SWI/SNF Regulates the Cellular Response to Hypoxia*

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Hypoxia induces a variety of cellular responses such as cell cycle arrest, apoptosis, and autophagy. Most of these responses are mediated by the hypoxia-inducible factor-1α. To induce target genes, hypoxia-inducible factor-1α requires a chromatin environment conducive to allow binding to specific sequences. Here, we have studied the role of the chromatin-remodeling complex SWI/SNF in the cellular response to hypoxia. We find that SWI/SNF is required for several of the cellular responses induced by hypoxia. Surprisingly, hypoxia-inducible factor-1α is a direct target of the SWI/SNF chromatin-remodeling complex. SWI/SNF components are found associated with the hypoxia-inducible factor-1α promoter and modulation of SWI/SNF levels results in pronounced changes in hypoxia-inducible factor-1α expression and its ability to transactivate target genes. Furthermore, impairment of SWI/SNF function renders cells resistant to hypoxia-induced cell cycle arrest. These results reveal a previously uncharacterized dependence of hypoxia signaling on the SWI/SNF complex and demonstrate a new level of control over the hypoxia-inducible factor-1α system.

The transcriptional response to hypoxia is mainly controlled by the hypoxia-inducible factor (HIF) 2 system (1). HIF is a heterodimeric transcription factor composed of α and β subunits. To date, there are three α subunits identified and several splice variants of the β subunit (also called ARNT) (1, 2). While HIF-1β is constitutively expressed and its levels remain unchanged, HIF-α subunits are extremely labile at normal oxygen levels. HIF-1α levels are controlled by a class of 2-oxoglutarate dioxygenases called prolyl-hydroxylases (PHDs). There are four PHDs identified so far, but only PHD1, PHD2, and PHD3 have been shown to control HIF-1α. These enzymes catalyze the hydroxylation of specific proline residues in the oxygen dependent degradation domain of HIF-α that target this subunit for ubiquitination by the Von Hippel Lindau (VHL) system and consequently proteasomal degradation (3, 4). However, when oxygen levels are reduced, or cofactors such as iron ions are not available, PHD activity is inhibited, allowing HIF-1α levels to increase, followed by its translocation into the nucleus and transactivation of target genes. Among the HIF-1α targets are PHD2 and PHD3, revealing a negative feedback loop for the system (5, 6).

Hypoxia induces a variety of possible cellular responses (2, 7). These include cell cycle arrest, via p21 and p27 induction (8, 9), autophagy (10, 11) or apoptosis (12). All of these cellular responses have been shown to be dependent and independent of HIF-1α (8–12). As such, HIF-1α transactivates genes such as BNIP3, a BH3-only Bcl-2 family member that can induce autophagy (2). On the other hand, Noxa, a pro-apoptotic protein, has also been shown to be a HIF-1α target (2). Autophagy can be considered both a survival and death pathway (13, 14). It is characterized by the formation of autophagosomes, which are used to recycle damaged organelles such as mitochondria. Light Chain 3 (LC3) proteins are cleaved during autophagy and the cleaved form associates with autophagosome. The presence of LC3 in autophagosomes as well as the conversion of LC3 to the lower migrating form LC3-II are indicators of autophagy.

To induce transcription of genes, transcription factors such as HIF-1α require access to particular sequences in the promoter and enhancer regions of target genes. For this to occur, chromatin must be in an open conformation, to facilitate the binding of large protein complexes. There are several ways of modulating chromatin structure, including histone tail modification, integration of histone variants and nucleosome repositioning resulting from the action of ATP-dependent remodeling complexes (15–17). SWI/SNF chromatin remodeling complexes represent an evolutionarily conserved subgroup of ATP-dependent enzymes. They can cause a spectrum of chromatin rearrangements in an ATP-dependent manner, which can both positively and negatively regulate gene expression (18). In humans, several different forms of SWI/SNF complexes have been described. These are composed of eight to twelve subunits, which include an ATPase: either BRG1 or BRM, and a host of BRG1/BRM associated factors (BAFs). BRG1 or BRM, BAF170, BAF155, BAF57, BAF60, BAF53, INI1, and actin, are generally shared between these complexes (19). Additional subunits define the character of particular subcomplexes, such as the PBAF complex, which also contains BAF180 and BAF200 (20). The BRG1 and BRM subunits are catalytic motor subunits while the additional subunits are important in targeting and modulating the activity of the ATPase (18, 19). Both BRG1 and BRM have been shown to associate with HIF-1α at the EPO complex.
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promoter and suggested to act as co-activators of HIF-1α in the induction of this particular gene (21). However, to what extent SWI/SNF is involved in the cellular response to hypoxia is currently unknown.

