Epigenetics 6:12, 1444-1453; December 2011; © 2011 Landes Bioscience

# Identification of a core member of the SWI/SNF complex, BAF155/SMARCC1, as a human tumor suppressor gene

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**Key words:** SWI/SNF, BAF155, SMARCC1, tumor suppressor gene, cancer epigenetics

Recent studies have established that two core members of the SWI/SNF chromatin remodeling complex, BRG1 and SNF5/INI1, possess tumor-suppressor activity in human and mouse cancers. While the third core member, BAF155, has been implicated by several studies as having a potential role in tumor development, direct evidence for its tumor suppressor activity has remained lacking. Therefore, we screened for BAF155 deficiency in a large number of human tumor cell lines. We identified two cell lines, the SNUC2B colon carcinoma and the SKOV3 ovarian carcinoma, displaying a complete loss of protein expression while maintaining normal levels of mRNA expression. The SKOV3 cell line possesses a heterozygous 4 bp deletion that results in an 855AA truncated protein, while the cause of the loss of BAF155 expression in the SNUC2B cell line appears due to a post-transcriptional error. However, the lack of detectable BAF155 expression did not affect sensitivity to RB-mediated cell cycle arrest. Re-expression of full length but not a truncated form of BAF155 in the two cancer cell lines leads to reduced colony forming ability characterized by replicative senescence but not apoptosis. Collectively, these data suggest that loss of BAF155 expression represents another mechanism for inactivation of SWI/SNF complex activity in the development in human cancer. Our results further indicate that the c-terminus proline-glutamine rich domain plays a critical role in the tumor suppressor activity of this protein.

## Introduction

A new era of cancer research is underway with the realization that the initiation and regulation of cancer involves more than the identification of oncogenes or tumor suppressor genes. The field of epigenetics has exploded in recent years with multiple studies demonstrating the involvement of DNA methylation and chromatin modification in cancer development. Both mechanisms regulate gene transcription by controlling the access of transcription factors to DNA. Chromatin disorders have been implicated in the devastating effects of solid tumors as well as myeloid leukemia, Rubinstein-Taybi Syndrome and malignant rhabdoid tumors.[1,2]

The proper functioning of the SWI/SNF chromatin remodeling complex is vital to appropriate cell cycle control and tumor suppression. Despite the seemingly small (5%) amount of genes whose regulation the complex affects, they are widely dispersed throughout the genome with more repression than activation.[3] The ubiquitously expressed multi-unit complex is composed of a small core including BRG1 or BRM, SNF5/INI1/BAF47, BAF155, BAF170 and variable associated complex members depending on cell type and stage of development. Most core members are vital to life and essential to development as homozygous knockout mice show embryonic lethality.[3-8]

Either BRG1 or BRM serve as the catalytic subunit of the complex. Approximately ten percent of human cancer cell lines show mutations or deletions of these genes.[2] Another core member, SNF5/BAF47/INI1, is an established tumor suppressor gene that is deleted in almost all malignant rhabdoid tumors.[9] The absence of SNF5 protein provides the diagnostic marker for these cancers. Genetically engineered mice also provide evidence for the tumor suppressor activity of these genes. Mice heterozygous for *Brg1* develop tumors resembling breast adenocarcinomas,[4,10] while heterozygous *SNF5* mice develop rhabdoid tumors histologically similar to their human counterparts.[5,7,8] Several studies have implicated that the SWI/SNF complex acts as a tumor suppressor via its role in cell cycle regulation. The SWI/SNF complex can control cellular proliferation by its association with known cell cycle checkpoint genes, such as BRCA1, cyclin E, p21, p53 and p16.[11-17]

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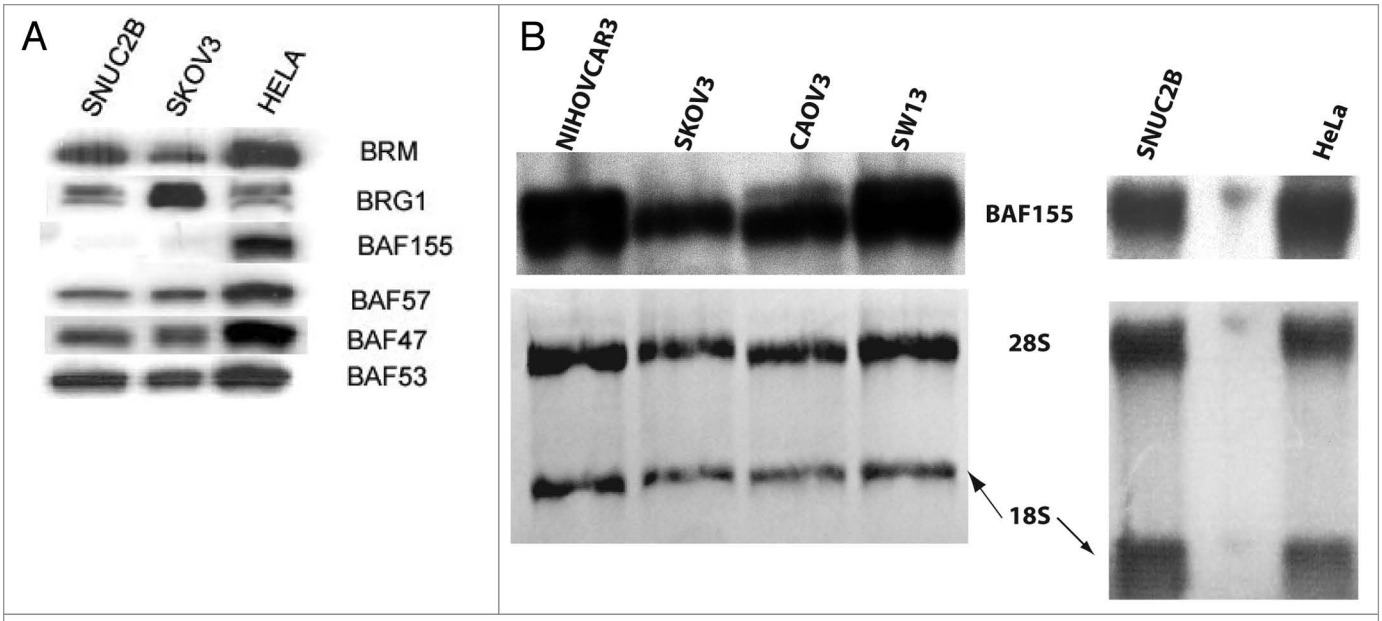
Submitted: 08/24/11; Revised: 10/14/11; Accepted: 10/21/11
DOI: 10.4161/epi.6.12.18492

