The SWI/SNF Chromatin Remodeling Subunit BAF57 Is a Critical Regulator of Estrogen Receptor Function in Breast Cancer Cells*

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Estrogen receptors (ERs) play critical roles in both normal mammary gland development and in the formation and progression of breast tumors, constituting a major therapeutic target for breast cancer treatment. We have previously described that ER transcriptional activity is potentiated by BAF57, a core subunit of the mammalian SWI/SNF chromatin remodeling complex. Here we provide evidence demonstrating an important role for BAF57 as regulator of ER functions in breast cancer cells. Different experimental manipulations leading to the abrogation of BAF57 expression and/or function severely reduced the expression of various endogenous ER target genes and blocked estrogen-stimulated proliferation in ZR-75-1 breast cancer cells. Moreover, using a structure-function analysis, we have defined the protein domains required for the functional interaction between ERα and BAF57, including a key region within the hinge of ERα that is essential for BAF57 recruitment and its function on ER-mediated transcription. Interestingly, we found that BAF57 is an ER subtype-selective modulator that specifically regulates ERα-mediated transcription. Taken together, our results suggest that targeting BAF57 could represent a new way to effectively inhibit the action of ERα.

Breast cancer is a leading cause of cancer mortality among Western women with an estimated annual incidence of 1 million cases worldwide (see Ref. 1 and references therein). Estrogen hormones play a critical role in the development and normal physiology of the mammary gland, and, whereas various factors contribute to the etiology of breast cancer, estrogens within the context of other signaling pathways are an important influence in the initiation and progression of breast tumors (reviewed in Ref. 2). Estrogen effects are mediated by the related estrogen receptors α (ERα) and β (ERβ), although accumulating evidence suggests that ERα is the main transducer of estrogen signals promoting cell proliferation in both normal and cancerous breast tissues (3–5).

Estrogens can act through nongenomic pathways, although most described estrogen actions are mediated by ERα and ERβ acting as ligand-dependent transcription factors. Estrogen receptors belong to the nuclear receptor superfamily (6), and they regulate many physiological processes in response to their natural ligand, 17β-estradiol (E2). Upon ligand binding, ERs bind to specific DNA sequences (EREs) present in the promoters of their target genes, triggering the recruitment of many cofactors that must overcome the barrier to transcription that represents the tightly packed chromatin fiber and recruit basal transcription factors and RNA polymerase II. The chromatin remodeling factors are usually divided into two distinct categories: first those enzymes responsible for the covalent modification of the histones, and second, ATP-dependent protein complexes that modify the location and association of nucleosomes with the DNA. Both classes of chromatin remodeling factors, acting together in a concerted manner, contribute to the generation of a dynamic chromatin structure around the promoters that ultimately will modulate gene transcription.

Several ATP-dependent chromatin complexes, including the mammalian SWI/SNF, ISWI, NURD, and WINAC, have been implicated in the regulation of the transcriptional activation mediated by NRs (7). The study of the detailed molecular mechanisms by which these complexes are recruited to target promoters and their functional interactions with other histone-modifying enzymes, the mediator complex, and the RNA polymerase II have been under extensive research during the past 10 years. SWI/SNF was the first chromatin remodeling complex directly involved in the regulation of several NRs, including ER. Transient transfection studies initially showed that the co-expression of BRM/SNFα and BRG1/SNFβ, the catalytic subunits of the human SWI/SNF complex, were able to potentiate the transcriptional activation by ER and other NRs (8, 9). Subsequently, in vitro purified chromatin-dependent transcription systems revealed that SWI/SNF complexes and another family of ATP-dependent chromatin complexes, ISWI, are selectively required for the ligand-dependent transactivation for different NRs (10–12).
The SWI/SNF family comprises a number of large multiprotein complexes containing between 8 and 10 subunits (reviewed in Ref. 13). These complexes always contain a single catalytic subunit, BRM/SNFα or BRG1/SNFβ, and several other variable BRG1-associated factors (BAFs) that contribute to the enzymatic activity of the complex and facilitate the recruitment to sequence-specific transcription factors, although the detailed functions of each individual subunit remain to be determined. We have previously identified an interaction between one of these subunits, BAF57, and ER, which is stimulated by estrogen and requires a functional hormone-binding domain. Moreover, BAF57 interacts directly with the p160 family of coactivators, and it is necessary for their ability to stimulate transcription by the ER, and BAF57 was shown to be recruited to estrogen-responsive promoters in a ligand-dependent manner (14).