Here we have investigated the role of SWI/SNF chromatin-remodeling complex in the response to hypoxia. We find that SWI/SNF is required for HIF-1α function and is important for hypoxia-induced cell cycle arrest.

EXPERIMENTAL PROCEDURES

Cells—U2OS osteosarcoma cell line was obtained from the European Collection of Cell Cultures and grown in Dulbecco’s Modified Eagle Medium (Lonza) supplemented with 10% fetal bovine serum (Gibco), 50 units/ml penicillin (Lonza), and 50 μg/ml streptomycin (Lonza) for no more than 30 passages. U2OS-HRE luciferase cells were a kind gift from Dr. Margaret Ashcroft (London, UK) and have been described previously (22). Control and BAF57 shRNA plasmids were obtained from Origene, and stable cell lines were created using the manufacturer’s instructions. These cells were maintained in 10% fetal bovine serum/Dulbecco’s modified Eagle’s medium supplemented with 50 units/ml penicillin, and 50 μg/ml streptomycin and 0.5 μg/ml puromycin (Sigma).

DNA Constructs—Expression plasmids for BAF57 (pCMV-XL5-BAF57) and BAF170 (pCMV-XL4-BAF170) were obtained from Origene. GFP-BRG1 was a kind gift from Prof. Tom Owen-Hughes (Dundee, UK) and has been described previously (23). The pGL-HRE luciferase construct was a kind gift from Dr. Margaret Ashcroft (London, UK) and has been previously described (22).

siRNA Transfection—siRNA duplex oligonucleotides were synthesized by MWG and transfected using Oligofectamine from Invitrogen and Interferin (Polyplus) per the manufacturer’s instructions. These cells were maintained in 10% fetal bovine serum/Dulbecco’s modified Eagle’s medium supplemented with 50 units/ml penicillin, and 50 μg/ml streptomycin and 0.5 μg/ml puromycin (Sigma).

RNA Isolation and Northern Blot Analysis—Semi-quantitative RT-PCR and PCR sequences are as follows: Control, AAAGGATTTCCTGGAGAGC. HIF-1α: For, CAT AAA GTC TGC AAC ATG GAA CAT and Rev, ATT TGA TGG GTG AGG AAT GGG TT; β-actin: For, GTG GGA GTG GGT GGA GGC and Rev, TCA ACT GGT CTC AAG TCA GTG; BAF57: For, AGT TGG TGC GGT CTG AAA GT and Rev, ATC GGA ATG TTC TCG TCG TC; BRG1: For, GAC GAG ACC GTC AAC CAG AT and Rev, TTT TCC TCC TCC TCC TCA CA.

Protein Expression Assays—Western blot, proliferation assays, transfections, and luciferase assays have been described previously (Refs. 26, 27 and references therein).

Statistical Analysis—ANOVA and Student’s t-tests were performed on the means, and p values were calculated. *, p ≤ 0.05 and **, p ≤ 0.10.

Other Experimental Procedures—Western blot, proliferation assays, transfections, and luciferase assays have been described previously (Refs. 26, 27 and references therein).

