### Abstract

**Purpose:** BAF57, a component of the switching-defective and sucrose nonfermenting (SWI/SNF) chromatin-remodeling complex conglomerate, modulates androgen receptor activity to promote prostate cancer. However, the molecular consequences of tumor-associated BAF57 expression have remained undefined in advanced disease such as castration-resistant prostate cancer and/or metastasis.

**Experimental Design:** Clinical human specimens of primary and metastatic prostate cancer were immunohistochemically examined for tumor-grade association of BAF57 expression. Global gene expression analyses were conducted in models mimicking tumor-associated BAF57 expression. Aberrant BAF57-dependent gene expression changes, bypass of androgen-mediated signaling, and chromatin-specific SWI/SNF complex alterations with respect to cytoskeletal remodelers such as integrins were validated. Cell migration assays were used to profile the biologic phenotypes conferred under conditions simulating tumor-derived BAF57 expression.

**Results:** Immunohistochemical quantitation of primary human specimens revealed that BAF57 was significantly and aberrantly elevated as a function of tumor grade. Critically, gene expression analyses showed that BAF57 deregulation circumvented androgen-mediated signaling, elicited α2 integrin upregulation, and altered other SWI/SNF complex components at the α2 integrin locus. BAF57-dependent α2 integrin induction conferred a prometastatic migratory advantage, which was attenuated by anti-α2 integrin antibody blockade. Furthermore, BAF57 was found to be markedly upregulated in human prostate cancer metastases of the lung, lymph node, and dura.

**Conclusion:** The findings herein, identifying tumor-associated BAF57 perturbation as a means to bypass androgen-signaling events that facilitate novel prometastatic phenotypes, link BAF57 upregulation to tumor dissemination. These data thereby establish BAF57 as a putative marker of metastatic potential that could be leveraged for therapeutic intervention.

### Introduction

Prostate cancer is one of the most frequently diagnosed noncutaneous male malignancies in the United States, constituting about 30% of all new cases of cancer diagnosed (1). In this tumor type, the transcriptional activity of the androgen receptor (AR) is required for tumor formation and progression. Therefore, the current standard of care for disseminated disease relies on the use of androgen-depletion strategies such as GnRH agonists to suppress testicular androgen synthesis, CYP-17 inhibitors to thwart intracrine androgen synthesis (2, 3), or AR antagonists such as bicalutamide. Although these modes of therapeutic intervention are initially effective, patients eventually develop recurrent tumors that constitute the advanced lethal stage of disease termed castration-resistant prostate cancer (CRPC, ref. 4). Although newer, more powerful AR antagonists such as enzalutamide (formerly MDV3100) show clinical response (5, 6), morbidity rates continue to daunt disease prognosis. Molecular profiling has determined that diverse mechanisms of inappropriate AR reactivation and function, such as AR amplification, splicing, posttranslational modifications, chromosomal translocations, AR interplay with a variety of coregulators such as chemokines (e.g., CXCL12), oncogenes (e.g., c-myc and c-src), and coactivators (e.g., CBP, SRC family)
Translational Relevance

Prostate cancer is a leading cause of cancer-related male mortality. Current treatments of nonorgan-confined disease provide only transient remission before progression to refractory advanced castration-resistant prostate cancer and/or metastasis. It is therefore pivotal to elucidate novel, clinically relevant targets for durable response. BAF57, a component of the SWI/SNF chromatin-remodeling complex, is a known transcriptional modulator, upregulated in advanced disease. Herein, functional investigation revealed that BAF57 perturbations supplanted androgen-mediated signaling and also elicited SWI/SNF complex alterations to induce metastatic migratory phenotypes. Further analyses showed that BAF57 facilitated prostate cancer cell migration through androgen receptor–dependent modulation of α2 integrin expression and function. Finally, evaluation of human metastases showed that BAF57 expression was significantly enhanced in disseminated disease, thereby linking BAF57 aberrations to lethal tumor phenotypes. For the first time, this study establishes an association between anomalous subunit-specific programs of oncogenic SWI/SNF transcriptional misregulation and clinically relevant outcomes.