In the present report, we investigate the importance of BAF57 for ER function in human breast cancer cells. We demonstrate that the levels of BAF57 in the cells are critical for the correct transmission of estrogen-dependent signals. Different experimental approaches leading to the reduction of BAF57 expression or its function specifically inhibited the expression of endogenous ER target genes and blocked estrogen-dependent cell proliferation. We also present evidence suggesting that BAF57 is a specific regulator for the ERα isoform, and we further characterize the domains in ER required for its functional interaction with BAF57.

EXPERIMENTAL PROCEDURES

Plasmids—The complete open reading frame of the full-length human BAF57 was PCR-amplified using cDNA from ZR-75-1 breast cancer cells and subcloned into pSG5 (Amer- sham Biosciences).

The following plasmids have been previously described: pSG5-mERα, pSG5-SRC1a, pSG5-SRC1α, RAC3, pGL3–2ERE–PS2–LUC, GST–BASF57(1–411), GST–N–BASF57(1–200), GST–C–BASF57(201–411), GST–NHR–L173(250), hERβ (14), pSG5–H1(261–275), pSG5–H2(253–307), pSG5–H3(245–307), pSp6–MOR–S122A, pMT2–MOR(121–599), pSp6–MOR(121–599), pGL3–2ERE–PS2–LUC (10 ng), and the expression vectors pSG5-mERα, pMT2–MOR(121–599), pSp6–MOR(121–599) (10 ng). Empty vectors were used to normalize DNA amounts.

Viral Stocks—The viral stocks were then used to transduce ZR-75-1 cells, and pooled siRNA duplexes using Lipofectamine 2000 (Invitrogen). RNA was extracted using Trizol (Gibco) and gene expression levels were normalized to L19 levels. Primer sequences may be obtained on request.

Generation of Stable Cell Lines by Using the Lentiviral Expression System—To generate the pLenti-hBAF57(ΔC) expression construct, the cDNA fragment corresponding to the residues 1–200 of human BAF57 was subcloned into pLenti6/V5-D-TOPO vector (Invitrogen). Lentiviral particles were produced in 293FT cells using the ViraPower™ lentiviral expression system (Invitrogen) following the manufacturer’s protocol. The viral stocks were then used to transduce ZR-75-1 cells, and independent colonies stably expressing the recombinant protein Lenti-hBAF57(ΔC) were isolated by blasticidin selection (8 μg/ml).

Proliferation Assays—Cells were plated into 24-well plates in phenol red-free, antibiotic-free medium with 5% dextran charcoal-stripped serum and infected with siRNAs using Lipofectamine 2000 (Invitrogen). 24 h after transfection, 17β-estradiol (Sigma) was added, and cell proliferation was measured at different time points. Growth medium and hormones were replaced every 2–3 days. Quantification of cell growth was determined in quadruplicates using the CellTiter One Solution Assay (Promega) and reading absorbance at 490 nm.

RESULTS

BAF57 Is Required for Estrogen-dependent Gene Expression in Breast Cancer Cells—To investigate the role of BAF57 as regulator of ER functions in ZR-75-1 breast cancer cells, we employed two different experimental strategies designed to selectively block BAF57 expression and/or function in these cells, namely RNA interference and the use of dominant negative mutants. Transfection with BAF57 siRNAs (siBAF57) specifically and effectively reduced BAF57 expression at both mRNA and protein levels (Fig. 1, A and B). As a control, a commercially available nontargeting siRNA (siCONTROL) with no
known homology to mammalian genes was used, and no change in BAF57 expression was observed (Fig. 1, A and B).