RESULTS

SWI/SNF components potentiate HIF-1α transcriptional activity—It was previously shown that BRG1 and BRM were important for HIF-1α-mediated induction of the EPO gene in Hep3B cells. Furthermore, both BRM and BRG1 could be found at the EPO promoter with HIF-1α (21). The results in that study suggested that BRG1 and BRM could act as coactivators for HIF-1α. However, this possibility has not yet been proven. To address this question, HIF-1α transcriptional activity was tested in the presence of higher levels of several SWI/SNF subunits (Fig. 1, A and B). U2OS cells were transfected with a luciferase reporter construct possessing 3 copies of the HRE consensus binding site in the presence or absence of BRG1, BAF170, or BAF57. In addition, these cells were exposed to 1% O2 for 24 h prior to harvest, and luciferase activity measured. As can be seen in Fig. 1, increasing the levels of BRG1, BAF170, and BAF57 (Fig. 1A) induced a significant increase in normoxic HIF-1α activity (Fig. 1B). As expected, the increase in HIF-1α activity was much more evident when cells were exposed to hypoxia. These results suggested that SWI/SNF is a general transcriptional coactivator for HIF-1α and also supports the
FIGURE 1. SWI/SNF components increase HIF-1α transcriptional activity. A, U2OS cells transfected with 1 μg of the indicated plasmids and exposed or not for 24 h in 1% O₂. Whole cell lysates were performed and Western blot analysis conducted for the levels of BRG1, BAF170, and BAF57. Actin was used as a loading control. B, U2OS cells were transfected with 0.5 μg of HRE-luciferase and 1 μg of the indicated plasmids, and exposed or not to 1% O₂ for 24 h prior to the luciferase assay. Graphics represent the mean plus S.D. of a minimum of three independent experiments, and results are expressed as fold activation/repression relative to control levels. ANOVA t-tests were performed on the means and p values were calculated. *, p ≤ 0.050 and **, p ≤ 0.010.

previous observed results when the EPO gene was analyzed in a different cell system (21).

SWI/SNF Components Are Required for Full HIF-1α Transcriptional Activity—Because overexpression studies can sometimes result in artifacts, we wanted to investigate the effects of modulating SWI/SNF levels in more physiological conditions. For this purpose, we used siRNA oligonucleotides directed toward several members of the SWI/SNF complex (Fig. 2A). In addition, we used a U2OS cell line, which possesses a stable integrated HIF-1α luciferase reporter, a cell line that has been extensively used to investigate HIF-1α function (22, 28). When several of the SWI/SNF components were depleted, it was possible to observe a reduction in normoxic HIF-1α activity. However, this effect was even more evident when cells were exposed to hypoxic stress by the addition of the hypoxia mimetic Desferroxamine (DFX) (Fig. 2B). Interestingly, depletion of BRM had no effect on HIF-1α activity in this cell line despite the effectiveness of the siRNA. These results support the gain of function experiments depicted in Fig. 1 and indicate that endogenous SWI/SNF is required for HIF-1α activity. Furthermore, these results suggest that BRG1 is the catalytic subunit responsible for modulating HIF-1α activity in these cells.

SWI/SNF Components Are Required for HIF-1α Expression and Activity—Our gain and loss of function experiments for members of the SWI/SNF complex indicated that this remodeling complex is important for HIF-1α activity. We next determined the effects of SWI/SNF depletion on the levels of endogenous HIF-1α target genes following exposure to hypoxia (Fig. 3). Consistent with the hypothesis that the SWI/SNF complex could act as a HIF-1α coactivator, the induction of Glut1 (a well known HIF-1α target) by hypoxia was compromised in cells depleted of BRG1 or BAF170 (Fig. 3A, 3rd line from the top). Unexpectedly, depletion of BAF170 and BRG1 resulted in a reduction in HIF-1α protein levels following hypoxia (Fig. 3A, top line). Although HIF-1α protein levels are mostly regulated by the activity of PHD2 in these cells (29), PHD2 levels were not increased above control (Fig. 3A, second line from the top), indicating that the mechanism behind HIF-1α reduction is not dependent on PHD elevation. In addition, depletion of BAF170 also resulted in reduction of BAF57 levels. This is in agreement with a recent study where the concept of a stoichiometric complex between BAF170, BAF57, and BAF155 was proposed, and the authors demonstrated that depletion of either of these proteins rendered them susceptible for proteasomal degradation (30).

We had previously observed that modulating BAF57 levels had a strong effect on HIF-1α activity (Figs. 1 and 2). Depletion of BAF57 and BAF155 (another member of the subcomplex) was performed, and the effects on HIF-1α and HIF-1α target levels analyzed (Fig. 3B). A severe impairment of HIF-1α expression following hypoxia could be observed in the absence of BAF57 (Fig. 3B, top line). Furthermore, Glut1 induction following hypoxia was also abolished (Fig. 3B, 3rd line from the top). This could be due to reduced levels of HIF-1α or to an inhibition of HIF-1α transcriptional activity (Fig. 2). Consistent with being a HIF-1α target gene (5), PHD2 levels were also reduced compared with control samples (Fig. 3B, 2nd line from the top). Interestingly, we could still detect increases in PHD2 in response to hypoxia, suggesting that either there is enough HIF-1α remaining to transactivate PHD2 or that other factors apart from HIF-1α can induce PHD2 following hypoxia. When BAF155 was depleted, BAF57 levels were also reduced, which could contribute to the partial reduction of HIF-1α expression (Fig. 3B, 4th and 5th line).

