–6) and MCF7-Brm-KD-p53-KD-13 (Fig. 2D, Brm1 panel, lanes 7–12). In addition, p53 was undetectable upon treatment with doxorubicin or camptothecin in MCF7-Brm1-KD-p53-KD-13 cells, suggesting that p53 was efficiently knocked down (Fig. 2D, p53 panel, compare lanes 3–6 with lanes 9–12). We found that induction of p21 by doxorubicin or camptothecin was completely abrogated in p53 knockdown MCF7 cells (2D, compare lanes 3–6 with lanes 9–12). Consistent with the data above, induction of p21, but not MDM2, was reproducibly decreased by Brm1 knockdown in MCF7 cells upon treatment with doxorubicin or camptothecin (2D, compare lanes 3 and 5 with lanes 4 and 6, respectively). Taken together, these data suggest that in MCF7 cells, Brm1 is necessary for DNA damage-mediated induction of p21 in a p53-dependent manner.

Knockdown of Brm Has No Significant Effect on p53-mediated Induction of p21 in MCF7 Cells—Unlike Brm1, little is known about the role of Brm in p53-mediated transcription. Thus, we examined p53 induction of p21 and MDM2 in MCF7 cells in which Brm is inducibly knocked down. We found that upon treatment with doxorubicin, p21 was induced in a time-dependent manner (Fig. 3A). However, the extent of p21 expression was not changed upon knockdown of Brm (Fig. 3A, compare lanes 3 and 5 with lanes 4 and 6, respectively). Likewise, Brm knockdown had no effect on p53 stabilization and induction of MDM2 (Fig. 3A). To confirm this, another cell line, MCF7-Brm-KD-24, was treated with doxorubicin for 4 and 8 h. We found that the extent of p21 induction was not affected upon Brm knockdown, although the extent of MDM2 induction was inhibited in the first 4 h following treatment with doxorubicin (Fig. 3B, compare lanes 3 and 5 with lanes 4 and 6, respectively). Because the phosphorylation status of p130 is known to be regulated upon Brm down-regulation, we examined the level of p130 in MCF7 cells with or without Brm knockdown in the presence or absence of doxorubicin treatment. The level of cyclin B protein was also examined as a control. We showed that the level of cyclin B was not affected by Brm knockdown (Fig. 3C). Interestingly, although the phosphorylation status of p130 in the absence of doxorubicin was not affected by Brm knockdown (Fig. 3C, p130 panel, compare lanes 1 and 2), the level of hyper-phosphorylated p130 was markedly increased upon Brm knockdown in the presence of doxorubicin treatment (Fig. 3C, p130 panel, compare lanes 3 and 5 with lanes 4 and 6, respectively). These data are consistent with previous reports (16, 34). Furthermore, upon treatment with Nutlin-3, the levels of p53, p21, and MDM2 remained unchanged by Brm knockdown (Fig. 3D, compare lanes 3 and 4). We would like to mention that upon treatment with Nutlin-3, the activity of p53 was substantially increased, whereas the level of p53 was only slightly increased, consistent with the earlier report (32). Taken together, these data suggest that knockdown of Brm alone does not affect p53-dependent transcription in MCF7 cells.

Brm Is Necessary for p53-mediated Induction of p21 in Brm1-deficient Cells—Although the extent of knockdown for Brm1 and Brm was comparable in MCF7 cells, knockdown of Brm1, but not Brm, diminished induction of p21-mediated by p53. However, most studies found that Brm1 and Brm have similar functions and, to some degree, can compensate for each other. Therefore, we reasoned that the lack of effect for Brm may be
p53 Activity Is Differentially Regulated by Brm and Brg1

To extend the above observations, we asked whether Brm has an effect on induction of p21 and MDM2 in a Brg1-deficient context. To accomplish this, Brml was stably knocked down in Brm knockdown MCF7 cells, and one representative cell line, MCF7-Brm-KD-Brg1-KD-12, was chosen for further studies. As shown in Fig. 4D, the level of Brm was decreased upon induction of Brm siRNA (Fig. 4D, Brm panel, compare lanes 2 and 3), whereas Brg1 was stably knocked down regardless of treatment with tetra-cycline (Fig. 4D, Brg1 panel, lanes 2 and 3). We showed that upon treatment with doxorubicin or Nutlin-3, p21, and MDM2 were induced, but the extent of their induction was substantially attenuated by Brm knockdown in MCF7 cells when Brg1 was stably knocked down (Fig. 4E, compare lanes 3 and 5 with lanes 4 and 6, respectively). However, the levels of p53 were not substantially affected by Brm knockdown (Fig. 4E, compare lanes 3 and 4). These data were consistent with the results detected in H1299 cells (Fig. 4C). However, we would like to note that although Brg1 can be efficiently knocked down by a constitutively expressed siRNA against Brg1, a minute amount of Brg1 is still expressed (Fig. 4D), which might be responsible for the relatively higher level of p21 expression in MCF7 cells (Fig. 4E) compared with H1299 cells (which is Brg1-null) (Fig. 4C) upon inducible Brm knockdown. Taken together, our data indicate that Brm is necessary for p53-dependent induction of p21 and MDM2 in the absence of Brg1.