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| Cell Line | BAF155:BRG1 (range) | BAF170:BRG1 (range) | SNF5:BRG1 (range) |
|---|---|---|---|
| SKOV3 | 1.01 (0.93-1.05) | 0.93 (0.85-0.98) | 1.08 (0.98-1.18) |
| SNUC2B | 1.19 (1.09-1.29) | 1.03 (0.98-1.07) | 1.14 (1.10-1.16) |

**Figure 1.** SKOV3 and SNUC2B cell lines lack BAF155 protein but not BAF155 mRNA. (A) Thirty μg of total cellular protein from each cell lines was separated by SDS-PAGE and characterized for expression of the indicated proteins by protein gel blot analysis. The actin-related BAF53 protein served as the loading control. (B) Thirty micrograms of mRNA of each cell line was analyzed by RNA gel blot and probed with a BAF155 cDNA probe. 18S and 28S bands served as loading controls. (C) Both cell lines lacking BAF155 protein expression were analyzed by QT-PCR expression for BRG1, SNF5, BAF155 and BAF170. The number of ΔCt cycles at 3 different concentrations of cDNA were assessed for each gene and normalized to the corresponding number for BRG1 amplification. The results represent triplicate results from 2 independent experiments.

The unique contribution of BAF155, another core member, to the complex remains ill-defined. Initially isolated as a gene highly expressed in thymus/low in periphery, BAF155 actually shows ubiquitous expression similar to the rest of the SWI/SNF complex. Also known as SWI3 in yeast, SRG3 (SWI3 related gene) in mouse, and MOIRA in Drosophila, BAF155 has been implicated to have a significant role in development. In mice, SRG3 is essential to early embryogenesis, as well as having a specific requirement for brain development and T-cell differentiation.[6] SRG3 also can be induced by androgen and subsequently transactivate AR in the prostate.[18] Reduction of SRG3 leads to a significant inhibition of GC-induced apoptosis.[19] The role of alterations in BAF155 expression/function in tumorigenesis remains unclear. Its loss could contribute to tumor development due to its location in region of chromosome band 3p21.31, that includes other suspected tumor suppressor genes, such as *SEM3B* and *FUS1*.[20,21] However, other studies have found increased expression of BAF155 mRNA in cervical intraepithelial neoplasia (CIN), prostate cancer and colorectal cancer.[22-25]

This study sheds light on the potential tumor-suppressor functions of BAF155 by characterizing 2 human tumor cell lines that lack BAF155 expression. Our studies demonstrate that re-expression of exogenous full length BAF155 induces senescence in these cell lines. In contrast, exogenous expression of BAF155 in a human cell line with endogenous expression had no little or no effect on cell growth. Furthermore, truncation of the c-terminus of BAF155 caused a significant loss of its tumor suppression activity. Our results establish another member of the SWI/SNF complex as a tumor suppressor gene that may contribute to its regulation of the cell cycle and cellular senescence.

## Results

**Loss of BAF155 expression in two carcinoma cell lines.** To gauge the frequency of BAF155 loss as well as to generate a cell culture model system to study BAF155 functions, we screened >100 human cancer cell lines by protein gel blot for BAF155 expression. We identified two carcinoma cell lines lacking BAF155 protein (**Fig. 1A**): SKOV3, an ovarian carcinoma cell line, and SNUC2B, a colorectal carcinoma cell line. Initial screening of nuclear extracts from these cells showed that other complex members, including BRG1 and SNF5, were present. We also screened whole cell extracts to determine whether BAF155 still appeared in the cytoplasm. We could not observe BAF155 expression under any protein extraction conditions in these two carcinoma cell lines. Both cell lines contained a full complement of all other SWI/SNF complex members, although SNUC2B's BRG1 ATPase domain has two point mutations.[26] However, as

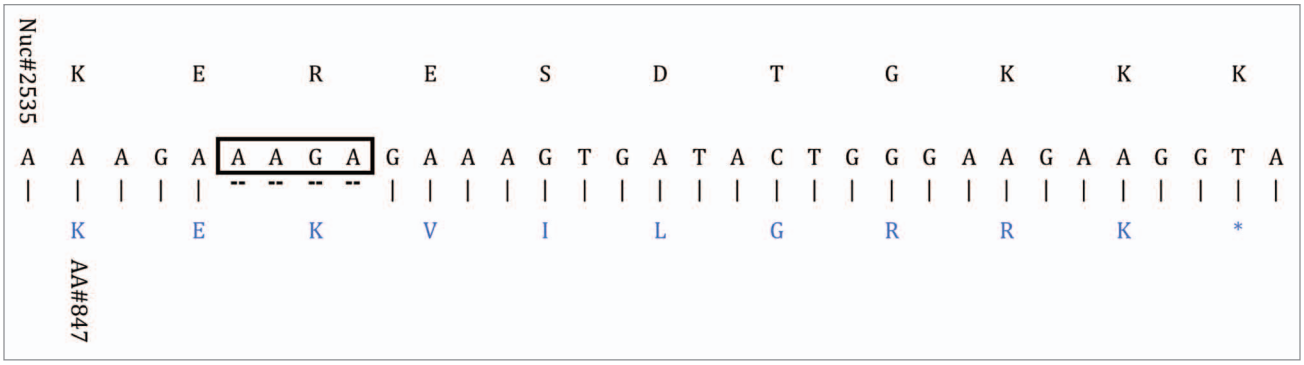



**Figure 2.** SKOV3 contains a truncating mutation in the BAF155 gene. Sequence of exon 24 of SKOV3 ovarian carcinoma cell line lacking protein expression. Individual exons were amplified by PCR, purified and sent to UNC-CH Genome Analysis Facility for analysis. This 4 bp deletion mutation leads to a stop codon at 2,666–2,669, yielding an 855AA truncated BAF155 protein. Result was confirmed three times.

previously reported, the expression of other complex members appeared reduced in the absence of BAF155 expression (**Fig. 1A**).[27] We then assessed mRNA levels by RNA gel blot analysis to determine if the cell lines failed to transcribe BAF155. As shown in **Figure 1B**, both cell lines expressed comparable levels of BAF155 mRNA to BAF155 positive cell lines. Because of the strong homology between BAF155 and BAF170, we confirmed these results with real time PCR. We did not observe any reduction in BAF155 or BAF170 expression in comparison to expression of the core ATPase, BRG1, by this method (**Fig. 1C**).