We extended our analysis to the other catalytic subunit, BRM (Fig. 3C). We could not detect any effect of BRM depletion on either HIF-1α levels (Fig. 3C, top line) or target gene activation (Fig. 3C, 2nd and 3rd lines from the top). These results indicate that BRG1 is the catalytic subunit responsible for the modulation of HIF-1α and that BRM cannot compensate for BRG1 in this particular cell line.
SWI/SNF Components Regulate HIF-1α Expression by Proteasomal-independent Mechanisms—Results presented in Fig. 3 indicate that HIF-1α expression requires the SWI/SNF chromatin remodeling complex. Given that we did not observe any increase in PHD2 levels, we hypothesized that SWI/SNF control over the HIF-1α system was distinct from the traditional proteasomal degradation mechanism. To test this possibility, we analyzed HIF-1α levels in the presence or absence of SWI/SNF components when the proteasome is inhibited (Fig. 4). As it can be seen in Fig. 4A, in normal conditions, HIF-1α levels are not detectable; however, in the presence of the proteasomal inhibitor MG132, it is possible to observe a robust stabilization of HIF-1α protein. As expected, when a HIF-1α siRNA knockdown was performed, no detectable HIF-1α could be seen, even in the presence of MG132 (Fig. 4A, last lane). When BRG1 was depleted, HIF-1α levels were decreased when compared with control (Fig. 4A). On the other hand, depletion of BRM resulted in no differences in HIF-1α levels. However, the most dramatic effects were obtained when BAF57 or BAF155 were depleted. Depletion of BAF57 or BAF155 resulted in the same reduction of HIF-1α as was observed with direct HIF-1α knockdown (Fig. 4A). These results suggest that SWI/SNF regulates HIF-1α expression through a mechanism that is not dependent on the proteasome. This possibility is also supported by gain of function experiments, where increased levels of BRG1, BAF170, and BAF57 in the presence of MG132 for a reduced period of time also result in increased stabilization of HIF-1α (Fig. 4B).

SWI/SNF Components Are Required for HIF-1α mRNA Expression—Recently, we and others (24, 31, 32) have shown that HIF-1α levels can be actively changed by regulation of HIF-1α mRNA production by mechanisms involving the NF-κB family of transcription factors. As depletion of BAF57 and BAF155 reduced HIF-1α protein levels to the same degree as depletion of HIF-1α itself (Fig. 4A), we next analyzed HIF-1α mRNA levels under conditions of reduced BRG1 and BAF57 availability (Fig. 5). It is possible to observe in Fig. 5A, that reduction of BRG1 or BAF57 resulted in a significant reduction of HIF-1α mRNA levels. This is also evident when cells were treated with hypoxia. The difference between BAF57 and BRG1 effects could be attributed to the relative siRNA efficiency. While the siRNA for BAF57 is highly efficient (90% reduction of BAF57 mRNA), BRG1 siRNA results in a 70% reduction of BRG1 mRNA (supplemental Fig. S1). Despite the difference in levels, these results suggest that BAF57 and BRG1 are required for HIF-1α mRNA expression. Because the SWI/SNF complex remodels chromatin in both positive and negative fashions, we investigated if the HIF-1α promoter was a target for SWI/SNF (Fig. 5, B–D). Using chromatin immunoprecipitation, we could detect hallmarks of active transcription such as H3 acetylation and RNA polymerase II occupancy (Fig. 5B). In contrast, when a region of chromosome 1, containing Alu repeats was analyzed, no detectable acetylated H3 could be observed. The absence of acetylated H3 is consistent with a previous report for this area of chromosome 1 (33). Acetyla-
tion of lysine residues in the H3 tails generates specific docking sites for bromodomain containing proteins (34–36). Bromodomains are found in chromatin-associated proteins (34, 36), including chromatin remodeling enzymes such as SWI/SNF. As such, when antibodies for BRG1, BAF57, and BAF155 were used, we could detect specific occupancy of these components at the HIF-1α promoter but not in a control region of the gene (Fig. 5C). Importantly, in the presence of hypoxia, we can still detect BRG1 occupancy at the HIF-1α promoter (Fig. 5D). The observation that SWI/SNF components are present at the HIF-1α promoter (Fig. 5C and D) combined with the effects on HIF-1α mRNA (Fig. 5A) and protein levels (Figs. 3 and 4) indicate that HIF-1α is a target of SWI/SNF and that this complex is fundamental for HIF-1α expression.

