Aberrant BAF57 Signaling Facilitates Pro-metastatic Phenotypes

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Running Title: BAF57 is a Marker of Metastasis in Prostate Cancer.
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Translational Relevance:
Prostate cancer (PCa) is a leading cause of cancer-related male mortality. Current treatments for non-organ confined disease provide only transient remission before progression to refractory advanced castration resistant prostate cancer (CRPC) 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 pro-metastatic migratory phenotypes. Further analyses showed that BAF57 facilitated PCa cell migration through AR dependent modulation of α2 integrin expression and function. Finally, evaluation of human metastases demonstrated 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.
Abstract

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

Experimental Design: Clinical human specimens of primary and metastatic PCa were immunohistochemically examined for tumor-grade association of BAF57 expression. Global gene expression analyses were performed 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 like integrins were validated. Cell migration assays were used to profile the biological 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 demonstrated 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 pro-metastatic migratory advantage, which was attenuated by anti-α2 integrin antibody blockade. Furthermore, BAF57 was found to be markedly overexpressed in human PCa 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 pro-metastatic 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 (PCa) is one of the most frequently diagnosed non-cutaneous 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 adrenal steroid 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) (4). While newer, more powerful inhibitors under clinical trials like MDV3100 are soon expected to gain traction (5), 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, post-translational modifications, chromosomal translocations, AR interplay with a variety of co-modulators such as chemokines (e.g., CXCL12), oncogenes (e.g., c-myc and c-src) and coactivators (e.g., CBP, SRC family) fuel the development of aggressive CRPC and subsequent metastasis (reviewed in (6)). Advanced PCa is lethal and successful disease management depends on identifying reliable biomarkers that signal the propensity to metastasize and utilizing potential new candidates for therapeutic targeting (7).

BAF57, a known AR co-modulator, is a subunit of the multimeric SWI/SNF complex. The complex consists of a central core ATPase (Brg1 or Brm) and several associated subunits termed Brg1-associated factors (BAFs) (reviewed in (8-10)). The primary function of this conglomerate involves chromatin remodeling, utilizing ATP hydrolysis, to facilitate context-dependent gene activation or repression. However, it is now appreciated that the functions of SWI/SNF extend beyond nucleosomal remodeling to include tissue-specific gene expression, lineage maintenance, neural and muscle differentiation, cell cycle regulation and tumor suppression (11-15). The combinatorial assembly of the various subunits is thought to underscore the diversity of SWI/SNF
functions (reviewed in (8-10)). Increasing evidence from a plethora of cancers suggest that perturbations of individual subunits contribute to disease progression (16).

In the case of PCa, Brg1 upregulation is documented in invasive human PCa samples (17), while Brm loss is correlated with prostatic hyperplasia (18) and BAF155 upregulation is associated with lymph node metastases of recurrent PCa (19). BAF57, a known steroid receptor activator and coregulator (20-22), is upregulated in PCa and endometrial cancer (ECa) (23, 24). Despite extant evidence for heightened BAF57 expression in aggressive PCa, the molecular and cellular consequences of this event have remained unexplored.

Here, molecular analyses reveal an unprecedented role for aberrant BAF57 in surmounting the requirement for androgen in conditions reminiscent of CRPC, coupled with robust induction of metastasis-specific cytoskeletal remodeling proteins such as integrins. BAF57-expression governed migratory phenotypes in the transition to advanced CRPC/metastasis that are concordant with the marked upregulation of BAF57 in clinical specimens of human metastatic PCa. BAF57 upregulation is also symptomatic of oncogenic alterations in SWI/SNF complex mediated transcription, exemplified by changes in expression and chromatin occupancy at the \( \alpha_2 \) integrin locus. Furthermore, these data substantively identify and establish BAF57 as a novel, critical effector of lethal disease phenotypes. The association with clinical samples of metastasis renders BAF57 an ideal candidate for the design of targeted therapies. This study is also the first assessment of subunit specific anomalies resulting in clinically relevant SWI/SNF dependent transcriptional outcomes.
Materials and Methods