**Ovarian carcinoma cell line SKOV3 expresses a mutation in the *BAF155* gene.** To determine the reason for the lack of BAF155 protein expression in the two deficient cell lines, we sequenced the BAF155 mRNA to search for mutations. In the SKOV3 cell line, we found a 4 base pair deletion in exon 24 leading to a stop codon at 2,566 bp (**Fig. 2**). This mutation would yield a truncated protein of 855 aminoacids, instead of the full-length protein of 1,105 aminoacids. The loss of the last 250 aminoacids would remove the leucine zipper and proline-glutamine rich domains as well as the antibody binding site used in the protein gel blot in **Figure 1A**. Although we did not observe a BAF155 product of any size on western gels, we cannot rule out production of a truncated protein by the SKOV3 cell line. Genomic sequencing of exon 24 revealed the presence of both mutant and wild-type alleles. Therefore, silencing of the wild-type allele had apparently occurred in this cell line. The primary sequence of SNUC2B has not yielded a mutation. We are currently sequencing the untranslated regions of mRNA to look for potential abnormalities in protein translation signals.

**Loss of BAF155 in the SNUC2B cell line is not due to proteasome degradation.** We next determined if the absence of BAF155 protein resulted from increased protein degradation. Cells were treated with lactacystin, a proteasome inhibitor, for up to 36 h, harvested for protein and analyzed by protein gel blot for BAF155 expression. In order to assess the inhibition of proteasome degradation, we assessed expression of c-JUN, a protein with a rapid degradation rate, as well as poly-ubiquitination of proteins using an anti-ubiquitin antibody. As shown in **Figure 3**, after lactacystin treatment, we did not observe re-expression of the BAF155 protein in the SNUC2B cell line. In contrast, both c-JUN and protein poly-ubiquitination significantly increased during the same time period (**Fig. 3**). Treatment with an alternative proteasome inhibitor, MG132, gave similar results (data not shown).

**BAF155-induced growth inhibition of carcinoma cells via senescence.** We next assessed the effects on cell growth after re-expression of BAF155 in the cell lines lacking endogenous expression. We used expression vectors that coded for either a full-length protein or one with a 332 aminoacid deletion of the c-terminus-BAF155Δ2 (**Fig. 4**). This size of this deletion approximates the one found in the SKOV3 cell line (**Fig. 2**). We have previously shown that the c-terminal deletion abrogates BAF155's ability to regulate the stability of another complex member, BAF57 (Chen, 2005).

We assessed the effects of full-length or truncated BAF155 protein expression upon long-term proliferation by a colony-forming assay. Colony forming assays showed a marked reduction in colony forming ability in BAF155 null cells after wild type BAF155 re-expression, but not when transfected with mutant BAF155 (**Fig. 4**). No effect on colony forming ability was seen in HeLa cells after transfection with either BAF155 expression vector. In contrast, the BAF155Δ2 truncation mutant was severely abrogated for its growth suppressive activity (**Fig. 4**). These results support the notion that if the deleted form of BAF155 is expressed in the SKOV3 cell line, its normal functions would be impaired.

To determine if the inhibition of growth was due to a senescent or apoptotic response, we investigated typical markers of each of these events in both cell lines at several time points following re-expression of BAF155. We did not observe an increase in DNA fragmentation, characteristic of early to mid apoptosis, by TUNEL staining at 2 d following BAF155 expression (**Table 4**). In contrast, approximately 11% of the BAF155-deficient cell lines expressed the senescence marker, β-galactosidase by 10 d, a 10X increase over the vector control (**Table 4**). These results appear similar to those observed upon re-expression of BRG1 in BRG1/BRM-deficient human tumor cell lines.[15,28-31]

**Cells lacking BAF155 expression retains the ability to support Rb-mediated cell cycle arrest.** We then determined whether the cell lines could undergo RB-mediated cell cycle arrest in the absence of BAF155 protein. Upon infection with $p16^{INK4A}$