The human tissue samples used for immunohistochemical analyses consisted of 2 tissue microarray (TMA) slides, one for primary tumor specimens and the other for metastatic specimens. Of the 60 clinical specimens (in triplicate) obtained after radical prostatectomy from patients, approximately 40 were determined to be acceptable for analyses by the pathologist. The metastatic TMA cores in triplicate were derived from 20 patients who died of metastatic disease and subsequently underwent rapid autopsy conducted under the aegis of the University of Michigan (Ann Arbor, MI). The TMA slides were generous gifts from Dr. Kenneth Pienta (University of Michigan) and Dr. David Reisman (University of Florida, Gainesville, FL). A clinical pathologist quantified the BAF57 staining for intensity and extent of scoring on a scale of 0–3. Final scores were computed using a composite of intensity score multiplied by the extent of staining score.

Transient transfections and mRNA analyses

To assess BAF57-mediated induction of AR activity, LNCaP cells were seeded at a density of 1.8 × 10⁶ cells on poly-L-lysine–coated 10-cm dishes and transfected overnight with 7 µg of pcDNA3.1 3x-FLAG or pcDNA3.1 (−) BAF57-FLAG and 1 µg of H2B-GFP as transfection marker in steroid-free medium with lipofectin as per manufacturer’s protocol. Following transfection, medium was changed to 5% charcoal dextran–treated (CDT) improved minimum essential medium (IMEM; steroid-depleted medium is used to mimic castration). Twenty-four hours later, 1 nmol/L dihydrotestosterone (DHT) or 0.1% ethanol vehicle was added for 12 hours. The TRizol
method was used to harvest mRNA. Subsequently, cDNA was generated and used for quantitative PCR (qPCR) analyses by SYBR-based chemistry. Oligonucleotides used in qPCR are provided in Supplementary Table S1.

Migration assay using integrin antibody-blockade
C4-2 cells seeded at a density of $2 \times 10^5$ were transfected using pBabe-puro-FLAG or pBabe-puro BAF57-FLAG and H2B-GFP as transfection marker in steroid-free medium with lipofectin as per manufacturer’s protocol. Following transfection, cells were supplemented with 5% CDT-supplemented IMEM and allowed to attain confluency for approximately 48 hours before the first wound was made. At initial wounding (time = $T_0$), P1E6 monoclonal antibody (Millipore; MAB1950) or immunoglobulin G (IgG; R&D Systems; MAB002) at 20 $\mu$g/mL was added and the area was imaged. Antibody treatments were replenished every 24 hours and the same area was imaged at $T_{24}$ and $T_{72}$ for GFP-positive cells.

Results
BAF57 is upregulated as a function of tumor grade
BAF57 was previously determined to be critical for androgen-dependent AR activity and shown to be upregulated in a preliminary study of clinical prostate cancer specimens (24). To define the role of BAF57 in disease progression herein, immunohistochemical analyses of the expression patterns of BAF57 were conducted using a larger cohort of prostate cancer specimens obtained from radical prostatectomy. Representative images of BAF57 expression (Fig. 1A) and quantification of BAF57 staining (Fig. 1B) revealed that nuclear BAF57 expression was upregulated in prostatic intraepithelial neoplasia (PIN, a precancerous proliferative lesion), when compared with nonmalignant benign prostatic hyperplasia (BPH; reviewed in ref. 26). The Gleason grading system is currently the most powerful prognostic indicator of disease progression. The primary and secondary score patterns are graded 1 to 5 and the 2 grades are added, with higher scores generally indicative of adverse outcomes.
(reviewed in ref. 27). Notably, BAF57 is also significantly upregulated as a function of increasing tumor grade (Fig. 1B) suggesting a strong correlation with adverse disease development and progression.

**BAF57 potentiates hormone-sensitive and castration-resistant AR activity**

Further screening of a panel of prostate cancer cell lines (Supplementary Fig. S1A) and samples of matched non-neoplastic and tumor tissue (Fig. 1C) from radical prostatectomy revealed a modest but consistent elevation of BAF57 protein expression. Therefore, isogenic cell model systems mimicking tumor-associated elevation of BAF57 expression were generated (Fig. 2A) using transient expression of BAF57 in both androgen-dependent AR-positive prostate cancer (LNCaP) and in the CRPC cell line C4-2. The C4-2 cells, derived from LNCaP, remain sensitive to androgen stimulation but are also capable of growth in castrated hosts (28). Because AR is a critical effector of prostate cancer progression, the impact of heightened BAF57 expression on AR activity was ascertained. As expected, BAF57 deregulation acted in concert with androgen DHT to enhance AR activity, reflected in an at least 2-fold induction of well-characterized AR target genes KLK3/PSA, TMPRSS2, and FKBP5 over vector control (Fig. 2B).