Tissue microarray. The human tissue samples used for immunohistochemical analyses consisted of two 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 performed under the aegis of the University of Michigan. The TMA slides were generous gifts from Dr. Kenneth Pienta (University of Michigan, Ann Arbor) and Dr. David Reisman (University of Florida, Gainesville). 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 $1.8 \times 10^6$ cells on poly-L-lysine coated 10cm dishes and transfected overnight with 7µg of pcDNA3.1 3x- FLAG or pcDNA3.1 (- ) BAF57-FLAG and 1µg 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) IMEM (steroid depleted medium is used to mimic castration). 24hrs later, 1nM DHT or 0.1% ethanol vehicle was added for 12h. The TRizol method was used to harvest mRNA. Subsequently cDNA was generated and used for q-PCR analyses by SYBR-based chemistry. Oligonucleotides used in q-PCR 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 ~48h before the first wound was made. At initial wounding (Time=\(T_0\)), P1E6 monoclonal antibody (Millipore, MAB1950) or IgG (R&D Systems, MAB002) at 20\(\mu\)g/ml was added and the area was imaged. Antibody treatments were replenished every 24h 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 (23). To define the role of BAF57 in disease progression herein, immunohistochemical analyses of the expression patterns of BAF57 were employed using a larger cohort of PCa 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 pre-cancerous proliferative lesion), when compared to non-malignant benign prostatic hyperplasia (BPH) (reviewed in (25)). 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 two grades are added, with generally higher scores indicative of adverse outcomes (reviewed in (26)). 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 (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 PCa (LNCaP) and in the castration resistant prostate cancer (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 (27). Since AR is a critical effector of PCa progression, the impact of heightened BAF57 expression on AR activity was ascertained. As expected, BAF57 deregulation acted in concert with androgen dihydrotestosterone (DHT) to enhance AR activity, reflected in an at least two-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 2C, 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 (28). Since BAF57 activates ligand-independent AR activity (Fig. 2B, 2C), it can be posited that BAF57 elevation in the absence of hormone initiates unique transcriptional programs that drive castration resistance and metastatic PCa. BAF57 is critical in androgen dependent PCa, modulating AR response to ligand and androgen-dependent cell proliferation (29). 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 performed in 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 12h before microarray analyses (Fig. S1B, S1C).

K-means clustering was performed (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 (30) 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 *FKBP5* (31). However, to avoid missing bonafide 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 (Fig. S2B) are the 558 transcripts that bypassed the requirement for hormone in the presence of BAF57 upregulation, with 28 uniquely BAF57 regulated transcripts (Fig.S2D) and 1323 hormone-sensitive transcripts. KEGG pathway and gene ontology analyses (Supplementary Table S2 and Fig. S2C respectively list the KEGG pathways and the individual transcripts in each pathway category) showed unexpected changes in actin cytoskeletal remodelers. Since changes in cytoskeletal signaling components are hallmarks of disease progression and are thought to activate aberrant signaling thereby facilitating metastasis (32), 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 PCa (33) revealed two potential BAF57 regulated candidates, Cathepsin C (CTSC) and Mucosa Associated Lymphoid translocase 1 (MALT1). Additionally, 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 PCa metastases (34, 35).

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 (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 (Fig. S3D). These studies suggest that integrins are targets of aberrant BAF57 signaling. While it is known that integrin alterations regulate tumor cell migration and invasion in cancer (36), these data offer the first molecular evidence of BAF57 deregulation in the potential control of integrin expression in cancer models.

Since 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 (Fig. S2G). These data are consistent with prior reports wherein detectable angiogenic markers were observed only in fairly advanced disease with well-established metastatic deposits (reviewed in (37)). 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, identified as potentially regulated by BAF57 deregulation, are heterodimeric cell surface receptors consisting of an alpha and beta 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 (38). 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 post-transcriptional (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, that frequently heterodimerizes with α2 integrin for signaling (Fig.S3A, S3C). These observations demonstrate 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 Androgen Receptor Occupied Regions (ARORs) (Fig. S3E, left panel). Since BAF57 regulates AR activity (29), a putative AR binding site along with an ARE was identified in the second intron of the ITGA2 gene and was further explored by ChIP q-PCR 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) while Brm showed a relatively modest occupancy pattern (Fig. S3E, right panel). Together, these data suggest that BAF57 elevation activates ITGA2 transcription by directing AR and the SWI/SNF chromatin-remodeling complex to the intronic locus. These data establish a critical link between SWI/SNF and α2 integrin levels in cancer progression.