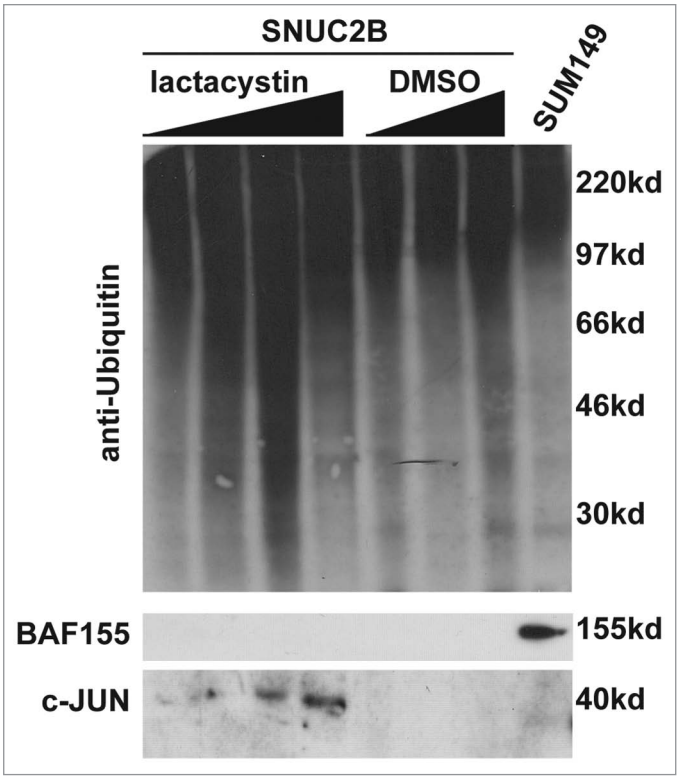
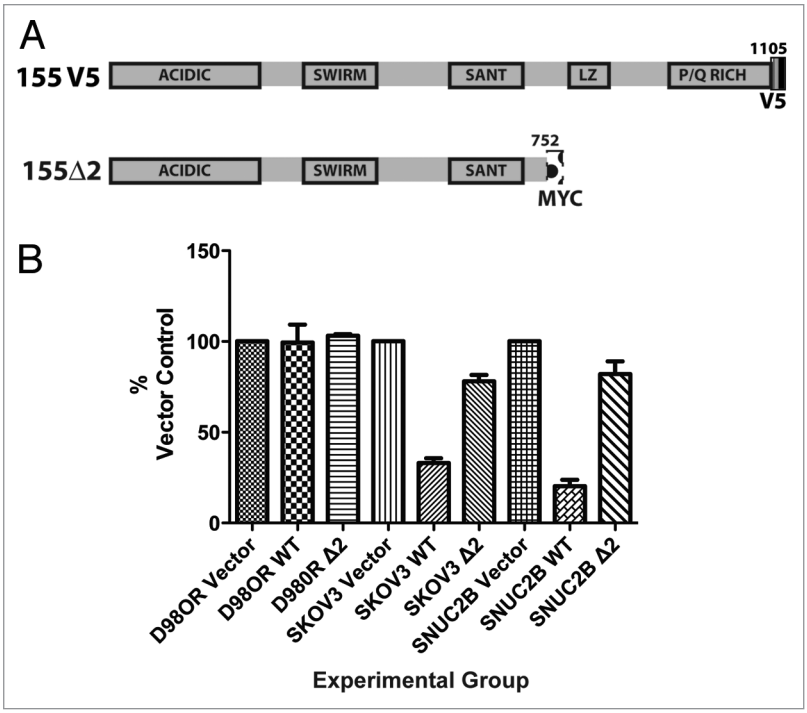



**Figure 3.** BAF155 protein is not degraded by the ubiquitin-proteasome pathway in the SNUC2B cell line lacking BAF155 expression. Increased time after lactacystin treatment inhibits proteasome degradation as demonstrated by the increased poly-ubiquination of protein and the increase of c-jun protein. Mock treatment with DMSO revealed the accumulation is due specifically to the lactacystin treatment. The SUM149 breast tumor cell line serves as a positive control for BAF155 protein expression.




encoding adenovirus, both cell lines demonstrated a significant inhibition in BrdU incorporation (**Fig. 5A**). Furthermore, each cell line showed decreased expression of the cell cycle protein CCNA (cyclin A) as well as the expected expression of the transfected $p16^{INK4A}$ (**Fig. 5B**). These data indicate that the SWI/SNF complex can efficiently regulate RB mediated cell cycle arrest even in the absence of full length BAF155.

## Discussion

The previously conceived notion of cancer etiology being nature OR nurture has been replaced with the knowledge that carcinogenesis often includes both genetic and environmental factors. This paradigm becomes more obvious as the field of epigenetics comes into focus to reveal its importance in cancer development and progression. Many diverse complexes, including the SWI/SNF complex, carry out the chromatin remodeling arm of epigenetic modification. In yeast, a fully-functional SWI/SNF complex includes all complex members[32,33] and the loss of any component causes subtle changes in complex activities. In mammalian cells, the SWI/SNF complex associates with and possibly regulates cell cycle control genes including pRB,[17,28] BRCA1,[12] $p21^{CIP1/WAF1}$,[15,16,34] $p16^{INK4A}$ [9] and Cyclins E and A.[17,29] A loss of any SWI/SNF member could contribute to the tumorigenicity of a cell given these connections to Rb function as well as other cell cycle regulators. This is indeed true for SWI/SNF complex member SNF5/INI1, as loss of SNF5 activity appears to abrogate the RB signaling pathway through a block in $p16^{INK4A}$ or $p21^{CIP1/WAF1}$ induction or increased expression of cyclin D1.[9]

In this report, we have revealed a role for another core SWI/SNF member, BAF155, in cell cycle checkpoint control and likely tumor suppression, via its leucine-zipper domain. We show expression of exogenous BAF155 in carcinoma cells lacking BAF155 consistently produces replicative senescence. This effect was also seen with low levels of BAF155 expression in the stable clones two weeks post-transfection. This low level of exogenous BAF155 was sufficient to cause substantial inhibition of colony forming ability. These data indicate a vital (direct or indirect) role for BAF155 in the control of carcinoma cells SKOV3 and SNUC2B. The leucine zipper or proline/glutamine rich domain of BAF155 would seem to control this effect, as transfection with the Δ2 mutant lacking these domains

**Figure 4.** Re-expression of BAF155 suppresses growth of BAF155-deficient cell lines. (A) Schematic of BAF155 expression vector contents used for re-expression studies in this and subsequent figures. Note the striped boxes indicate the identifiable tag for each insert. The delta-2 mutant BAF155 also has added a nuclear localization signal. (B) Results of the 14 d colony forming assay. Cells were carried under selection for 12 d beginning 2 d post-transfection. Bar values were calculated by a ratio of stained colonies from the BAF155 transfected cells present at day 14 to the colonies of vector control transfected cells for each cell line.