Intriguingly, under conditions mimicking castration, BAF57 induced a modest but significant enhancement in AR target gene expression in both hormone-sensitive and CRPC models (Fig. 2B and C, respectively). These findings provide the first evidence of a distinct potential for BAF57 deregulation in facilitating inappropriate ligand-independent AR transcriptional programs that are known to promote disease progression.

**BAF57 overexpression supplants the requirement for androgen and uniquely upregulates genes linked to tumor cell migration and invasion**

Evidence in the literature points to the existence of a differential transcriptional program in CRPC, wherein the function of AR, the major effector of disease progression, is to modulate gene sets that favor CRPC growth and survival (29). Because BAF57 activates ligand-independent AR activity (Fig. 2B and C), it can be posited that BAF57 elevation in the absence of hormone initiates unique transcriptional programs that drive castration resistance and metastatic prostate cancer. BAF57 is critical...
in androgen-dependent prostate cancer, modulating AR response to ligand and androgen-dependent cell proliferation (30). However, the mechanism of BAF57 action in CRPC has not been studied. To discern the BAF57-induced transcriptional changes in a CRPC setting, unbiased global expression analyses were conducted in hormone therapy sensitive, androgen-dependent cells under conditions of transient SMARCE1/BAF57 upregulation. For these studies, cells were transfected transiently with plasmids encoding BAF57 or control along with GFP under steroid depleted conditions, then stimulated with DHT or vehicle for 12 hours before microarray analyses (Supplementary Fig. S1B and S1C).

K-means clustering was conducted (Supplementary Fig. S2A) to determine transcripts that display similar expression patterns in the presence and absence of ligand. CRPC is driven by AR activation in low-ligand environments (31) and the first pattern parsed out transcripts wherein BAF57 elevation supplanted the necessity for hormone in gene activation and contained clinically relevant biomarkers such as TMPRSS2 and FBKPS (32). However, to avoid missing bona fide BAF57-regulated targets, additional analysis was conducted using a false discovery rate (FDR) of 25% and a 2-fold change. Depicted in the Venn diagram (Supplementary Fig. S2B) are the 558 transcripts that bypassed the requirement for hormone in the presence of BAF57 upregulation, with 28 uniquely BAF57-regulated transcripts (Supplementary Fig. S2D) and 1,323 hormone-sensitive transcripts. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Gene Ontology analyses (Supplementary Table S2 and Supplementary Fig. S2, respectively, list the KEGG pathways and the individual transcripts in each pathway category) showed unexpected changes in actin cytoskeletal remodelers. Because changes in cytoskeletal signaling components are hallmarks of disease progression and are thought to activate aberrant signaling thereby facilitating metastasis (33), α6 integrin (ITGA6) and Rho-associated coiled kinase 2 (ROCK2) were identified as focal points in cancer signaling networks mediating tumor cell motility and migration. Comparison with metastasis-associated gene signatures for prostate cancer (34) revealed 2 potential BAF57-regulated candidates, cathepsin C (CTSC) and mucosa-associated lymphoid translocase 1 (MALT1). In addition, BAF57-regulated α2 integrin (ITGA2) was chosen for validation, given its association with lymph node metastases and putative roles in bone remodeling, processes that characterize prostate cancer metastases (35, 36).

Validation studies revealed that BAF57 elevation caused significant induction (P ≤ 0.05) of at least 2-fold in ITGA2, ITGA6, CTSC, and ROCK2 (Fig. 3A) but not MALT1 (Supplementary Fig. S2E), in conditions mimicking castration in both hormone therapy-sensitive and CRPC models. Conversely, BAF57 ablation depleted ITGA2 transcript in C4-2 cells (Fig. 3C) but not ROCK2 in LNCaP (Supplementary Fig. S3D). These studies suggest that integrins are targets of aberrant BAF57 signaling. Although it is known that integrin alterations regulate tumor cell migration and invasion in cancer (37), these data offer the first molecular evidence of BAF57 deregulation in the potential control of integrin expression in cancer models.