We then analyzed the effect of BAF57 depletion on the expression of endogenous ER target genes, monitoring mRNA levels by quantitative reverse transcription-PCR (QRT-PCR). The mRNA expression levels of a number of well characterized ER target genes (pS2, cathepsin D, progesterone receptor, BRCA1, BRCA2, and bcl-2) increased in ZR-75-1 cells in response to treatment with 17β-estradiol (E2) for 24 h, as shown in Fig. 1C. The E2-dependent activation of all of these genes was markedly impaired by transfection with siBAF57 (Fig. 1C), indicating that BAF57 is important for the transcriptional activity of endogenous ER. No effects were observed with siCONTROL, confirming the specificity of siBAF57 (Fig. 1C). Interestingly, we detected a significant increase in the levels of both BAF57 mRNA and protein upon E2 stimulation (Fig. 1, A and B), suggesting that BAF57 itself could be a novel target for estrogens.

To corroborate our findings following BAF57 depletion, we examined the effects of dominant negative mutants of BAF57 (14) on the expression of estrogen target genes. To accomplish this, we used lentiviral transduction to generate ZR-75-1-derived cell lines stably expressing a carboxyl-terminal truncated BAF57, BAF57(ΔC). Individual blasticidin-resistant clones were isolated, and BAF57(ΔC) expression was examined by QRT-PCR (Fig. 2A). Using a set of primers located within the C-terminal region of BAF57, designed to detect both endogenous BAF57 and exogenous BAF57(ΔC), we observed a 2-fold increase in BAF57 expression levels in three independent clones ZR-BAF57(ΔC) as compared with the parental cell line that presumably results from the ectopic expression of BAF57(ΔC). To determine whether expression of the transgene BAF57(ΔC) has any influence on endogenous full-length BAF57, we compared mRNA levels of endogenous BAF57 in the clones ZR-BAF57(ΔC) versus the parental cells using a set of primers located within the N-terminal region of BAF57, that only detect full-length BAF57. We observed that endogenous BAF57 mRNA expression was slightly reduced in the ZR-BAF57(ΔC) clones as compared with that of parental ZR-75-1 cells. Strikingly, exogenous expression of BAF57(ΔC) in the stable cell lines leads to a dramatic reduction of endogenous BAF57 protein as monitored by Western blot analysis (Fig. 2B).

Next we analyzed the effects of the stable expression of the dominant negative form of BAF57 on the mRNA levels of endogenous ER target genes by QRT-PCR. We observed that E2-dependent activation of a number of ER target genes (pS2, cathepsin D, progesterone receptor, and bcl-2) was consistently attenuated in three independent ZR-BAF57(ΔC) clones as compared with the E2 response in control ZR-75-1 cells (Fig. 2C). Similarly, the ability of E2 to increase BRCA1 and BRCA2 expression was impaired, but surprisingly there was a 3-fold increase in basal expression (Fig. 2C). Thus, both approaches demonstrate the importance of BAF57 in the regulation of E2-dependent transcription in breast cancer cells.

**BAF57 Is Essential for ER-dependent Proliferation**—Since we observed that E2-stimulated gene expression depends on BAF57, we next examined its importance on E2-dependent cell proliferation. For this purpose, ZR-75-1 cells were transfected with either siBAF57 or siCONTROL, and cell proliferation in response to E2 was determined at different time points. As expected, E2 stimulated the growth of ZR-75-1 cells, whereas no proliferative effects were observed in the absence of ligand (Fig. 3, A and B). Mock- and siCONTROL-transfected cells remained proliferating in response to hormone along the entire 7-day monitored period. However, specific BAF57 knockdown had an inhibitory effect on E2-stimulated proliferation, and eventually siBAF57-transfected cells stopped proliferating after 3 days (Fig. 3B). After 7 days, E2-dependent cell growth was reduced by 50% in siBAF57-transfected cells compared with mock controls. As an additional control to demonstrate the specificity of siBAF57 effects observed, we monitored the proliferation of the BAF57-negative breast cancer line BT549. The lack of expression of BAF57 protein in this human breast ductal...
cell line was previously determined by Western blot analysis in a screening performed with the aim to characterize SWI/SNF protein expression in human breast cancer cell lines (18). In this case, transfection with siBAF57 did not affect proliferation of BT549 compared with mock cells (Fig. 3C). In both cell types, transfection of siCONTROL slightly reduced cell proliferation, possibly due to toxic nonspecific effects (Fig. 3, B and C).

