The SMARCA2/4 ATPase Domain Surpasses the Bromodomain as a Drug Target in SWI/SNF-Mutant Cancers: Insights from cDNA Rescue and PFI-3 Inhibitor Studies


Abstract

The SWI/SNF multisubunit complex modulates chromatin structure through the activity of two mutually exclusive catalytic subunits, SMARCA2 and SMARCA4, which both contain a bromodomain and an ATPase domain. Using RNAi, cancer-specific vulnerabilities have been identified in SWI/SNF-mutant tumors, including SMARCA4-deficient lung cancer; however, the contribution of conserved, druggable protein domains to this anticancer phenotype is unknown. Here, we functionally deconstruct the SMARCA2/4 paralog dependence of cancer cells using bioinformatics, genetic, and pharmacologic tools. We evaluate a selective SMARCA2/4 bromodomain inhibitor (PFI-3) and characterize its activity in chromatin-binding and cell-functional assays focusing on cells with altered SWI/SNF complex (e.g., lung, synovial sarcoma, leukemia, and rhabdoid tumors). We demonstrate that PFI-3 is a potent, cell-permeable probe capable of displacing ectopically expressed, GFP-tagged SMARCA2-bromodomain from chromatin, yet contrary to target knockdown, the inhibitor fails to display an antiproliferative phenotype. Mechanistically, the lack of pharmacologic efficacy is reconciled by the failure of bromodomain inhibition to displace endogenous, full-length SMARCA2 from chromatin as determined by in situ cell extraction, chromatin immunoprecipitation, and target gene expression studies. Furthermore, using inducible RNAi and cDNA complementation (bromodomain- and ATPase-dead constructs), we unequivocally identify the ATPase domain, and not the bromodomain of SMARCA2, as the relevant therapeutic target with the catalytic activity suppressing defined transcriptional programs. Taken together, our complementary genetic and pharmacologic studies exemplify a general strategy for multidomain protein drug-target validation and in case of SMARCA2/4 highlight the potential for drugging the more challenging helicase/ATPase domain to deliver on the promise of synthetic-lethality therapy.

Introduction

Epigenetic dysregulation plays a fundamental role in the development of cancer (1). Large-scale genome sequencing has uncovered recurrent somatic mutations and copy-number (CN) changes in histone-modifying enzymes and chromatin remodeling complexes supporting a causal role for altered epigenetic states in tumorigenesis (2–4). Although the mechanistic consequences of these alterations remain poorly understood, it is appreciated that such events promote acquisition of cell oncogenic capabilities through deregulation of nucleosome-dynamics, gene transcription, DNA replication, and repair (5). Indeed, chromatin regulators are emerging as therapeutic targets and inhibitors of histone-modifying enzymes, as well as bromodomains, which “read” the histone marks, have recently shown efficacy in preclinical and clinical settings through their ability to reverse oncogenic transcriptional programs (6–8).

The Switch/Sucrose Non Fermentable (SWI/SNF) is a multi-subunit chromatin remodeling complex that consists of one of two mutually exclusive helicase/ATPase catalytic subunits, SMARCA2 and SMARCA4. Together with core and regulatory subunits, SMARCA2/4 couple ATP hydrolysis to the perturbation of histone-DNA contacts. This sculpting of the nucleosomal landscape at promoters provides access to transcription...
factors and cognate DNA elements facilitating both gene activation and repression (9). Because various SWI/SNF subunits are mutated or lost at high frequency in human tumors (2–4, 10), this complex has garnered considerable attention (11). A tumor-suppressive role has most strongly been demonstrated in childhood malignant rhabdoid tumors, in which the SMARCB1 (Snf5) subunit is biallelically inactivated in nearly all cases (10). Accordingly, knockout of mouse Smarcb1 results in fully penetrant and lethal cancers with 11 weeks median onset (12). In human synovial carcinoma, recurrent chromosomal translocations, which are diagnostic of the malignancy, result in oncogenic fusions (SS18-SSX) that alter the composition/function of the SWI/SNF complex (13).