**Tumor-associated BAF57-induced pro-metastatic phenotypes are modulated by the AR signaling axis.**

As BAF57 deregulation promoted SWI/SNF mediated regulation of α2 integrin, it became imperative to investigate the biological impact of this event. Wound-healing assays using the BAF57 model systems previously described (Fig. 2) identified BAF57-dependent PCa cell migration over time (Fig. S4A, S4B, S4C). Furthermore, invasion assays in LNCaP cells (Fig. S4D) furnished complementary evidence for BAF57-conferred migratory advantage. Migration was determined to be independent of proliferation (Fig. S4E). To validate that the cell migratory effects upon BAF57 induction were indeed governed by α2 integrin, studies employing a validated monoclonal blocking antibody (39, 40) directed against integrin α2 were performed. Consistent with the findings above, antibody blockade resulted in a quantitative (Fig. 4A) impairment in the movement of GFP-positive, BAF57-deregulated cells towards the wound area at the designated time points (depicted in Fig. S4G, representative images). This indicated a requirement for α2 integrin mediated cell motility that was also recapitulated in hormone therapy sensitive model systems (Fig. S4F). Thus, these data provide compelling evidence for BAF57 driven α2 integrin upregulation culminating in migratory, pro-metastatic phenotypes.

To interrogate whether the AR can impact BAF57 mediated migratory processes, wound-healing experiments were performed in the presence and absence of a second-generation AR antagonist MDV3100 that has been FDA approved for the treatment of CRPC (5). These studies (Fig. 4B, S3F respectively) revealed that thwarting AR activity using MDV3100 could impair BAF57-governed migratory and transcriptional processes. Wound healing experiments were also performed 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 towards modest increments in migration (Fig. S4C), suggesting that in CRPC models, BAF57 can co-operate with hormone to activate pro-migratory pathways. In summary, BAF57 appears to co-operate with the AR signaling axis to govern pro-metastatic phenotypes.

**BAF57 deregulation predisposes to metastasis.**

It is well established that SWI/SNF perturbations are associated with tumor development (8, 10, 16, 42-44). However, since BAF57 elevation conferred a pro-metastatic 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 carried out in lung, lymph node, dura and liver metastases of CRPC (Fig. 5A and 5B, Fig. S5B; clinical parameters, where made available, for the autopsy specimens obtained, are described in Supplementary Table 1) revealed that with the exception of liver metastases, BAF57 was significantly (p<0.01) elevated at other sites of metastatic disease. Interestingly, multiple sites of metastasis examined from the same patient displayed variability in BAF57 expression levels (Fig. S5A); the overall average across patients with liver metastases remained at a low score of 2, while lymph node, lung and dura metastases across patients showed a higher average score ranging between 5.5 and 6.5 (Fig. S5B). This indicates that despite variations within the same individual, elevated BAF57 protein expression in metastatic disease, relative to primary PCa is fairly consistent (Fig. S5C) and likely points to an invasive and aggressive cancer phenotype. Thus, these data imply that BAF57 expression in metastatic PCa 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 pro-metastatic 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 pro-metastatic phenotypes.
Discussion

The current study is the first comprehensive molecular assessment of a discrete pro-metastatic function for BAF57. Data in support are as follows: 1) BAF57 elevation is significantly maintained in high-grade human prostate tumors, 2) BAF57 deregulation potentiates inappropriate ligand independent AR activity and also bypasses the requirement for androgens in the induction of genes linked to cell migration and metastasis, 3) 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, 4) BAF57 elevation confers a migratory advantage to cancer cells that can be mechanistically linked to α2 integrin, and 5) 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 utility of SWI/SNF as a possible therapeutic target in aggressive PCa.