Similar results were obtained when we monitored proliferation of the ZR-BAF57(ΔC) clones. The expression of the dominant negative BAF57 mutant inhibited E2-dependent cell proliferation over 50% compared with parental ZR-75-1 cells (data not shown). These findings indicate that BAF57 is an essential regulator of ER-dependent growth in ZR-75-1 cells.

Inhibition of BAF57 Function Leads to Down-regulation of the Proto-oncogene c-fos—E2 stimulates G1 to S phase progression in breast cancer cells by activating ER and increasing expression of a number of cell cycle genes. One important target of E2 is the proto-oncogene c-fos (19), which exerts a direct influence on cell proliferation in breast cancer (20, 21). To examine whether blocking BAF57 could be affecting c-fos expression, we compared c-fos mRNA levels upon E2 stimulation in stable ZR-BAF57(ΔC) clones versus ZR-75-1 control cells using QRT-PCR. Treatment with E2 induced a rapid and transient increase in c-fos expression in ZR-75-1 cells that reached maximum at 1 h and decreased to basal levels after 24 h with E2 (Fig. 4A). Stable expression of the dominant negative form of BAF57 dramatically reduced both basal and E2-stimulated c-fos expression (Fig. 4A). We performed transient transfection experiments with siBAF57 to confirm that BAF57 function is important for the expression of c-fos. Transfection of siBAF57 completely abolished the increase in c-fos expression observed in mock-transfected cells treated with E2 for 1 h (Fig. 4B). These results indicate that BAF57 plays a crucial role in the transcription of c-fos proto-oncogene.

Identification of Functional Domains Required for BAF57-ER Interaction—We have previously reported that the mechanism by which BAF57 interacts with ligand-activated ERα differs from that shown for p160 coactivators, since BAF57 protein structure does not contain any LXXLL motifs. Initial mapping studies using ERα deletion mutants demonstrated that whereas a functional AF2 domain is required for ligand-dependent binding of BAF57 to ERα, this region by itself is not sufficient to account for BAF57 interaction (14). Furthermore, we detected a strong direct interaction between BAF57 and an ERα deletion mutant comprising the AF1 domain, the DBD, and the hinge region. The additional observation that isolated AF1 failed to interact with BAF57 revealed the importance of the central hinge region of ERα for the recruitment of BAF57. To further characterize the molecular determinants of the BAF57-ERα interface, we carried out a combination of in vitro binding analysis and functional studies using various ERα and BAF57 mutants.

To analyze the possible requirement of the DBD domain for BAF57 interaction, we used two different ERα DBD mutants, EAAE-ERα and C241A/C244A, which contain mutations in the first and second zinc finger, respectively, that disrupt DNA binding (16). In GST pulldown assays using purified and immobilized GST-BAF57 and in vitro translated ERα DBD mutants, we observed that the two DBD mutants retained a ligand-dependent association with BAF57 similar to that of the wild-type receptor (Fig. 5A). These results indicate that a functional DBD is not required for the in vitro binding of BAF57 to ERα.

We evaluated the importance of the ERα hinge region for BAF57 interaction in another set of GST pulldown experiments, by using three different ERα hinge deletion mutants, H1 (Δ261–275), H2 (Δ253–307), and H3 (Δ245–307). The in vitro binding of the hinge mutants to BAF57 was in all cases severely impaired, as compared with WT ERα (Fig. 5B). The mutant H1
defines the shortest sequence in the hinge region, corresponding to residues 261–275 of mERα/H9251, found to be essential for BAF57 recruitment. These in vitro observations were validated in vivo by performing transient transfection experiments in BT549 cells. This breast carcinoma cell line lacks BAF57 protein, and we previously reported that the ability of SRC1e to potentiate transcriptional activation of ERα in BT549 cells was markedly impaired unless exogenous BAF57 is co-expressed (14). Reporter assays performed in BT549 cells confirmed that expression of ectopic BAF57 potentiates SRC1e-dependent activation of wild-type ERα (Fig. 5D). However, in the H1 mutant receptor, BAF57 expression did not significantly increase SRC1e coactivation (Fig. 5D). Transfections with H2 and H3 mutants revealed that these receptors are transcriptionally inert (data not shown). Taken together, these data suggest that the hinge region of ERα plays a critical role in the estrogen-dependent recruitment of BAF57 and also in the in vivo effects on ERα-mediated transcription.