Pointing to the broader relevance of SWI/SNF in cancers are frequent inactivating mutations in accessory subunits, including ARID1A in ovarian and endometrial carcinomas (14, 15), and PBRM1 in renal cell carcinomas (16).

Context-specific molecular vulnerabilities that arise during tumor evolution represent an attractive class of drug targets; however, the frequency and spectrum of somatic lesions often confound efforts to identify such therapeutic targets solely based on genomic information (17). To address this challenge, functional, unbiased chemical, and genetic loss-of-function (LOF) platforms, which use either drug-like small-molecules or siRNA/shRNA libraries, hold the promise to systematically identify nonobvious target-genotype interactions that might impact clinical decisions (17–19). Recently, using genetic LOF approaches, three groups have independently identified SMARCA2 as an essential gene in SMARCA4-deficient lung cancer (20–22) proposing a synthetic lethality therapeutic approach. However, it remains unclear whether small-molecule inhibitors of the SMARCA2 bromodomain or ATPase domain can mimic the reported RNAi phenotypes resulting from paralog dependency in SWI/SNF (11, 23).

Several subunits in the SWI/SNF complex contain bromodomains, which are evolutionary conserved protein–protein interaction modules that bind acetyl-lysine on proteins and histone tails (6, 24). Bromodomains are druggable and following the antitumor activity of JQ1 (6), there is interest in developing small-molecule inhibitors against other bromodomains, which could contribute to either assembly or targeting of the SWI/SNF complex to specific genomic loci providing an intervention drug target rationale. However, because bromodomains are frequently found in large protein complexes (and often flanked by additional domains involved in chromatin-binding and protein–protein interactions), RNAi-mediated depletion alone does not reveal the contribution of individual domains to the LOF phenotype, representing a specific challenge for drug-target validation.

In this report, we conduct complementary cDNA rescue and pharmacologic studies to explore whether the bromodomain of SMARCA2/4 represents a tractable target in SWI/SNF-mutant cancers. We characterize the PFI-3 bromodomain inhibitor in biochemical assays and across preclinical models with altered SWI/SNF complex (lung, synovial sarcoma, leukemia, and rhabdoid tumor cells) and discover that bromodomain function of SMARCA2/4 is dispensable for tumor cell proliferation, while the catalytic ATPase activity is essential.

Materials and Methods

Bioinformatics

Genome sequencing data and CN information were downloaded from cBioPortal (Supplementary Table S1). Cell line genomic annotation was from the Sanger (www.cancerxgene.org) and the Broad Institutes (www.broadinstitute.org/ccle). Outlier sum statistics (26) and standard software packages for sequence analysis were used.

Cell lines

Cells obtained from the ATCC were cultured accordingly: RPMI-1640 (A549, H1299, H157, H520, H460, HeLa, and TPH-1); Iscove’s Modified Dulbecco’s Medium (MV-4;11); McCoy’s 5a Modified Medium (A-204 and G-401) and supplemented with 10% FBS (Gibco). The Aska and Yamato cells (Osaka Medical Center) were grown in DMEM (20% FBS). All Cell lines were mycoplasma negative (LookOut Mycoplasma Kit PCR, Sigma) and maintained at low passage (<3 month) after thawing from master vials (IACS and Pfizer BioBanks) subjected to short tandem repeat (STR) profiling of polymorphic loci (Promega PowerPlex 16 system) with a >80% match criteria for cell line authentication.

Immunoblotting

Analysis was performed on whole-cell lysates (Supplementary Information) using primary antibodies: SMARCA4 (Abcam, #108318), SMARCA2 (Abcam, #15397), HA-Tag (Cell Signaling Technology, #2367), or Tubulin (Cell signaling Technology, 3873), and secondary antibodies (Li-Cor, #926-68020, #926-32111).

Assays

PFI-3 (PF-06687252) is available from SGC (http://www.thescg.org/chemical-probes/PFI-3). Bromodomain selectivity was measured using ligand binding, site-directed competitive assays (BROMOscan, DiscoverRx; ref. 27). Cell potency was measured using in situ cell extraction, CellTiter-Glo (Promega) and clonogenic assays (Supplementary Information).