The multi-subunit 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 (BAFs). Recent studies have detailed loss, mutation or expression changes of various subunits in several different cancers (16). PCa has also been characterized by changes in several SWI/SNF subunits. BAF155 upregulation has been correlated with metastatic and recurrent PCa (19) while Brm loss is a marker of prostatic hyperplasia (18) and Brg1 elevation is associated with invasive PCa (17). BAF57, a known AR co-modulator is aberrantly overexpressed in a small cohort of clinical human specimens of PCa (23). However, pathological 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 PCa 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 PCa 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, 1B and 1C). Also, the consistent elevation of BAF57 in metastatic human PCa specimens from lung, lymph node and dura reinforces a propensity for metastasis (Fig. 5A, 5B). Moreover, the current study is in consonance with a recent report linking elevated BAF57 expression and myometrial/lymphovascular space invasion in endometrial cancer (24), 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 PCa will enhance the clinical utility of BAF57 as a putative marker of disseminated disease. The cBio Cancer Genomics portal, maintained by the Memorial Sloan Kettering Cancer Center is an open-access database with comprehensive molecular and clinical data tumor samples spread across twenty different cancer studies (45). Data from metastatic PCa 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 (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 (46), 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, while DHT co-operated with BAF57 to drive migration (Fig. 4B, S4C). These data are in agreement with other studies showing androgen driven metastatic pathways in PCa (41). 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 (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 (Fig. S1D) and differential SWI/SNF occupancy patterns at the ITGA2 locus in PCa cells (Fig. 3D, 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 (a) global imbalances in SWI/SNF components, (b) mistargeting of these complexes and (c) re-configuring of transcription (8). 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 co-depletion 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 cBio 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 genome-wide BAF57 and/or SWI/SNF binding at regulatory loci in CRPC and metastatic PCa 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 PCa samples. BAF57 perturbation confers a migratory advantage to PCa cells that can be attributed to BAF57-reliant α2 integrin signaling (Fig. 4A, S4G). Cell migration is an important precursor to dissemination of disease and metastasis formation (32). Integrin signaling is crucial to various cytoskeletal remodeling processes such as migration and survival that characterize metastasis. More importantly, integrin upregulation in PCa has been linked to lymph node metastases and aggressive disease. It is proposed that a subpopulation of PCa cells harboring integrin upregulation exhibit cancer stem cell like characteristics conferring metastatic potential (7). 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 pathological evidence for BAF57 deregulation in PCa 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 utility 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 PCa 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.
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REFERENCES


**Figure Legends**

**Figure 1. BAF57 is upregulated in advanced tumors.** (A) Representative images of BAF57 immunohistochemical (IHC) staining performed on a tissue microarray (TMA) slide containing human tumor tissues derived from radical prostatectomy. Spleen is a metastatic specimen, serving as the negative control for BAF57 staining. (B) Quantification of the average BAF57 IHC score in the TMA samples. ‘N’ is the number of samples analyzed, BPH (N=9); PIN (N=10), 3+3PCa (N=9); 4+4PCa (N=5) and 4+5PCa (N=5). The average score obtained for the incipient stage of disease BPH was approximately 3, while scores between 7-9 were obtained for advanced PCa and the mean values ± SD were chosen for representation. Statistical comparisons were made using the Bonferroni multiple comparison test with *= p<0.05 and **=p<0.01. (C) Representative immunoblots (left panel) of BAF57 expression and RAN as loading control from matched non-neoplastic (N) and tumor (T) specimens (N=5) obtained from radical prostatectomy. Li-Cor quantified BAF57 immunoblot expression (right panel) was obtained by normalizing BAF57 in each tumor to RAN and with non-neoplastic tissue set to 1. An approximate 3.5 fold increase in BAF57 relative to normal tissue was observed in tumor compared to non-neoplastic tissue.

**Figure 2. BAF57 potentiates both hormone sensitive and castration resistant AR activity.** (A) Representative immunoblot of transient BAF57 overexpression in GFP sorted LNCaP (left panel) and C4-2 cells (right panel). Quantification of BAF57 expression was obtained after normalization to loading control and with vector set to 1 in each case. (B) & (C) The expression of AR target gene transcripts KLK3/PSA, TMPRSS2 and FKBP5 relative to housekeeping gene 18S, was determined in BAF57-transfected LNCaP or C4-2 cells respectively, with or without 1nM DHT stimulation for 12h. The data represent an average of at least two to five independent
biological replicates (mean ± SD) by qPCR. Untreated vector controls are set to 1. Statistical significance was determined using the t-test, with **=p<0.01, *=p<0.05.