Because tumor metastasis is generally accompanied by changes in angiogenic markers, targets from the microarray, HIF1A and DPP4 were also tested along with traditional angiogenesis markers such as VEGF. However, no significant changes in any of these angiogenic genes were observed upon BAF57 deregulation (Supplementary Fig. S2G). These data are consistent with prior reports wherein detectable angiogenic markers were observed only in advanced disease with well-established metastatic deposits (reviewed in ref. 38). Combined, these data suggest a potential initiating role, independent of angiogenesis, for BAF57 deregulation in facilitating metastatic gene expression.

Tumor-associated BAF57 mediates ligand-independent upregulation of α2 integrin

Integrins are heterodimeric cell surface receptors consisting of an α and β subunit that contain extracellular ligand-binding domains and a cytoplasmic domain for recruitment of downstream signaling proteins to initiate signal transduction pathways regulating proliferation, survival, and migration (39) and identified as potentially regulated by BAF57 deregulation. Given that high-grade disease is prone to metastasis and BAF57 is not only deregulated in high-grade disease but also induced genes associated with cell migration and invasion, a functional link between these observations was explored. As shown, BAF57 not only upregulated ITGA2 transcript (Fig. 3A), but also induced modest increases in protein expression (Fig. 3B), in both hormone-sensitive (~1.3-fold for BAF57 relative to vector untreated and 1.5-fold for DHT-treated vector samples) and CRPC models. However, the ITGA2 transcript, unlike the protein, was not subject to androgen regulation in parental LNCaP cells, suggesting that the observed androgen-dependent regulation of α2 integrin protein may be posttranscriptional (Supplementary Fig. S3B). Disruption of BAF57 function in CRPC models further established that α2 integrin expression is contingent upon BAF57, wherein knockdown of BAF57 revealed concomitant decreases in ITGA2 transcript and protein levels (Fig. 3C). Similar results were observed with the β1 integrin (ITGB1) subunit, which frequently heterodimerizes with α2 integrin for signaling (Supplementary Fig. S3A and S3C). These observations show a specific diminishment of integrin function upon loss of BAF57 and indicate an unprecedented function for BAF57 in the control of cytoskeletal effectors in metastatic disease.

To gain mechanistic insight into the specificity governing BAF57-regulated integrin activation, the ITGA2 gene locus was evaluated for potential androgen response elements (ARE) and AR-occupied regions (AROR; Supplementary Fig. S3E, left). Because BAF57 regulates AR activity (30), a putative AR-binding site along with an ARE was identified in the second intron of the ITGA2
gene and was further explored by chromatin immunoprecipitation qPCR analyses in steroid-depleted conditions. Antibodies directed against AR, SWI/SNF core ATPase components Brg1 and Brm revealed that BAF57 deregulation enhanced AR and Brg1 occupancy at this locus (Fig. 3D), whereas Brm showed a relatively modest occupancy pattern (Supplementary Fig. S3E, right). Together, these data suggest that BAF57 elevation activates ITGA2 transcription by directing AR and the SWI/SNF chromatin-remodeling complex to the intronic...
validate that the cell migratory effects upon BAF57 induc-
tion were indeed governed by α2 integrin, studies were
conducted using a validated monoclonal-blocking antibo-
dy (40, 41) directed against integrin α2. Consistent with
the findings above, antibody blockade resulted in a quan-
titative (Fig. 4A) impairment in the movement of GFP-
positive, BAF57-deregulated cells toward the wound area
at the designated time points (depicted in Supplementary
Fig. S4G, representative images). This indicated a require-
ment for α2 integrin-mediated cell motility that was also
recapitulated in hormone therapy–sensitive model systems
(Supplementary Fig. S4F). Thus, these data provide comp-
pelling evidence for BAF57-driven α2 integrin upregulation
culminating in migratory, prometastatic phenotypes.