RNAS, plasmids, gene expression, and chromatin immunoprecipitation

SMARCA2/4 shRNA (SIGMA TRC-collection) and siRNA (ON-TARGET PLUS Dharmacon) sequences are listed in Supplementary Table S2. ATPas-dead (K785A) and bromodomain mutations (Y1497F and N1540W) were made in human SMARCA4 cDNA (GeneCopoeia #GC-T3533) using site-directed mutagenesis (QuickChange, Agilent Technologies) with equivalent mutations in SMARCA2 (GeneCopoeia #GC-Z4424). All cDNAs, subcloned into lentiviral vectors, were sequence verified and virus generation, infection, and generation of stable cell lines were conducted following standard procedures (Supplementary Information). Gene expression (Affymetrix) data and methods have been deposited with NCBI (GSE699088). Chromatin immunoprecipitation (ChiP) and qPCR were conducted as previously described (28).

Results

Genomic alterations in SWI/SNF across human tumors

To build upon recent meta-analysis (2–4), we first examined both SWI/SNF mutation and CN variation drawing on a larger set of patient tumors (n = 10,038) from 45 genome sequencing
studies (Supplementary Table S1). Clearly, genomic alterations in the 20 canonical SWI/SNF subunits are highly prevalent (Fig. 1A) occurring in 15% of all cancers (3, 4, 10). Cancers with the highest frequency of lesions in SWI/SNF subunits are rhabdoid tumors, female cancers, including ovarian, uterine, cervical and endometrial, lung and gastric adenocarcinoma, melanoma, esophageal, and renal clear cell carcinoma (Fig. 1A and Supplementary Table S1). A tumor-suppressive role of the SWI/SNF complex in these contexts has been recognized based on the high frequency of inactivating mutations, which is further underscored by mouse genetic studies (10, 29). In contrast, SWI/SNF mutations do not emerge as significant recurrent alterations in glioblastoma, thyroid cancer, multiple myeloma, and acute myeloid leukemia (AML). In AML, SMARCA4 may instead be an oncogene driving cMYC transcription in concert with BRD4 (7, 11, 30, 31). As such, it appears that cellular and tissue context defines the tumor-suppressive or oncogenic functions of the SWI/SNF complex (5, 11, 23).

SMARCA4 deficiency is prevalent and mutually exclusive to SMARCA2 CN loss in lung cancer

In primary human lung adenocarcinoma (LUAD), about half of the SMARCA4 mutations are deleterious (nonsense and frame-shift mutations) and occur at a 7% frequency in The Cancer Genome Atlas patient samples (Fig. 1B). Overall, SWI/SNF complex components are mutated in 71 of 229 patients with an average mutation rate of approximately 1.7 per sample. In addition, two copy loss of SMARCA4 is observed in 14 out of 299 LUAD cases adding to the fraction of SMARCA4-deficient tumors. Because heterozygous SMARCA4 knockout mice are haploinsufficient and tumor prone (29), we also analyzed copy-number driven mRNA expression and conclude that loss of one allele is also sufficient to decrease SMARCA4 expression (Fig. 1C). Focusing on LUAD and lung squamous cell carcinoma (LUSC), mapping of the genomic annotation onto individual patient samples revealed that loss of SMARCA2 and SMARCA4 is largely mutually exclusive (Fig. 1D and Supplementary Fig. S1A). Moreover, with respect to gene expression, outlier statistics identifies SMARCA4, along with ARID1A, as the most significantly altered subunits in the SWI/SNF complex in LUAD (Fig. 1E) with similar profiles observed for LUSC (Supplementary Fig. S1B). At the genome level, SMARCA4 ranks in the top 5% of all genes with negative outlier sum statistics and its bimodal expression profile (Fig. 1F) clearly defines a SMARCA4-deficient patient population.