FIGURE 3. BAF57 is required for estrogen-dependent proliferation in breast cancer cells. A–B, ZR-75-1 cells mock-transfected or transfected with BAF57 or control siRNAs were treated with either vehicle or 10 nM E2. Ligand-independent (A) and ligand-dependent proliferation (B) were both monitored at different time points by using the MTS assay. C, no effects of silencing BAF57 expression were observed on BT549 ligand-independent cell proliferation.

FIGURE 4. BAF57 is involved in the transcriptional regulation of c-fos. A, ZR-75-1 cells or ZR-BAF57(H9004) cells stably expressing dnBAF57 (C6) were treated with vehicle (−) or 10 nM E2 for 30 min, 1 h, 2 h, or 24 h. c-fos mRNA expression was analyzed by QRT-PCR. B, ZR-75-1 cells mock-transfected or transfected with BAF57 siRNAs were treated with vehicle (NH) or 10 nM E2 for 1 h, and c-fos expression was determined by QRT-PCR.
BAF57 Is a Critical Regulator of ER Function

Our mapping experiments defined a complex interaction surface between BAF57 and ERα that requires an intact, transcriptional competent AF2 domain and residues in the hinge region (14) (Fig. 5, B and D). The isolated AF1 domain does not bind BAF57 in vitro. However, ERα AF1 domain can activate transcription synergistically with AF2 contributing to the binding of p160 coactivators (22–24), and we initially identified BAF57 as an SRC1-interacting protein. To determine whether AF1 has a role in the regulation of BAF57 recruitment to ERα, we performed GST pulldown experiments with an ERα deletion mutant containing the hinge region and the AF2 domain but devoid of AF1. Although the isolated AF1 domain itself does not bind to BAF57, the deletion of this domain had profound effects in the in vitro recruitment of BAF57. The AF1 deletion mutant exhibited strong constitutive ligand-independent binding to BAF57, and the increase in the interaction induced by E2 was almost negligible (1.3-fold; Fig. 5C), as compared with wild-type ERα (4.8-fold; Fig. 5C). Interestingly, the expression of exogenous BAF57 in BT549 cells was unable to potentiate SRC1-dependent coactivation of the AF1 deletion mutant regardless of whether E2 was present or not (Fig. 5D). These results suggest that AF1 might repress the interaction between ERα and BAF57 in the absence of ligand but is necessary for optimum ligand-dependent recruitment of SRC1 or other cofactors to form a fully functional holoreceptor. Phosphorylation in serine 122 in the AF1 domain of mouse ERα or the equivalent serine 118 in the human orthologue is important for the regulation of the ligand-independent activity of ERα (25). A point mutant of mouse ERα containing a serine to alanine change in that residue (S122A) was recruited in vitro to GST-BAF57 in a similar manner as the wild type receptor (Fig. 5C). In addition, SRC1 coactivation of this mutant in BT549 cells was potentiated by exogenous BAF57 to the same levels as those observed with wild type receptor (Fig. 5D). These results indicate that phosphorylation of Ser122 is not required for a functional interaction between BAF57 and ERα.

In an attempt to define the BAF57 regions required for the interaction with ERα, we generated various BAF57 deletion mutants, as shown in Fig. 5E. In GST pulldown experiments, we observed that the estrogen-dependent binding of ERα to GST-BAF57 was compromised in the absence of the N-terminal HMG domain, the central NHRLI domain, or the C-terminal domain, indicating that the interaction requires an intact BAF57 structure.