**FIGURE 3. SWI/SNF components control HIF-1α levels and activity.**
A, U2OS cells were depleted of BAF170 and BRG1, B, BAF155 and BAF57 or C, BRM by siRNA and incubated for the indicated times at 1% O2 prior to harvest. Western blot for the indicated proteins was performed, and actin was used as a loading control.

**FIGURE 4. SWI/SNF components are necessary and sufficient for HIF-1α expression.**
A, U2OS cells were transfected with the indicated siRNA oligonucleotides. MG132 was added 3 h prior to cells lysis, and Western blot analysis was performed with the indicated antibodies. B, U2OS cells were transfected with 1 μg of the indicated plasmids. MG132 was added 1.5 h prior to cells lysis, and Western blot analysis was performed with the indicated antibodies.

**BAF57 Is Required for SWI/SNF and NF-κB Function at the HIF-1α Promoter**—In all of our experiments, the most dramatic changes of HIF-1α levels were observed following depletion of one SWI/SNF component, in particular, BAF57. BAF57 does not possess catalytic activity although studies have demonstrated that it possesses DNA binding and promoter targeting activity (19). It has been shown to be important for estrogen and androgen receptor functions and recently also in thyroid hormone responses (37–39). Given its DNA binding ability and our own results concerning alteration of HIF-1α levels, we tested if BAF57 was necessary for SWI/SNF function at the HIF-1α promoter (Fig. 6). For this purpose, we created stable U2OS cells where BAF57 was selectively reduced (Fig. 6A). These cells behaved similarly to cells with transient BAF57 depletion, where HIF-1α expression is reduced even following prolonged exposure to hypoxia (Fig. 6A, top panel). Using these BAF57 knockdown cells, we performed ChIPs for other SWI/SNF components at the HIF-1α promoter. When compared with control cells, BAF57-depleted cells presented a reduced recruitment of BAF155 and BRG1 to the HIF-1α promoter (Fig. 6B, quantified in supplemental Fig. S2A).

We had previously observed that RNA polymerase II subunits could be detected at the HIF-1α promoter (Fig. 5B), suggestive of active transcription. As BAF57 depletion resulted in lower HIF-1α mRNA, we investigated if this was also evident in RNA polymerase recruitment. When BAF57 was depleted, we observed around 50% reduction in RNA polymerase II recruitment to the HIF-1α promoter (Fig. 6C, quantified in supplemental Fig. S2A). Interestingly, acetylated H3 levels at the HIF-1α promoter were not affected by BAF57 depletion, indi-
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![Graph](image)

**FIGURE 5. HIF-1α is a novel target of SWI/SNF.** A, U2OS cells were transfected with the indicated siRNA oligonucleotides. Cells were exposed or not to 1% O2 for 4 h, and RNA was extracted 48 h after transfection. RT-PCR was performed, and mRNA levels for HIF-1α were measured. The graph depicts quantification of a minimum of three independent experiments using Image J software. ANOVA t-tests were performed on the means, and p values were calculated. *, p ≤ 0.050 and **, p ≤ 0.010. B, ChIPs were performed on cell extract from U2OS, and the HIF-1α promoter was analyzed for levels of acetylated H3 and RNA polymerase II. Rabbit IgG was used as a negative control and ALU repeat on chromosome 1 was used as a control region. C, ChIPs were performed as in B, using the indicated SWI/SNF components. PCR was performed for regions in the HIF-1α promoter, HIF-1α coding region, and ALU repeat on chromosome 1. D, U2OS cells were treated or not with 1% O2 for 4 h prior to cross-linking, and ChIPs were performed using a BRG1 antibody. PCR was performed for regions in the HIF-1α promoter and HIF-1α coding region. N, normoxia; H, hypoxia.

dating that BAF57 is not required for the recruitment of histone acetylases (not shown and quantified in supplemental Fig. S2A).