To interrogate whether the AR can impact BAF57 medi-
at ed migratory processes, wound-healing experiments
were carried out in the presence and absence of a sec-
ond-generation AR antagonist MDV3100 (enzalutamide)
that has been U.S. Food and Drug Administration (FDA)
approved for the treatment of CRPC (5, 6). These studies
(Fig. 4B and Supplementary Fig. S3F, respectively)
revealed that thwarting AR activity using MDV3100 could
impair BAF57-governed migratory and transcriptional
processes. Wound-healing experiments were also carried
out in C4-2 cells in the presence or absence of DHT, to
delineate the effect of androgen on BAF57–dependent
migratory propensities. These data show a tendency
toward modest increments in migration (Supplementary
Fig. S4C), suggesting that in CRPC models, BAF57 can
cooperate with hormone to activate promigratory path-
ways. In summary, BAF57 seems to cooperate with the AR
signaling axis to govern prometastatic phenotypes.

BAF57 deregulation predisposes to metastasis
It is well established that SWI/SNF perturbations are
associated with tumor development (9–11, 17, 42–44).
However, as BAF57 elevation conferred a prometastatic
migratory advantage to cancer cells through α2 integrin,
it was postulated that BAF57 expression patterns might also
be perturbed in clinical metastatic disease. Quantitative
analyses of immunohistochemical expression of BAF57
conducted in lung, lymph node, dura, and liver metastases of
CRPC (Fig. 5A and B and Supplementary Fig. S5B)
revealed that with the exception of liver metastases, BAF57
was significantly (P < 0.01) elevated at other sites of met-
astatic disease. Interestingly, multiple sites of metastasis
examined from the same patient displayed variability in
BAF57 expression levels (Supplementary Fig. S5A); the
overall average across patients with liver metastases
remained at a low score of 2, whereas lymph node, lung,
and dura metastases across patients showed a higher aver-
age score ranging between 5.5 and 6.5 (Supplementary Fig.
S5B). This indicates that despite variations within the same
individual, elevated BAF57 protein expression in metastatic
disease, relative to primary prostate cancer is fairly consis-
tent (Supplementary Fig. S5C) and likely points to an
invasive and aggressive cancer phenotype. Thus, these data
imply that BAF57 expression in metastatic prostate cancer is
likely to be a key player in disease progression. In summary,
the data presented herein assign a compelling role for aberrant BAF57 expression in driving not only AR activity but also prometastatic integrin signaling contributing to advanced disease (Fig. 5C). The study substantiates a paradigm wherein heightened BAF57 expression and SWI/SNF deregulation serve to regulate the cytoskeletal machinery directly impacting the development of prometastatic phenotypes.

Discussion

The current study is the first comprehensive molecular assessment of a discrete prometastatic function for BAF57. Data in support are as follows: (i) BAF57 elevation is significantly maintained in high-grade human prostate tumors, (ii) BAF57 deregulation potentiates inappropriate ligand-independent AR activity and also induces ITGA2 transcription by eliciting changes in SWI/SNF complex components and function. α2 integrin induction facilitates migratory and prometastatic phenotypes that can be inhibited by α2 integrin blocking antibody.

Figure 5. BAF57 deregulation predisposes to metastasis. A, representative images of BAF57 IHC staining conducted on a TMA of metastatic tissue cores derived at rapid autopsy. B, quantification of the average BAF57 IHC staining in metastatic tissue. BPH values from Fig. 1B are provided as a nonneoplastic reference. N refers to the number of samples, some originating from multiple biopsies and locations within the same patient, analyzed for each category; BPH (N = 9), liver (N = 16); lymph node (N = 23); lung (N = 6); and dura (N = 6). Statistical comparisons were made using the Bonferroni multiple comparison test with **, P < 0.01. C, model for SWI/SNF deregulation in driving advanced cancer progression by modulating precursors of metastasis. The data presented herein suggest that BAF57 deregulation potentiates ligand-dependent and -independent AR activities and also induces ITGA2 transcription. α2 integrin induction facilitates migratory and prometastatic phenotypes that can be inhibited by α2 integrin blocking antibody.
of genes linked to cell migration and metastasis, (iii) BAF57 attenuation compromises α2 integrin expression and Brg1 and Brm are enriched at the α2 integrin-regulatory locus implicating BAF57-directed SWI/SNF–dependent transcriptional control, (iv) BAF57 elevation confers a migratory advantage to cancer cells that can be mechanistically linked to α2 integrin, and (v) BAF57 expression is conspicuously elevated in human clinical specimens of metastasis. Combined, these findings establish the prognostic value of BAF57 in the development of metastasis and the use of SWI/SNF as a possible therapeutic target in aggressive prostate cancer.