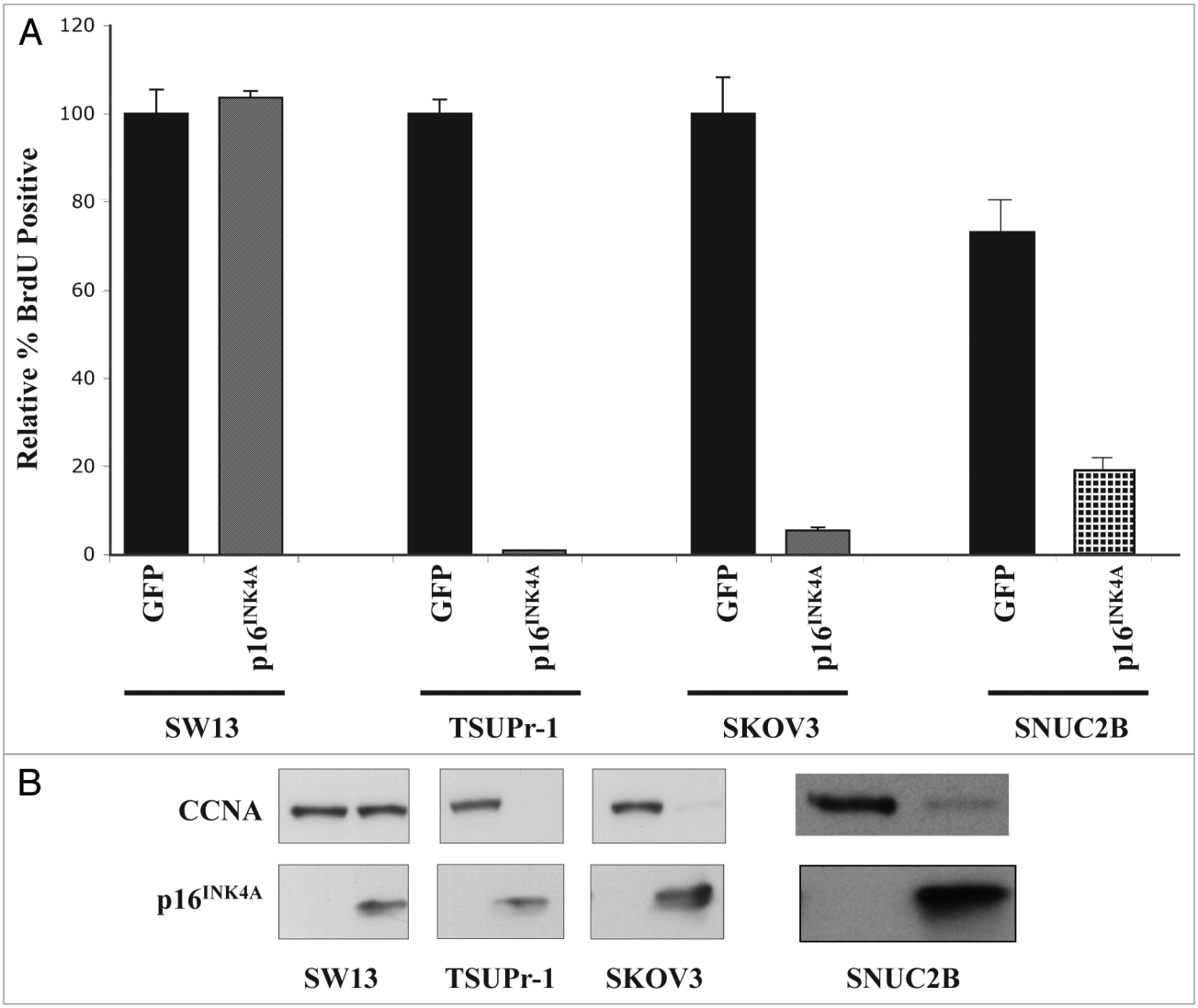



**Figure 5.** BAF155-deficient cell lines retain Rb-mediated growth sensitivity. (A) Thirty six hours after infection with adenoviruses expressing either GFP or $p16^{INK4A}$, cells were analyzed for incorporation of BrdU and graphed as a ratio of BrdU positive cells in the GFP control cells for each cell line. The TSU Pr-1 prostatic carcinoma cell line served as a positive control for $p16^{INK4A}$ growth inhibition and the BRG1/BRM-deficient SW13 adrenal carcinoma cell line served as a $p16^{INK4A}$ non-responsive control. (B) SKOV3 and SNUC2B cells were infected with GFP or $p16^{INK4A}$ encoding adenoviruses. Protein gel blot run from protein extracted 36 h later.

inactivated its ability to suppress growth in the two carcinoma cell lines.

BAF155 is the third core member of the SWI/SNF complex to demonstrate tumor suppressive capabilities related to cell cycle control. **Table 5** summarizes the tumor suppressive capabilities of the core members of the SWI/SNF complex. All core members are required for the basic task of remodeling nucleosomes, but the necessity for their presence in specific tumor suppressive functions of the complex varies. It appears that RB-mediated cell cycle arrest requires only one of the catalytic core subunits, BRG1 or BRM. The specific functions served by SNF5 and BAF155 seem dispensable for this particular tumor suppressive activity of the SWI/SNF complex. In the case of BAF155 loss, increased expression of its highly related paralog BAF170 may substitute as recently demonstrated by Yan et al. However, re-expression of any one core member above does induce growth arrest via senescence, a key characteristic of many tumor suppressors. This implies SWI/SNF complex may participate in more than one pathway to halt cell cycle progression. Additional mutational or inactivation studies are required to determine the pathways SNF5 and BAF155 operate through to halt tumor cell progression.

The lack of protein expression in SNUC2B without a mutation in the primary sequence or apparent degradation by the proteasome can be reconciled in a number of ways. The region of chromosome 3 where BAF155 is found is home to a cluster of suspected tumor suppressor genes. It is of note that somatic mutations have rarely been found in genes in the 3p21 region (only 5 of >200 primary human cancers) although many tumors show inactivation of these genes.[36-38] Further, the non-expressed but non-mutated 3p21 genes are found inactivated by other

epigenetic mechanisms, such as chromosome instability, aneuploidy, promoter methylation, haploinsufficiency, altered RNA splicing, or defects in transcriptional, translational or post-translational processes. Some are inactivated by rather exotic epigenetic means. For example, the FUS1 gene may express mRNA with no apparent mutations without any protein by protein gel blot, IHC or microtissue array, similar to BAF155 in SNUC2B. Also, like so many 3p21 genes, FUS1 is a likely tumor suppressor gene, shown to be lost in >50% of lung cancers cell lines tested. It was eventually discovered that wild type FUS1 could be found as an N-myristoylated protein. Significant loss of expression or a myristoylation defect of the FUS1 protein was found in deficient lung cancer cell lines.[39] A more extensive proteasome-degradation inhibitor may be necessary to ensure that degradation is not the cause of BAF155 protein absence in SNUC2B. Another possibility is a mutation in the large non-coding exon accounting for the last 1,500 bp of the BAF155 mRNA. Mutations within this region could result in significant changes in the tertiary structure of the mRNA leading to inefficient protein translation. We are currently determining the sequence of this region to assess this prospect.