Brml Promotes p53 Binding to the p21 Promoter through Chromatin Remodeling—To gain an insight into the role of Brm and Brg1 in the p53 pathway, we sought to examine whether knockdown of Brm and Brg1 has an effect on p53 to activate the p21 promoter. To test this, a luciferase reporter under the control of the p21 promoter with two p53-responsive elements was co-transfected with p53 into MCF7 or H1299 cells. We found that knockdown of Brml or Brg1 had no effect on p53 to activate the p21 promoter in MCF7 cells (Fig. 5A). Likewise, knockdown of Brm also had no effect on induction of p21 promoter and MDM2 in the absence of Brg1.

Chromatin Remodeling

Our data indicate that Brm is necessary for p53-dependent induction of p21 and MDM2, which has been confirmed by the previous studies. Furthermore, our data also show that Brg1 is not necessary for p53-dependent induction of p21 and MDM2 in the absence of Brm. Taken together, our data suggest that Brm is the only functional ATPase present in the SWI/SNF complex, which must be recruited by p53 for gene induction.

Due to the possibility that Brg1 is preferentially utilized by p53 for chromatin remodeling, the activity of Brm for p53 is compensated by Brg1. However, because Brg1 is frequently lost in cancers, Brm would become the only ATPase present in the SWI/SNF complex, which must be recruited by p53 for gene induction.

Our data also show that p53 activity is subject to extensive regulations. Because the levels of p53 protein induced by DNA damage were not affected by knockdown of Brm or Brg1, we performed a ChIP assay to

FIGURE 4. Brm is necessary for p53-mediated induction of p21 in Brg1-deficient cells. A, Western blots were prepared with extracts from H1299 and MCF7 cells and analyzed with antibodies against Brm, Brg1, and actin. B, Western blots were prepared using extracts from H1299 cells that were uninduced (−) or induced (+) to knock down Brm for 48 h and then transiently transfected with an empty pcDNA3 or pcDNA3 expressing HA-tagged p53 for 24 h. The blots were analyzed with antibodies against MDM2, HA, p21, and actin. C, Western blots were prepared using extracts from MCF7 cells that were uninduced (−) or induced (+) to knock down Brm for 48 h and then transiently transfected with an empty pcDNA3 or pcDNA3 expressing HA-tagged p53 for 24 h. The blots were analyzed with antibodies against MDM2, HA, p21, and actin. D, Western blots were prepared using extracts from MCF7-Brm-KD-9 cells that were uninduced (−) and from MCF7-Brm-KD-Brg1-KD-12 that were uninduced (−) or induced (+) to knock down Brm for 72 h. The blots were analyzed by antibodies against Brm, actin, and Brg1. E, Western blots were prepared using extracts from MCF7-Brm-KD-Brg1-KD-12 cells that were uninduced (−) or induced (+) to knock down Brm for 72 h followed by mock treatment or treatment with 0.5 μg doxorubicin (Dox) for 6 h or 2.0 μM Nutlin-3 for 4 h. The blots were analyzed with antibodies against Brm, MDM2, p53, p21, and actin. ctrl, control.
p53 Activity Is Differentially Regulated by Brm and Brg1

FIGURE 5. Knockdown of Brg1, but not Brm, decreases p53 binding to the p21 promoter. A, chromatin remodeling activity is not required for p53 to activate transiently transcribed exogenous p21 promoter. pGL2-p21 luciferase reporter and pcDNA3 or pcDNA3-HA-p53 were co-transfected with pRL-CMV into MCF7-Brm-KD-9 cells that were uninduced (-) or induced (+) to knock down Brm for 48 h or MCF7-Brg1-KD-5 cells that were uninduced (-) or induced (+) to knock down Brg1 for 48 h. The luciferase assay was carried out 24 h following transfection as described under “Experimental Procedures.” B, the experiment was carried out similarly as A except H1299-Brm-KD-13 and H1299-Brm-KD-15 cell lines were used. C and D, schematic presentation of the p21 (C) and MDM2 (D) promoters with the locations of the p53-responsive elements and PCR primers used for ChIP assay. E, knockdown of Brg1 decreases the ability of p53 to bind to the p53-responsive element in the p21 promoter (upper panels) but not to that in the MDM2 promoter (lower panels). MCF7-Brg1-KD-5 cells were uninduced (-) or induced (+) to knock down Brg1 for 72 h followed by mock treatment or treatment with 0.5 μM doxorubicin for 4 and 8 h. ChIP assays were performed as described under “Experimental Procedures.” F, knockdown of Brg1 has no significant effect on p53 DNA binding activity. The experiment was performed as in E except that MCF7-Brg1-KD-9 was used.