**FIGURE 5. Functional domains involved in BAF57-ERα interaction.** A, in vitro translated [35S]methionine-labeled wild-type mERα or the mERα DBD mutants EAAE-ERα and C241A/C244A were incubated with either GST alone or GST fusion protein of BAF57 immobilized onto Sepharose beads in the presence of vehicle (−) or 100 nm E2 (left). Shown is a schematic representation of the DBD mutants used in the assay (right). B, in vitro binding of GST fusion protein of BAF57 to [35S]-labeled full-length mERα or mERα hinge deletion mutants in the presence of vehicle (−) or 100 nm E2. C, in vitro binding of GST fusion proteins of BAF57 to [35S]-labeled full-length mERα or the indicated AF1 mutants. Below each panel, the percentage of the input pulled down (counts/min) for each assay is shown. D, transcriptional activity of WT mERα or the mutants H1(Δ261–275), 121–599, and S122A in BT549 cells transfected with expression vectors for mERα or the indicated mutants. SRC1(−), BAF57, the 2×EREp52-Luciferase reporter, and the Renilla control vector pRL-CMV. Luciferase activities were normalized to the Renilla luciferase activities. The values are expressed relative to the activity of WT mERα in the presence of E2. The results shown represent the average of two independent experiments assayed in quadruplicate ± S.D.

| Domain          | E2 (% of WT) | 121–599 (% of WT) | S122A (% of WT) |
|-----------------|-------------|-------------------|-----------------|
| AF1             | 13 ± 1      | 12 ± 3            | 6 ± 1           |
| AF2             | 10 ± 1      | 9 ± 1             | 6 ± 1           |
| AF1-DBD         | 25 ± 3      | 15 ± 2            | 11 ± 2          |
| AF2-DBD         | 25 ± 3      | 15 ± 2            | 11 ± 2          |

top). Below each lane, the percentage of the input pulled down (counts/min) for each assay is shown. A Coomassie-stained gel shows the levels of the bacterially expressed GST fusion proteins used in the assay marked with an asterisk (right, bottom).
**BAF57 Is a Critical Regulator of ER Function**

![Graphs](image)

**FIGURE 6. BAF57 is an ER subtype-selective coactivator.** A and B, transient transfections in BT549 cells with increasing amounts of expression vectors for ERα (A) or ERβ (B) along with SRC1a and hBAF57, the reporter 2×EREpS2-Luciferase, and the Renilla control vector pRL-CMV. The values are expressed relative to the activity of ER (2 ng, α or β) alone in the presence of E2. C, transient transfections in COS-1 cells with expression vectors for ERα (left) or ERβ (right) along with SRC1a or SRC3 and BAF57, the reporter 2×EREpS2-Luciferase, and the Renilla control vector pRL-CMV. The values are expressed relative to the activity of ER (α or β) alone in the presence of E2. D, transient transfections in COS-1 cells with expression vectors for ERα (left) or ERβ (right) along with SRC1a and BAF57, the reporter 2×EREpS2-Luciferase, and the Renilla control vector pRL-CMV. The values are expressed relative to the activity of ER (α or β) alone in the presence of E2. In all cases, after transfection, cells were washed and treated for 24 h with either vehicle (NH) or 10 nm E2. Luciferase activities were normalized to the Renilla luciferase activities. The bars represent the means ± S.D. of at least two independent experiments performed in quadruplicate.

independently, by using BT549 cells transiently transfected with either ERα or ERβ expression vectors. As previously shown, SRC1a modestly enhanced E2-dependent transcription by ERα, and this effect was further potentiated in the presence of ectopic BAF57 (Fig. 6A). However, ectopic BAF57 has no effect on ERβ-mediated transcription (Fig. 6B). A similar result was observed for all p160 coactivators tested (SRC1a and RAC3; Fig. 6C). To further confirm the isoform specificity of BAF57 effects, we performed additional transient transfection experiments in the BAF57-positive cell line COS-1. Whereas exogenous expression of BAF57 in BT549 cells devoid of the endogenous protein potentiates estrogen-stimulated gene transcription, we have found that it suppresses transcription in cells expressing BAF57 (14). Interestingly, we found that such suppression by ectopic BAF57 is specific for ERα (Fig. 6D, left) and that the ability of SRC1a to stimulate the transcriptional activity of ERβ was unaffected by BAF57 overexpression (Fig. 6D, right). The data support the contention that BAF57 is specifically involved in the regulation of the activity of ERα isoform.