The importance of SKOV3's lost leucine-zipper domain in BAF155 may be its ability to bind with other SWI/SNF complex members. It is known that leucine zipper domains in general allow the proteins possessing them to dimerize, with themselves (homodimerize) or with other proteins possessing a leucine zipper domain (heterodimerize). This has been suggested for BAF155 and BAF170. Chen and Archer recently demonstrated another function for BAF155's leucine zipper domain.[40] This domain was shown to be necessary for BAF155's interaction with BAF57, stabilizing BAF57 in the cell. In fact, similar functions have been shown for several of BAF155's domains. The SANT domain of BAF155 has been shown to be necessary for BRG1 binding and stabilization[41] and BRM binding.[42] The SWIRM domain of BAF155 was required for BAF60a and SNF5 binding and stabilization.[41] Therefore, it has been proposed BAF155 is the scaffolding protein for the SWI/SNF complex, linking BRG1, and the therefore the remodeling capabilities of the complex, to the other subunits which interact with transcription factors or other targeting molecules. Our data would not seem to totally support this notion, as we observe the presence of the other SWI/SNF members in cells in the absence of detectable expression of BAF155 protein (**Fig. 1A**). However, we also note reduced levels of BAF57 and BAF47 (SNF5) proteins in the BAF155-deficient cell lines compared with the BAF53 protein loading control. Furthermore, the recent reports demonstrating mutations in the SWI/SNF complex components PBRM1 (BAF180) in renal cell carcinoma and especially ARID1A (BAF250A) in an aggressive form of ovarian carcinoma offer another mechanism whereby BAF155 loss could lower stability of these tumor suppressor proteins.[43-45] Thus, our results appear consistent with a role for BAF155 in complex stabilization. A determination of the region(s) of BAF155 required for growth suppression and cellular senescence will provide further insight into this mechanism.

Another confounding issue not addressed in previous studies comes from the existence of the closely related SWI3 homolog, BAF170. The BAF155 and BAF170 proteins share significant sequence and domain homology. Therefore, BAF170 may take over as the scaffold in these cells, allowing the complex to form. Clearly, other functions specific to BAF155 must exist whose loss leads to a cancerous phenotype that we see reversed upon exogenous BAF155 expression (**Figs. 3 and 4**). While some SWI/SNF members appear vital to development (e.g., BRG1, SNF5 and BAF155), others remain dispensable (BRM) as demonstrated by the embryonic lethality of mice null for those subunits.[4-8,46] Many of the studies performed to support the scaffold theory were done in immature cells-MEFs, developing thymocytes, or actively growing, transformed, immortalized cancer cell lines.[40,41] It is possible BAF155 serves the purpose of scaffolding during development but may share this function with BAF170 upon cellular differentiation. Further characterization of BAF155 and BAF170 expression in developing or differentiating cells (such as the MEFs or developing thymocytes previously tested) would address these issues.

In conclusion, we submit an additional SWI/SNF core subunit, BAF155/SMARCC1 as a likely tumor suppressor gene due to its loss in two carcinoma cancer cell lines including a hemizygous mutation, its genomic location in the tumor suppressor cluster region of chromosome 3p21 and because of its ability to cause a replicative senescence in deficient cancer cell lines upon its re-expression. As mentioned earlier, BAF155 may be targeted as part of a tumor suppressor gene cluster on 3p21 to be epigenetically silenced, thereby affecting all SWI/SNF regulated genes, which are often cell cycle related. The next step in the determination of potential effectors of BAF155 loss in cancer cells is exploration of epigenetic silencing mechanisms such as promoter hypermethylation, aberrant acetylation patterns and myristoylation in normal developing cell lines such as MEFs or developing thymocytes known to have highly regulated BAF155, as well as in differentiated cells, cancer cells and "normal" cells such as normal human fibroblasts. Further studies into the inactivation of SWI/SNF complex members in the development of human cancer should provide important new targets for treatment intervention, potential biomarkers for early detection and novel insights into the role of altered nucleosome positioning in gene silencing.

## Materials and Methods

**Cell lines.** All cell lines were grown at 37°C in a 5% $CO_2$ incubator with RPMI 1640 medium base supplemented with 10% FBS with the exception of SNUC2B which was grown in 20% FBS. Cell lines listed were originally acquired from ATCC except the HeLa derivative D98oR, that was originally isolated by Weissman and Stanbridge.[47] All cell lines were tested for mycoplasma and found to be negative.

**DNA transfection.** Transfection of expression vectors were performed by the Lipofectamine 2000 (Invitrogen) or Effectine (Qiagen) method according to the manufacturer's instructions. Expression vectors are listed in **Table 1**. Twenty-four hours after transfection, the cells were equally divided into eight 100 mm dishes. After an additional 24 h, growth medium containing

**Table 1.** Source and description of expression vectors

| Plasmid | Content of plasmid | Source |
|---|---|---|
| pcDNA3/BAF155-V5 | V5 tagged full length BAF155 | 40 |
| pCMV/myc/nuc/ BAF155-Δ2 | Myc tagged truncation mutant of BAF155 | 40 |
| β-gal control vector | β-galactosidase (control) CMV promoter | Open Biosystems RHS3708 |
| pCMV | Empty vector control | 40 |

**Table 2.** Antibodies used for studies

| Antibody | Brief description | Source |
|---|---|---|
| BAF155 (H-76) | Rabbit polyclonal, (AA 889–913) | Santa Cruz sc-10756 |
| β-ACTIN | Rabbit polyclonal, | Sigma A2066 |
| BRG1 (G-7) | Mouse monoclonal | Santa Cruz sc-17796 |
| BRM | Rabbit polyclonal | Abcam ab15597 |
| SNF5 | Mouse monoclonal | Transduction Labs, 612110 |
| BAF57 | Rabbit polyclonal | Gift of Karen Knudsen, Thomas Jefferson Medical School |
| BAF170 | Rabbit polyclonal | Gift of W. Wang, National Institute of Aging. |
| C-JUN | Rabbit polyclonal | Oncogene Science PC07 |
| UBIQUITIN | Mouse monoclonal | Santa Cruz sc-8017 |
| C-MYC (9E10) | Mouse monoclonal | Santa Cruz sc-40 |
| V5 | Mouse monoclonal | Invitrogen R961-25 |
| CCNA | Rabbit polyclonal | SCBT H-432 |
| $p16^{INK4A}$ | Mouse monoclonal | BD PharMingen G175–1239 |

600 μg/ml neomycin was added to the dishes. The cells were allowed to grow for 14 d. The dishes were then fixed with ethanol, stained with Coomassie Blue and assessed for colony number by visual inspection. The data represent the results of three independent experiments.