Recently, we demonstrated that all NF-κB subunits can be found at the HIF-1α promoter and that NF-κB is important for basal HIF-1α gene transcription (24). To determine if NF-κB and SWI/SNF bind cooperatively to the HIF-1α promoter, we examined NF-κB occupancy in the absence of SWI/SNF. Using BAF57-depleted cells, ChIPs were performed for several of the NF-κB subunits. Compared with control cells, depletion of BAF57 resulted in a decrease of RelB and p52 NF-κB subunit presence at the HIF-1α promoter (Fig. 6D). This result suggests that SWI/SNF is required for NF-κB occupancy at the HIF-1α promoter, possibly by maintaining the NF-κB binding site in an accessible state, although direct protein-protein interaction cannot be ruled out.

Because the general assumption is that SWI/SNF controls a great number of genes, we examined, if lack of NF-κB occupancy at the HIF-1α promoter was simply the result of a reduction in the expression of the NF-κB subunits themselves. However, Western blot analysis on BAF57-depleted cells for all of the NF-κB family members demonstrated that there is no clear difference in any of the NF-κB subunits, indicating that BAF57 and SWI/SNF do not control basal expression of the NF-κB genes (supplemental Fig. S2B). Taken together, these results indicate that BAF57 is required for SWI/SNF and NF-κB function at the HIF-1α promoter.

**BAF57 Depletion Results in Impairment of Hypoxia-induced Cell Cycle Arrest**—Hypoxia can induce a variety of cellular responses depending on the severity and extent of the hypoxia exposure (2, 7). An initial response includes a reduction in proliferation with a correspondent cell cycle arrest at the G1/S transition, a response that has been suggested to be mediated by HIF-1α (reviewed in Ref. 7). As such, we investigated if BAF57 was involved in hypoxia-induced proliferation arrest (Fig. 7). When exposed to hypoxia, a reduction in proliferation could already be observed at 24 h, but was most pronounced at 48 h. Importantly, when treated with hypoxia, BAF57-depleted cells did not reduce their proliferation (Fig. 7A), indicating that BAF57 is important for hypoxia-induced cell cycle arrest. We then compared the proliferation capacity of control and BAF57 depleted cells. Surprisingly, BAF57 depletion promoted cell proliferation (Fig. 7B). This was also evident when these cells were analyzed by a clonogenic assay (supplemental Fig. S3A). Given that BAF57 depletion promotes proliferation in normoxia, this is possibly a HIF-1α-independent function of BAF57 because HIF-1α-depleted cells do not demonstrate the same increase in clonogenicity (supplemental Fig. S3B).

Taken together, these results demonstrate the importance of SWI/SNF and BAF57 in mediating the cellular response to hypoxia through HIF-1α-dependent and -independent mecha-
nisms. In addition we have uncovered that SWI/SNF is necessary for the expression of HIF-1α mRNA, which is absolutely required for induction of HIF-1α protein and function following hypoxic stress.

DISCUSSION

In this study we have investigated the role of the SWI/SNF chromatin remodeling complex in the cellular response to hypoxia. Surprisingly we have found that HIF-1α itself is a SWI/SNF target (Figs. 3–5) and that modulation of SWI/SNF levels has significant consequences in the levels of HIF-1α expressed in cells (Figs. 3 and 4), even following prolonged hypoxia exposure. We have identified a critical SWI/SNF subunit, BAF57, that is required for targeting the complex to the HIF-1α promoter as well as recruitment of NF-κB and RNA polymerase II (Figs. 5 and 6). Additionally and importantly, we find that SWI/SNF is required for an important cellular response to hypoxia, cell cycle arrest (Fig. 7), by mechanisms that are partly dependent on HIF-1α.