**DISCUSSION**

The mammalian SWI/SNF chromatin remodeling complexes are important for transcriptional regulation mediated by several nuclear receptors, including ER, although the detailed mechanisms of recruitment of these complexes to specific target genes, the function of each individual subunit, and the molecular mechanisms of coordination with other nuclear receptor coregulators are not fully elucidated. We have previously shown that BAF57, a core subunit present in all mammalian SWI/SNF complexes, interacts directly with the ER in a ligand-regulated manner, is recruited to estrogen-responsive promoters in the presence of estrogens, and is necessary for the ability of p160 proteins to act as coactivators for ER in transfected cells (14). In this report, we provide evidence to indicate that BAF57 is crucial for the function of ER in human breast cancer cells. Both depletion of BAF57 protein by means of siRNA and inhibition of BAF57 function by using dominant negative BAF57 mutants severely decreased ER-dependent transcription from endogenous estrogen target genes. Moreover, we observed a dramatic inhibition of estrogen-dependent cell proliferation when compromising BAF57 functions in ZR-75-1 human breast cancer cells. Collectively, these data demonstrate that BAF57 is a critical regulator of ER activity and plays a role in the control of estrogen-dependent proliferation in breast cancer cells. Estrogen stimulation of mammary epithelia is a major factor involved in the development and progression of breast cancer, and ER represents a key regulator of cell proliferation and, more importantly, a strong predictor of response to hormonal therapy (28). More than 50% of all human breast tumors are ER-positive, and their growth is dependent upon the presence of an active estrogen-ER complex. Mechanisms that involve the functional inactivation of ER may play an important role in the loss of hormone responsiveness, contributing to the development of hormonal resistance, which is a common problem that limits the long term effectiveness of the current therapies using anti-estrogens for breast cancer treatment (29). Since BAF57 is required for ER activity, alterations in BAF57 expression or function could interfere with the normal ER function and have a major impact in estrogen response, leading to a tumor insensitive to hormones.

Our results suggest that the levels of BAF57 protein in the cell must be tightly regulated in order to have a correct transmission of estrogen signals. Stable expression of a dominant negative form of BAF57 induced a drastic reduction in the amount...
of full-length wild-type BAF57 protein in all clones tested, in accordance with other reports that demonstrated that some core subunits of SWI/SNF, including BAF57, are subject to a tight control maintaining the proper stoichiometric levels of all components within multimeric complexes (30). Biochemical purification of mammalian SWI/SNF complexes indicated that no free BAF57 subunits are present in the cells, implying that most, if not all, BAF57 proteins are assembled into SWI/SNF complexes (30, 31). Given that BAF57 interacts directly with various transcription factors, such as ER, the androgen receptor (AR), and other NRs, and it has been proposed as interface for the recruitment of SWI/SNF complexes to specific target promoters (14, 30, 33), the maintenance of constant physiological levels of BAF57 could be essential for SWI/SNF-dependent activation of specific target genes. In addition, an excess of free BAF57 protein might exert a dominant negative effect, interfering with NR signaling and other SWI/SNF-dependent processes. This suggests that the inhibition of ER transcriptional activity may occur due to an impaired recruitment of functional SWI/SNF complexes or because SWI/SNF complexes lacking BAF57 do not remodel chromatin properly, although both possibilities are not mutually exclusive. More experiments will be required to establish whether the effects on ER activity are caused only by aberrant recruitment of SWI/SNF complexes to the promoters of estrogen-dependent genes or if there are additional alterations in other SWI/SNF functions.