**Protein gel blots.** For protein preparation, subconfluent cells were removed by trypsinization, washed once in PBS and total proteins were extracted using 8 mol/L urea as described previously in reference 34. Protein concentration was quantified by the Bio-Rad protein assay (Bio-Rad Labs, Hercules, CA) per manufacturer's instructions. Proteins (30 μg) were separated by electrophoresis on 4–20% or 7.5% SDS-polyacrylamide gels (Cambrex, Rockland, ME) and electrotransferred onto Immobilon-P membranes (Millipore, Billerica, MA) according to the manufactures' instructions for Biomax ML film (Kodak, Cedex, France). All antibodies were used per manufacturer's instructions unless otherwise specified in the figure legend. The source of antibodies used for protein gel blot and immunohistochemistry is outlined in **Table 2**.

**DNA extraction from cell lines for sequencing.** Genomic DNA was extracted using the Qiagen DNeasy kit per manufacturer's instructions. For cDNA, mRNA was first extracted with the Qiagen RNeasy kit and then reverse transcribed using the Qiagen RT-MMLV kit according the manufacturer's instructions.

**Sequencing.** Sequencing primers were designed according to the NCBI database sequence of BAF155 to amplify individual exons according to the primers listed in **Table 3**. BAF155 sequencing samples were submitted to UNC-CH sequencing facility according to their instructions.

**Protein stability.** Cells were treated with Lactacystin (Calbiochem #426100) or MG132 (Sigma #C2211), dissolved in dimethysulfoxide (DMSO), for the times indicated in the figure. Total cellular proteins were then harvested in 8 M urea and assessed for specific protein expression by protein gel blot analyses as outlined above.

**RNA gel blotting.** Expression of BAF155 mRNA was determined by standard RNA gel blot protocol. Total cellular RNA was extracted using the Qiagen RNeasy kit. Fifteen micrograms of total RNA were run on 1% agarose/formaldehyde gels and were gravity transferred to positively charged nylon membranes. The membranes were stained for ribosomal 28s proteins for equal loading. BAF155 probes were $^{32}$P-radiolabeled by random primer extension, hybridized to membranes and subsequently rinsed to remove nonspecific radioactivity. Hybridization bands were visualized by autoradiography.

**Real-time PCR.** Total RNA was isolated from each cell line using the Qiagen RNA extract kit (Qiagen, Valencia, CA) according to manufacturer's protocol and analyzed by the TaqMan (Applied Biosystems, Foster City, CA) quantitative real-time reverse-transcription PCR using β-actin as a reference gene in each reaction. Primers used were as follows: BAF155: CAC CCC AGC CAG GTC AGA T (forward) and TGC AAC AGT GGG AAT CAT GC (reverse); BAF170: GAG AAG CAC TGG AGT ATC AGA (forward) and ATC TCC GCA TAC TTC AGC TG (reverse); BRG1: GAT AAA GGA GCG CAT TCG CA (forward) and TTC TGG CAC AGG AGC ATG AC (reverse); and SNF5: CAG AAG ACC TAC GCC TTC AG (forward) and GTC CGC ATC GCC CGT GTT (reverse). The probes used were CAG GCC CAG GTT CCA TGA TGC CCG (BAF155), CAG CAG CTC CTG GCC GAC AGA CAA G (BAF170), CAA GTA CCG CAG CCT CAA CGA CCT