In good concordance with cell line annotation at the Sanger and the Broad Institutes, SMARCA4 protein expression was nondetectable by Western blot analysis in approximately 20% (12/50) of lung cancer lines (Fig. 1G and Supplementary Fig. S1E). Of eleven SMARCA4-mutant cell lines, only one (NCI-H2286) displayed measurable protein expression (Supplementary Fig. S1C) consistent with the binding constant ($K_d=89$ nmol/L) measured by isothermal titration calorimetry (www.thescg.org/chemical-probes/). PFI-3 is a selective, potent, and cell-permeable SMARCA2/4 bromodomain inhibitor

To explore pharmacologic inhibition of the SMARCA2 bromodomain, we next evaluated the small-molecule inhibitor PFI-3 (Fig. 3A) discovered through a collaboration between the Structural Genomic Consortium and Pfizer. Biochemically, we determined that PFI-3 binds avidly to both SMARCA2 and SMARCA4 bromodomains (BROMOscan $K_i$'s between 55 nmol/L and 110 nmol/L) consistent with the binding constant ($K_d=89$ nmol/L) measured by isothermal titration calorimetry. Using recombinant purified bromodomains, we discovered that PFI-3 binds with similar avidity to both the short and long isoform of SMARCA2 revealing that the alternatively-spliced 18 amino acid insert (34) does not impair PFI-3 binding (Fig. 3A). Moreover, profiling against 32 bromodomains at DiscoverRx (27) confirmed exquisite selectivity versus other subfamilies (Fig. 3C and Supplementary Table S3) expanding the PFI-3 selectivity information obtained using differential scanning fluorimetry (DSF). In summary, we find that there is a good concordance between the ligand competition (BROMOscan) and the direct biophysical binding (DSF) assays.
Figure 1.
Genomic analysis of the SWI/SNF complex in human cancer. A, percentage distribution of lesions (mutations and CN changes) in SWI/SNF components across tumors profiled by The Cancer Genome Atlas and other laboratories (Supplementary Table S1). B, SWI/SNF mutation spectrum in LUAD (n = 229 tumors; 121 mutations). C, correlation of SMARCA4 CN with gene expression (RSEM, RNA-Seq Expression by Expectation Maximization). D, CN loss of SMARCA2/4 is mutually exclusive in LUAD (left; n = 493) and LUSC (right; n = 490). Oncoprint (www.cbioportal.org): blue, high CN loss (GISTIC 2.0 threshold value of $-2$); red, high CN gain (GISTIC 2.0 threshold value of $2$); green, mutations. E, SMARCA4 has the highest negative outlier sum statistics among SWI/SNF components (LUAD; n = 598). F, histogram showing bimodal distribution of SMARCA4 gene expression (LUAD; n = 548) highlighting the predicted patient "responder" population (red). G, protein expression and SMARCA4 genomic annotation across lung cancer cell lines: mutation (\*\*\*), copy-number loss (\*\*), and gene silencing (\*\*\*).
and note that in addition to targeting SMARCA2/4, PFI-3 also has activity (~70% inhibition at 2 μmol/L) against the structurally related fifth bromodomain from PBRM1, another SWI/SNF subunit.

In cell-based chromatin-binding assays, using in situ cell extraction techniques to remove non-chromatin bound proteins, we observed dose-dependent displacement of GFP-tagged SMARCA2 bromodomain (i.e., 132 amino acid residues) by PFI-3 (Fig. 3D and E). Notably, the inhibitor showed prolonged cell-target engagement with similar potency (IC50 ~ 5.78 μmol/L) following 2 and 24 hours treatment (Supplementary Fig. S3). As a negative control, JQ1 did not inhibit the binding of ectopically expressed SMARCA2 bromodomain, but selectively displaced GFP-tagged BRD4 (Fig. 3D, and data not shown). Taken together, our cell-biochemical data cooperate the accelerated fluorescence recovery after photobleaching (FRAP) reported for PFI-3 (35), and we conclude that PFI-3 is a selective, cell-permeable probe suitable to study the inhibition of SMARCA2/4 bromodomains in cells.