To date, only one study has revealed a connection between HIF-1α and SWI/SNF function (21). Wang et al. demonstrated that BRM and BRG1 are required for HIF-1 induction of the EPO gene in Hep3B cells. They found that BRG1 and BRM occupy the EPO promoter, and that overexpression of these SWI/SNF components potentiates HIF-1α-mediated gene activation. However, in that study, no analysis of HIF-1α levels was presented. We observed a reduction in HIF-1α target transactivation. This could be due to the impairment in HIF-1α levels but it is also possible, that a dual action of SWI/SNF on the HIF system takes place. SWI/SNF could be required for HIF-1α gene transcription (Figs. 3 and 4) and also act as a coactivator for HIF-1α target gene induction (Figs. 1 and 2). We verified whether SWI/SNF components were present at some of the known HIF-1α targets and whether a direct interaction could be detected in cells. However, we could not detect recruitment at these promoters or any significant interaction between these proteins (data not shown). It is possible, however, that specific HIF-1α target genes are also SWI/SNF targets. This possibility would only be determined using genome-wide approaches. Interestingly, BRM depletion had no effect on activity or levels of HIF-1α in our cells, indicating that BRG1 is the catalytic subunit present at the HIF-1α promoter (Figs. 2 and 3).

We observed that depletion of BAF57 resulted in impaired binding of other components of the SWI/SNF complex to the HIF-1α promoter, this is consistent with BAF57 being able to directly bind to DNA (19). Our data suggest that BAF57 is required for SWI/SNF targeting to the HIF-1α promoter (Fig. 6).

FIGURE 6. BAF57 is required for SWI/SNF and NF-κB function at the HIF-1α promoter. A, U2OS cells were stably transfected with control and BAF57 shRNA plasmids. Control and two clones (1 and 2) were analyzed by Western blot with the indicated antibodies. Cells were exposed to 1% O₂ for 2, 4, and 24 h. B, U2OS cells stably transfected with control and shRNA BAF57 plasmids were harvested for ChIPs. Occupancy by BAF57, BAF155, BRG1, and BRM was analyzed by PCR at the HIF-1α promoter and control region (control). C, polymerase II occupancy at the HIF-1α promoter and control region were determined by PCR. D, RelB and p52 occupancy at the HIF-1α promoter were determined by PCR. C, control; 1, clone 1; and 2, clone 2 of shBAF57 cells.

FIGURE 7. BAF57 is required for hypoxia-induced arrest in proliferation. A, U2OS cells stably transfected with control shRNA or shBAF57 plasmids were exposed to hypoxia (1% O₂), and proliferation was measured using AlamarBlue. ANOVA t-tests were performed on the means, and p values were calculated. *, p < 0.050 and **, p < 0.010.
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Given that BAF57 is a component of SWI/SNF, it would be expected that its depletion would interfere with a great number of genes. However, critical genes that are regulated by protein stability such as the tumor suppressor p53 and HIF-2α were not altered by BAF57 depletion (supplemental Fig. S4). In addition, transgenic studies have shown that BAF57 and BRG1 can modulate different genes important for CD4/CD8 cell lineages (40), giving rise to the idea of specificity. Furthermore, BAF57 has been specifically associated with androgen and estrogen responses, where it conveys functional specificity to the SWI/SNF complex (37, 38). Microarray studies on BAF57 depleted cells in the presence or absence of O₂ would be necessary to determine the extent of BAF57 involvement in the gene expression profile obtained.

The significance of our results is also suggested in both developmental, as well as, pathological processes. HIF-1α-deleted mice are embryonic lethal with defects in neural tube formation, cardiovascular malformations, and increased cell death in the cephalic mesenchyme (41, 42). Very recently, the genetic knock-out of the mouse homologue of BAF155, SRG3, was published (43). The mice show peri-implantation lethality. Rescue experiments revealed that SRG3 is required for angiogenesis and visceral endoderm development in the yolk sac, with genes specifically reduced including Angiopoietin1, Tie2, and EphrinB2 (43). Interestingly, this phenotype shares high similarities specifically reduced including Angiopoietin1, Tie2, and EphrinB2 (43). A recent study, where conditional deletion of BRG1 was previously unrealized function for BAF57, which suggests that BAF57 can also perform tumor suppressor functions. Overall, our study demonstrates the active involvement and requirement of SWI/SNF in the cellular response, which can be both dependent and independent of HIF-1α.

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