**Table 3.** Primers used for determining BAF155 sequence in cDNA and genomic DNA

| cDNA Primer# | Primer sequence (forward) | Primer sequence (reverse) |
|---|---|---|
| 1 | ACG ACG GGC TGC GAC GAT G | CAC CGA ATC CAG CTG GGG AC |
| 2 | TTG GTG CAG AAC AAT TGT TTG ACC | CAA GAA TGT GAC ATA AGG CGC |
| 3F | GCC AGT CAG AAG TCC AGA AAG AAG | |
| 7R | | GAG TGC TGG TGT GCC TGT TG |
| 9R1 | | CGT GGA GGT TCC CTG CAT C |
| 16 | CAA TGC CAG GAA ACA TCT TAG GA | TCC TAA GAT GTT TCC TGG CAT TG |
| 122 | CGC AGG CCT AGC TGT TTA TC | TCG ATA AAC AGC TAG GCC TGC G |
| 290 | GTG CAG CTT CTT CAG TTC C | TTC CTG GAA CTG AAG AAG CTG CAC C |
| 425F | CAA GTA TAA AAA TGA ACA GGG ATG G | |
| 545F | ATC TCG AAT GGA TCG TAA TG | |
| 660F | CAC ATT TAC CCA TAT TCT TCC TCA C | |
| 744R | | AAT GCA CTA ACA CTT GCT TCT CTT T |
| 859F | TGG ACA CTG ATA TTT TCA ATG AAT G | |
| 1001R | | TGC TTT TCT ATC TCT TCT TTC TGG A |
| 1167F | CCA ACA CCT GTA CCC AAT ATA GAA G | |
| 1191R | | TCT TTC TTT AGG TTC ACA TTT TTG G |
| 1538F | GTA TCG TCT AAA CCC CCA AGA GTA T | |
| 1562R | | ATA CTC TTG GGG GTT TAG ACG ATA C |
| 1812F | CAG ATG CTA AAT TTT CCT GAG AAA A | |
| 1866R | | AGT AAA TGT CAG TAC GGA GAC CAA A |
| 2496 | GAA AAG GAA CAG GAT AGT GAA GTG A | TCA CTT CAC TAT CCT GTT CCT TTT C |
| 2822R | | GGA AAC TAT CAT GGA CAG AGA GAA A |
| 2833R | | TCT AGA GCT TCT TTC TCT CTG TCC A |
| 3123R | | CTG CAA CAG TGG GAA TCA TG |
| 3340R | | CTG CAT CTT CCA GGC TAA GG |
| **Exon** | **Forward Primer** | **Reverse Primer** |
| Exon 1 | TTG CAT ATT CGT GGT TCT AAA TTC | GAG GCC AGC TGC CGC CT |
| Exon 16 | GCA CAT GAA GTT GAT TCT TTA G | CAA CCC CTA AAT GGC ATA AC |
| Exon17 | AGA GGC TAA CTC AGC CAT TT | GCC ATT TGT TAC TAT CCA GTC |
| Exon 18 | CTG AGC TCC ATC TTC AGC AT | ACT AAG ATG TGC TGG ATT GC |
| Exon 19 | CTG AGC TCC ATC TTC AGC AT | GGT GAC ACA CAG CGA GAC C |
| Exon 20 | TCT TGT TAC CTG ACA TGA AAG C | ATG AAT GTA ACG GCT GGT AA |
| Exon 21 | AAG TTA GAT TGC TGT TCC AT | TAT ACT GAT CAT TGT ACT CAC T |
| Exon 22 | CAG ACA CTC TCA CAG CTG TA | GGT CTG AAA GGC ATG CTA TC |
| Exon 23 | CGA GCT GTT CTC TAC AAG TCA C | AAG TGT AGC CAC CAG CAT TA |
| Exon 24 | GAG AGT GGG CAC GCT AGT A | CAA TAC GAG CAT TCA TGC CT |
| Exon 25 | AGC TTG AAT GAT CTT GTA CC | TGG AAA AGT GAC CAA GGT T |
| Exon 26 | CTG GTG TCA TTT CTT CCA CT | CCC TAA ATC TCA CAT GCC TAC |
| Exon 27 | GTT GAT CCC TTT CTT ACA CCC | AAC GGA CCC TGA GATA ATG C |
| Exon 28 | TCC AGC AAT AAC GAG TTA CTC | CAC GGC TTG GAG CTG TGA G |

A (BRG1) and ACC CTC TGC CCA CAG TGG AGA TTG (SNF5).

**Apoptosis detection—TUNEL assay.** Apoptosis via DNA fragmentation was measured using the "In Situ Cell Death Detection Kit, TMR red" from Roche (catalog # 12 156 792 910). The kit was used according to manufacturer's instructions following transfection with plasmids, at times post-transfection as indicated in each figure. At least 200 cells were counted in each of at least three independent experiments for each condition.

**Senescence detection via β-galactasidase.** Senescence-associated β-galactosidase was performed as previously described in reference 48, with modifications of Kramer et al. Briefly cells

**Table 4.** Effect of BAF155 reexpression on replicative senescence and apoptosis

| Cell Line | Transfection | Average apoptosis (%) (48 h) | | Average βGal positive (%) (10 d) | |
|---|---|---|---|---|---|
| | | Mean | Std. Dev. | Mean | Std. Dev. |
| SNUC2B | vector | 0.06 | 0.0032 | 1.4 | 0.8 |
| | BAF155 | 0.07 | 0.0075 | 11.0 | 0.1 |
| | BAF155-Δ2 | 0.06 | 0.0074 | - | - |
| | β-Gal | - | - | 20.5 | 0.2 |
| | | | | | |
| SKOV3 | vector | 0.03 | 0.0015 | 1.9 | 1.4 |
| | BAF155 | 0.04 | 0.0044 | 11.0 | 2.5 |
| | BAF155-Δ2 | 0.04 | 0.0191 | - | - |
| | β-Gal | - | - | 22.3 | 3.8 |
| | | | | | |
| HeLa | vector | 0.08 | 0.0050 | 1.5 | 0.7 |
| | BAF155 | 0.08 | 0.0074 | 1.5 | 0.7 |
| | BAF155-Δ2 | 0.08 | 0.0069 | - | - |
| | β-Gal | - | - | 20.4 | 2.3 |

TUNEL Apoptosis detection. Cells were analyzed 48 h post co-transfection with GFP and one of the following: empty vector control, V5 tagged full length BAF155, or myc tagged Δ2 truncation mutant BAF155. A ratio of TUNEL positive (broken DNA ends = apoptosing) cells to GFP positive cells (presumed to express BAF155 as well) were averaged and normalized to empty vector control cell.

**Table 5.** Comparison of tumor suppressor properties of SWI/SNF complex core components

| SWI/SNF core subunit | Required for RB-mediated arrest? | Can cause growth arrest? | Induces senescence? | Involved in apoptotic signaling? |
|---|---|---|---|---|
| BRG1/BRM | yes | yes | yes | no |
| SNF5 | no | yes | yes | no |
| BAF155 | no | yes | yes | no |

Summary of the tumor suppressor capabilities of SWI/SNF core members. Data compiled from this study and previous studies as discussed in the text.

were washed with PBS, fixed for 3 min with 3% formaldehyde/PBS, washed three times with PBS, and incubated overnight in a 37°C incubator in SA-β-gal solution 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, 2 mM $MgCl_2$ and 1 mg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal in PBS). Plates were visualized with bright field microscopy on a Zeiss 1M 35 microscope. Results are representative of duplicate experiments.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.


### Acknowledgments

This work was supported, in part, by R01CA91048 and R01CA138841 (B.E.W.), by the Intramural Research Program of the National Institute on Aging (grant AG000650-10), National Institutes of Health (W.W.), by the Intramural Research Program of the National Institute of Environmental Health Sciences (Z01 ES071006-11), National Institutes of Health (T.K.A.) and by R01CA127387 (E.S.K.). Dr. Delbove was supported, in part, by a training grant from the National Institutes of Health T32ES007017.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