Figure 3. BAF57 overexpression bypasses androgen requirement to uniquely upregulate α2 integrin and other cytoskeletal remodelers. (A) Relative expression of ITGA2, ITGA6, CTSC and ROCK2 transcripts identified by microarray analyses in LNCaP (upper panel) and in C4-2 cells (lower panel), with and without BAF57 overexpression in the absence of hormone was determined using an average of three to five independent experiments by qPCR. *=p<0.05 by t-test. Vector transfected controls are set to 1. (B) Representative immunoblot for α2 integrin protein expression and Lamin-B (loading control) upon BAF57 transfection in LNCaP in the presence and absence of hormone (upper panel) and in steroid-free conditions in C4-2 (lower panel). Fold change in expression is indicated relative to Lamin-B with vector transfected set to 1. (C) Relative expression analyses from two independent experiments of SMARCE1/BAF57 (upper-left panel) and ITGA2 transcripts (upper-right panel) and representative immunoblots of respective protein expression after BAF57 knockdown in C4-2 cells (lane 2, lower-left and lower-right panels, respectively). (D) Schematic (upper panel) of ITGA2 gene locus containing a putative ARE in the second intron, with q-PCR ChIP analyses in steroid-free conditions in C4-2 for AR and Brg1 occupancy (lower panels) at the ITGA2 locus. Results represent the average of at least two independent replicates and represent % input. ***=p<0.001, **=p<0.01 by t-test.

Figure 4. BAF57 deregulation promotes cell migratory behavior dependent on α2 integrins and the AR signaling axis. (A) The relative migration index in C4-2 cells at 24 hours and 72 hours in the presence and absence of α2 integrin antibody or IgG control, as described under Materials and Methods. Data represented is the average of three independent experiments (mean ± S.D.). Vector control at 24hr is set to 1. ***=p<0.001, **=p<0.01 *=p<0.05 using ANOVA. (B) Wound healing assays in C4-2 cells following MDV3100 treatment. Transfected C4-2 cells were treated with either vehicle or 10μM MDV3100 at the start of the wounding experiment, the 0h time point and the wound area was monitored for 48h subsequently for migrating cells. The
data quantified constitutes an average of two independent migration experiments with multiple technical replicates (mean ± SD). Untreated vector control at 48hr is set to 1.

**Figure 5. BAF57 deregulation predisposes to metastasis.** (A) Representative images of BAF57 IHC staining performed 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 non-neoplastic 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 metastasis precursors. The data presented herein suggest that BAF57 deregulation potentiates ligand-dependent and ligand-independent AR activities and also induces ITGA2 transcription by eliciting changes in SWI/SNF complex components and function. α2 integrin induction facilitates migratory and pro-metastatic phenotypes that can be inhibited by blocking α2 integrin antibody.
Figure 1

A

\[ \text{Spleen} \quad \text{BPH} \quad \text{PIN} \]

\(\alpha\)-BAF57

Gleason 3+3 4+4 4+5

\(\alpha\)-BAF57

B

![Graph showing BAF57 IHC Score](image)

C

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![Bar chart showing Relative BAF57 Expression](image)

Legend:
- Normal
- Tumor
Figure 2

A

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Relative Expression

B

KLK3/PSA

LNCaP

FKBP5

1nM DHT - - +

Relative Expression

C

KLK3/PSA

C4-2

TMPRSS2

FKBP5

1nM DHT - - +

Relative Expression
Figure 3

A. LNCaP

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B. LNCaP

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C. C4-2

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D. C4-2

ITGA2 gene locus

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AGAACAATCTGAGCTCATT

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*** | **
Figure 5

A. CRPC metastases

- Liver
- Lymph Node
- Lung
- Dura

B. BAF57 IHC Score

BPH, Liver, Lymph Node, Lung, Dura

C. Schematic representation of BAF57 elevation in the primary tumor leading to deregulation of BRG1 and AR, and activation of KLF3/TMPRSS2/FKBP5 and ITGA2.

Basal & stimulated AR activation

Migration Metastasis

α2 integrin antibody

α2 integrin induction
Aberrant BAF57 Signaling Facilitates Pro-metastatic Phenotypes

Sucharitha Balasubramaniam, Clay E.S. Comstock, Adam Ertel, et al.

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