The multisubunit SWI/SNF chromatin-remodeling complex exerts regulatory control over cell cycle, gene transcription, and genomic integrity. This complex consists of a central core ATPase subunit, either Brg1 or Brm and numerous associated accessory subunits termed Brg1/Brm–associated factors (BAF). Recent studies have detailed loss, mutation, or expression changes of various subunits in several different cancers (17). Prostate cancer has also been characterized by changes in several SWI/SNF subunits. BAF155 upregulation has been correlated with metastatic and recurrent prostate cancer (20), whereas Brm loss is a marker of prostatic hyperplasia (19) and Brg1 elevation is associated with invasive prostate cancer (18). BAF57, a known AR comodulator is aberrantly overexpressed in a small cohort of clinical human specimens of prostate cancer (24). However, pathologic signaling pathways driven by aberrant BAF57 expression and its clinical value as a metric of the metastatic phenotype have so far remained unresolved. Thus, this study fills an important gap in elucidating the detailed signaling outcomes and metastatic potential of SWI/SNF dysregulation in the prostate cancer context along with identifying potential drug targets.

In this study, immunohistochemical analysis of aberrant BAF57 elevation in a large cohort of clinical specimens of human primary and metastatic prostate cancer offsets the constraints posed by sample availability and size. Herein, nuclear staining of BAF57 revealed that high BAF57 expression correlated with aggressive disease (Fig. 1A–C). Also, the consistent elevation of BAF57 in metastatic human prostate cancer specimens from lung, lymph node, and dura reinforces a propensity for metastasis (Fig. 5A and B). Moreover, the current study is in consonance with a recent report linking elevated BAF57 expression and myometrial/lymphovascular space invasion in endometrial cancer (25), which established that BAF57 overexpression is an independent prognosticator of poor survival. Thus, future studies determining detailed clinical survival and disease recurrence parameters connected with BAF57 overexpression in prostate cancer will enhance the clinical use of BAF57 as a putative marker of disseminated disease. The Bio Cancer Genomics portal, maintained by the Memorial Sloan-Kettering Cancer Center (New York, NY) is an open-access database with comprehensive molecular and clinical data tumor samples spread across 20 different cancer studies (45). Data from metastatic prostate cancer samples from this repository showing elevated BAF57 transcript profiles (data not shown) are consistent with the immunohistochemical patterns of BAF57 elevation obtained in our study.

BAF57 overexpression models surmounted the requirement for androgen to potentiate clinically relevant AR target genes such as TMPRSS2 and FKBP5 (Fig. 2B) with no concomitant increases in AR protein (Supplementary Fig. S1D). Given the advent of new AR signaling inhibitors such as enzalutamide (formerly MDV3100) that prolong the survival benefit for men with metastatic CRPC (6), these regimens were evaluated for efficacy in the context of deregulated BAF57 signaling–driven inappropriate AR activation. The AR antagonist effectively suppressed the BAF57-mediated enhancement of AR target genes and caused a modest blockade of BAF57-dependent migratory phenotypes, whereas DHT cooperated with BAF57 to drive migration (Fig. 4B and Supplementary Fig. S4C). These data are in agreement with other studies showing androgen-driven metastatic pathways in prostate cancer (46). However, the efficacy of AR antagonists, such as MDV3100, in specifically suppressing integrin signaling powered metastatic pathways, remain to be determined.