PFI-3 does not phenocopy the growth-inhibitory effects of SMARCA2 knockdown in lung cancer

Armed with PFI-3 and motivated by the context-specific phenotype of SMARCA4-deletion (Fig. 2), we evaluated PFI-3 in the SMARCA4-deficient responder lines (A549, H1299, H157), but observed no antiproliferative effects in either 3-day cell viability (Fig. 4A) or long-term clonogenic assays (Fig. 4B and Supplementary Fig. S4). Because SWI/SNF is a multisubunit complex containing numerous chromatin-interacting domains, we speculated that selective SMARCA2 bromodomain inhibition by itself is not sufficient to dislodge the endogenous SWI/SNF complex from chromatin. To elaborate on this, the binding of endogenous (full-length) SMARCA2 to chromatin was monitored by immunofluorescence in A549 cells using SMARCA2 knockdown as a specificity control (Fig. 4C–E). Even high concentrations of PFI-3 (30 μmol/L; 1 and 24 hours) were unable to displace the SMARCA2 protein from chromatin (Fig. 4C and D). Again, we cross-validated the in situ cell extraction assay using the reference inhibitor, JQ1, which potently inhibited chromatin-binding of endogenous BRD4 (Fig. 4C, bottom) but not SMARCA2 (data not shown). Taken together, these data suggest that the bromodomain of SMARCA2 is dispensable for chromatin binding and SWI/SNF oncogenic activity in lung cancer.

PFI-3 treatment of synovial sarcoma cells and target gene promoter occupancy studies

As alterations in SWI/SNF have been implicated in disease progression of synovial sarcomas (13), we also evaluated the pharmacologic activity of PFI-3 in Yamato and Aska cells. These cells harbor the hallmark recurrent chromosomal translocation t(X;18)(p11.2;q11.2), which fuses the SS18 gene, an integral subunit of SWI/SNF complex, to one of the three SSX genes (SSX1, SSX2, and SSX3) (13).
SSX2, and SSX4), observed in >95% of patients (36). Incorporation of the SS18-SSX fusion protein into the SWI/SNF complex results in eviction and degradation of the tumor-suppressor SMARCB1. The altered SWI/SNF complex binds to the SOX2 locus, resulting in aberrant SOX2 expression, which is essential for proliferation of synovial sarcomas (13). Accordingly, Yamato and Aska cells show high levels of Sox2 expression (37). Hence, we hypothesized that PFI-3 may inhibit the altered SWI/SNF complex and impair cell growth, but we did not observe inhibition of cell proliferation in either 4-day viability (Fig. 5A) or long-term proliferation assays (Fig. 5B). We then assessed SOX2 expression and found that PFI-3 treatment (day 3 and day 6) failed to reduce SOX2 transcript levels at pharmacologically relevant concentrations (Fig. 5C and Supplementary Fig. S5).

To elaborate on the lack of inhibitor-induced phenotype, we examined SWI/SNF binding at the transcriptionally active SOX2 promoter. SMARCA4 ChIP demonstrated high SWI/SNF complex occupancy at the SOX2 promoter in Yamato cells, as previously reported (13), while no enrichment was observed at the MYOD1 locus, a transcriptionally silent locus as confirmed by RNA Polymerase II ChIP (Fig. 5D and Supplementary Fig. S5D). Importantly, we also examined whether PFI-3 treatment could impair binding of the SWI/SNF complex to the SOX2 locus, but observed only a minor change in SOX2 promoter occupancy, suggesting inefficient inhibition of SWI/SNF binding (Fig. 5D). These data are consistent with the in situ cell extraction results for PFI-3 (Fig. 4C and D) showing that SMARCA2/4 bromodomain inhibition cannot displace the multisubunit SWI/SNF complex from chromatin.