DISCUSSION

Several studies have shown that Brm and Brg1 are equivalent to or compensatory for each other in transcriptional activation and repression (10–14). However, whether Brm and Brg1 are able to differentially regulate a specific transcription pathway in vivo has not been systematically analyzed. In this study, we demonstrated that Brm and Brg1 can differentially regulate p53-dependent p21 transcription. Knockdown of Brg1, but not Brm, impairs p53-dependent p21 transcription in MCF7 cells. However, when Brg1 is absent, knockdown of Brm also inhibits p21 transcription. Next, we examined whether the dependence of p53 on Brg1 to induce p21 has an effect on the ability of p53 to induce cell cycle arrest. To test this, colony formation assay was performed, and it showed that knockdown of Brg1 led to decreased cell proliferation, consistent with the previous report that mice deficient in Brg1 are embryonic lethal and MDM2 panels, compare lanes 3 and 5 with lanes 4 and 6, respectively. In contrast, Brm knockdown had no effect on the extent of p53 binding to either the p21 or MDM2 promoter (Fig. 5F, p21 and MDM2 panels, compare lanes 3 and 5 with lanes 4 and 6, respectively). This was consistent with the requirement of Brg1 for p53 induction of p21 in MCF7 cells. Taken together, we found that Brg1 is necessary for p53 transcriptional activity by modulating p53-DNA interaction.

examine whether Brm or Brg1 regulates p53 binding to the p21 promoter. To test this, p53 was activated in MCF7 cells treated with doxorubicin, and the p53-DNA complexes were immunoprecipitated with anti-p53 (DO-1) as well as mouse IgG as a negative control. To visualize the enriched DNA fragments, PCR was performed to amplify the region spanning the p53-responsive elements in the p21 and MDM2 genes with primers shown in Fig. 5, C and D, respectively. We showed that the extent of p53 binding to the p21 and MDM2 promoters was increased in MCF7 cells upon doxorubicin treatment (Fig. 5, E and F, p21 and MDM2 panels), whereas no DNA fragment was enriched by control antibody (Fig. 5, E and F, IgG panels), suggesting that the p53-DNA binding is specific. Interestingly, we found that Brg1 knockdown markedly inhibited p53 binding to the p21 promoter but not to the MDM2 promoter (Fig. 5E, p21 (15). As a result, the effect of Brg1 on p53 to induce cell cycle arrest would be countered by the strong inhibition of cell proliferation by Brg1. Thus, future studies are needed to examine the role of p53 in the Brg1 tumor suppression.

The functional difference of Brm and Brg1 in the regulation of p21 transcription raises several possibilities. The first possibility is that Brm and Brg1 differ in their ability or efficiency of chromatin remodeling. The in vitro ATPase activity of Brg1 was found to be 4-fold higher than that of Brm. The Brg1 complex was also found to remodel chromatin more efficiently than the Brm complex. Thus, the effect of Brg1 knockdown on the extent of chromatin remodeling is likely to be greater than that by Brm knockdown. If efficient p21 transcription requires a “threshold” of chromatin remodeling, knockdown of Brg1, but not Brm, is likely to
inhibit chromatin remodeling to an extent below the threshold that is required for p21 transcription. Likewise, if Brm is not present, knockdown of Brm readily diminishes the chromatin remodeling to a level below the threshold. The second possibility is that the Brm- and Brg1-containing complexes have differential properties that may account for their distinct functions in p21 transcription. For example, Brm or Brg1 can serve as an ATPase for the BAF-BAP25 complex, whereas Brg1 is the sole ATPase for the PBAF-BAF180 complex. As a result, Brg1 can compensate for Brm loss within the BAF complex, but Brm cannot replace Brg1 in the PBAF complex. Knockdown of Brm will completely inactivate PBAF complex along with partial loss of BAF function. In contrast, knockdown of Brg1 will completely inactivate PBAF complex along with partial loss of BAF functions. Thus, it is likely that PBAF is preferentially recruited by p53 for p21 transcription. Consistent with this hypothesis, we found little, if any, effect of Brg1 knockdown on MDM2 transcription. The third possibility is that Brm and Brg1 are differentially recruited to the p53-regulated promoters, such as p21, because p53 and/or p53 co-activators may interact differentially with Brg1 and Brm. Although Brg1 and Brm do not contain any canonical DNA-binding domains, they can be recruited to a promoter via interaction with transcription factors, such as c-Myc, EKLF, and the nuclear receptors. We would like to mention that we failed to detect any direct physical interaction between endogenous p53 and Brg1 or Brm (data not shown). However, it remains possible that a co-activator in the SWI/SNF complex may mediate an indirect interaction between p53 and Brg1 or Brm.

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