Recapitulation of BAF57 perturbations in the Ishikawa cells, a model of endometrial cancer (Supplementary Fig. S2F) also resulted in the induction of α2 integrin and supports the premise of a common concerted program of SWI/SNF subunit-specific signaling underlying oncogenic transcriptional aberrations. The observed alterations in Brg1 levels (Supplementary Fig. S1D) and differential SWI/SNF occupancy patterns at the ITGA2 locus in prostate cancer cells (Fig. 3D and Supplementary Fig. S3E) indicate that heightened BAF57 expression may have important repercussions for the combinatorial assembly of subunits underpinning the functional specificity of the SWI/SNF complex. As such, changes in BAF57 expression may result in (i) global imbalances in SWI/SNF components, (ii) mistargeting of these complexes, and (iii) reconfiguring of transcription (9). All or some of these events could disrupt normative transcriptional programs, accelerating cancer progression. This argument can be buttressed by other studies showing that SRG3, a murine homolog of BAF155 affects stabilization and expression of Brg1, SNF5, and BAF60a (47); BAF57 depletion in HeLa cells leads to codepletion of BAF180, and resultant transcriptional misregulation (48); loss of SNF5/INI1/BAF47–dependent cancer progression relies on the necessary presence of Brg1-containing SWI/SNF complexes (49). The Bio portal resource also provides evidence in prostate tumors for concurrent upregulation of transcripts for SMARCE1 (encoding BAF57), SMARCA4 (encoding Brg1), and SMARCC1 (encoding BAF155) and concerted upregulation of SMARCE1 and SMARCC1 in uterine corpus endometrioid carcinoma (45). Future studies interrogating genomewide BAF57 and/or SWI/SNF binding at regulatory loci in CRPC and metastatic prostate cancer will assist in clarifying how malignancy-related subunit-specific anomalies resulting in SWI/SNF aberrations disrupt the normal interplay
between chromatin and AR that could be invoked for biomarker/therapeutic assessments. These data interpreted in conjunction with SWI/SNF enrichment at the ITGA2 locus underscore the clinical importance of concurrent SWI/SNF alterations. Such changes may be symptomatic of subunit-specific misregulated transcription supporting SWI/SNF–dependent induction of metastasis related gene expression programs.

The involvement of SWI/SNF in regulating genes pertaining to metastasis and invasion has been reported in human rhabdoid tumors wherein SNF5 (encoding for INI1/BAF47) loss or downregulation stimulates expression of genes connected with invasion and metastasis, such as SPP1, MMP12, NCOA3, TFRC, and RSU1 (50). Malignant melanomas harboring Brg1 elevation display invasive behaviors (51). Brg1 transcript upregulation is also a hallmark of gastric carcinomas with lymph node metastases (52). The current study is the first report of the role of BAF57 upregulation in human metastatic prostate cancer samples. BAF57 perturbation confers a migratory advantage to prostate cancer cells that can be attributed to BAF57-reliant α2 integrin signaling (Fig. 4A and Supplementary Fig. S4G). Cell migration is an important precuror to dissemination of disease and metastasis formation (33). Integrin signaling is crucial to various cytoskeletal remodeling processes such as migration and survival that characterize metastasis. More importantly, integrin upregulation in prostate cancer has been linked to lymph node metastases and aggressive disease. It is proposed that a subpopulation of prostate cancer cells harboring integrin upregulation exhibit cancer stem cell–like characteristics conferring metastatic potential (8). Hence, BAF57-mediated control of α2 integrin signaling has important ramifications for therapeutic response and resistance. SWI/SNF involvement in metastasis phenomena is only now beginning to be understood and the current study is the first to establish molecular and pathologic evidence for BAF57 deregulation in prostate cancer metastasis. Developing new models to further extend mechanistic understanding of the metastatic processes of tumor cell migration encompassing intravasation into the lymphatic or circulatory system, anoikis, extravasation, cell adhesion, and modulation of extracellular cell matrix interactions will extend the use of BAF57 as a putative marker of disseminated disease and afford opportunities for therapeutic intervention.

Collectively, these data support a model (Fig. 5C) of aberrant BAF57 signaling as a means by which primary prostate cancer is able to surmount the barrier to metastasis by reconfiguring transcription, culminating in metastatic gene expression programs. A clinical profile of enhanced BAF57 expression as an activator of integrin signaling circuits predisposing to metastasis along with aberrant SWI/SNF activity may potentially be mined for rational drug design in advanced prostate cancers.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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References
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Aberrant BAF57 Signaling Facilitates Prometastatic Phenotypes

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