In cells stably expressing BAF57ΔC, we see the combined effects of the expression of a dominant negative BAF57 and the concomitant reduction of wild-type BAF57 levels. This might explain why we observe different effects in the expression of BRCA1 and BRCA2 compared with the RNA interference approach, due to specific effects induced by the expression of BAF57ΔC and/or because the reduction of BAF57 levels is more dramatic than that caused by siRNAs. The induction of BRCA1 and BRCA2 expression in response to estrogens was decreased in all BAF57ΔC clones tested, but interestingly their basal expression level was augmented. The chromatin remodeling activities of SWI/SNF have been associated with both gene repression and activation, and it has been described that human SWI/SNF negatively regulates the BRCA1 promoter (34). Our results confirm that SWI/SNF is involved in basal repression of BRCA1 expression but also in the estrogen-dependent activation of its promoter. Thus, SWI/SNF could function in both activation and repression of BRCA1, depending on the upstream signals, similarly to the reported dual regulation of β-catenin/TCF target genes (reviewed in Ref. 35). In addition, we have uncovered a novel role for SWI/SNF in the regulation of BRCA2 expression. The participation of BAF57 in the regulation of a number of key genes with a role in the development and progression of breast tumors reinforces the idea that the loss of normal BAF57 functions could contribute to breast cancer etiology. Thus, BAF57 depletion leads to the inhibition of cell cycle regulatory genes such as BRCA1, BRCA2, and c-fos and also the antiapoptotic gene bcl-2, suggesting that probably both cell cycle arrest and induction of apoptosis are contributing to the reduced estrogen-dependent cell proliferation observed in breast cancer cells deficient in BAF57 function.

BAF57 has also been shown to regulate p160 coactivator function. All three members of the family (SRC1, SRC2/TIF2/GRIP1, and SRC3/RAC3/AIB1) exhibit reduced activity in the absence of BAF57 (14) and may contribute to impaired ER activity. Therefore, an inhibition of p160 function may be one of the mechanisms by which a reduction in BAF57 protein level impairs ER activity. This is particularly relevant for SRC3, an oncogene frequently amplified and overexpressed in breast cancers (36). SRC3 is an integrator of estrogen and growth factor signals, and it has a clear proliferative role in the mammary gland (reviewed in Ref. 37). If SRC3–BAF57 interactions are important for normal ER action in vivo, mutations in BAF57 or alterations in its normal expression might directly affect estrogen-dependent cell proliferation. Our mapping studies indicate that the interaction between BAF57 and ER is complex and several functional domains in both proteins participate. BAF57 binding to ER differs from that of p160 coactivators, since BAF57 lacks LXXLL motifs and also interacts with different surfaces on ER, allowing the possibility that both BAF57 and the p160 coactivators could simultaneously bind to ER.

The effects on estrogen action are surely accompanied by other perturbations in estrogen-independent signals in the cell. It has been recently reported that stable re-expression of BAF57 in BT549 cells reduced the transformed characteristics of these cells, inducing cell cycle arrest and apoptosis, suggesting that BAF57 is a tumor suppressor in the absence of estrogen signals (38). SWI/SNF complexes regulate the activity of many transcription factors, and, depending on the cellular context, alterations in its functions might increase or reduce the transformed phenotype. Whole genome DNA array experiments will help to distinguish the subset of genes regulated by SWI/SNF that are estrogen-dependent, providing us with new putative candidate genes involved in the origin and progression of breast cancer. An interesting finding was that expression of the BAF57 gene itself seems to be regulated by estrogens, although it remains to be identified whether there is a direct regulation of the promoter through consensus EREs or via indirect regulation of the gene.

The human BAF57 gene maps to chromosome 17q21, in close proximity to BRCA1, a locus associated with frequent loss of heterozygosity and allelic imbalance in breast cancer (39). However, mutations in BRCA1 are rare events in sporadic breast cancer with loss of heterozygosity in the region (40), suggesting that other genes in this region may function as tumor suppressors. The genetic link, together with the important role regulating the activity and expression of several tumor suppressor genes and oncoproteins and cell cycle and apoptosis in breast cancer cells, makes BAF57 an attractive candidate as target for the design of therapeutic and diagnostic strategies for breast cancer. This same rationale also applies to an analogous cancer type subject to hormonal regulation such as prostate cancer, since BAF57 function was found to be essential for AR activity and AR-dependent proliferation in prostate cancer cells (41). AR and ER represent two major therapeutic targets, and, in light of these data, BAF57 emerges as a common potential target for therapeutic intervention in both breast and prostate cancers. In addition, our preliminary results with transfected cells suggest that BAF57 effects are specific for ERα, the isoform that mediates estrogen mitogenic effects (3, 4, 42). The confir-
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Expansion of this specificity in vivo would suggest that targeting BAF57 functions may provide new treatments designed to block specifically ERα activity without inhibiting possible ERβ-mediated antiproliferative and proapoptotic effects (3, 4, 43) and minimizing side effects.

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