PFI-3 treatment of SMARCA4-dependent rhabdoid cancer or leukemia cells

Rhabdoid tumors are distinctly characterized by biallelic inactivation of SMARCB1, a core subunit of the SWI/SNF complex (10), and genetic studies have demonstrated that oncogenesis mediated by SMARCB1 loss is dependent on the residual activity of SMARCA4-containing SWI/SNF complex (38). To establish a benchmark for PFI-3 treatment of A-204 and G-401 rhabdoid tumor cells, we identified two shRNAs that produced effective (>80%) SMARCA4 protein knockdown (Fig. 5E) and confirmed inhibition of cell viability in both short-term proliferation and long-term clonogenic assays (Fig. 5F and G). In contrast with the RNAi phenotype, pharmacologic bromodomain inhibition did not impact the growth of rhabdoid cancer cells (Fig. 5H).

**Figure 3.**

PFI-3 is a potent, selective, and cell permeable bromodomain inhibitor of SMARCA2/4. A, chemical structure of PFI-3 and biochemical potency (BROMOScan Kd’s). B, BROMOScan dose–response curves using recombinant purified bromodomains. C, PFI-3 selectivity (2 µmol/L) across 32 bromodomains (DiscoverRx). D, in situ cell extraction of HeLa cells expressing GFP-tagged SMARCA2 bromodomain (green) cotreated with SAHA (5 µmol/L) and PFI-3 (or DMSO control) for 2 hours with Hoescht nuclear counterstain (red). HeLa control cells expressing GFP-tagged BRD4 treated (2 hours) with JQ1. E, displacement of the SMARCA2 bromodomain from chromatin (IC50) quantified based on mean GFP signal per nucleus (SD; n = 6).
Finally, we extended our phenotypic evaluation of PFI-3 to leukemia as previous RNAi studies have shown that AML cells depend on SMARCA4 to support oncogenic transcriptional programs (30, 31). Similar to our findings across lung, synovial sarcomas, and rhabdoid tumor cell lines, PFI-3 treatment did not afford an anticancer phenotype in THP-1 and MV4-11 leukemic cells (Supplementary Fig. S6) highlighting the critical importance of pharmacologic drug target validation as a follow-up to RNAi-mediated knockdown studies.

Synthetic lethality of SMARCA2 knockdown is linked to the catalytic ATPase activity

To genetically validate the PFI-3 results, we next used a 3’UTR targeting shRNA (shS2) to knockdown endogenous SMARCA2 in H1299 cells engineered to express either wild-type (WT), ATP-binding pocket deficient (K755A; ref. 20) or bromodomain mutant (N1482W; ref. 39) forms of SMARCA2 (Fig. 6A). Ectopic expression of either SMARCA2 WT or the bromodomain binding-deficient mutant (BRD-Mut), but not the ATPase-dead form (ATP-Dead), completely rescued the RNAi-mediated LOF phenotype (Fig. 6B and C). Likewise, A549 cells reconstituted with SMARCA4 WT or BRD-Mut (N1540W, Y1497F), but not ATP-Dead (K785A), were able to grow upon SMARCA2 knockdown (Fig. 6D–F). We also subjected the isogenic matched-pair cell lines to in situ cell extraction and discovered that the SMARCA2 mutants bound chromatin similarly to that of WT (Fig. 7A and B). Hence, failure of the ATP-Dead construct to rescue is due to lack of catalytic activity and not due to gross impairment in chromatin binding. Altogether, our genetic assessment clearly demonstrates that SMARCA4-deficient cancer cells do not require a functional SMARCA2/4 bromodomain for growth. Instead, we unequivocally identify the catalytic activity of the ATPase domain as the appropriate, albeit more challenging, small-molecule drug target.

Genome-wide microarray analysis of SMARCA2/4 rescue experiments

To examine the dependency on ATPase activity, we generated microarray expression data (GSE69088) for the above cDNA rescue experiments. Unsupervised clustering of the top variable genes revealed three distinct expression profiles that were robust to the gene set size while clustering (Fig. 7C and D). In the absence of SMARCA2 knockdown (shLuc), all H1299 derivative lines clustered together (Group 1) irrespective of the nature of the ectopically expressed SMARCA2 constructs. On the other hand, the SMARCA2 knockdown cells (shSMARCA2) showed strong differential gene expression defining two distinct clusters: cells rescued with either WT or BRD-Mut (Group 2) versus cells expressing either vector control (Ctrl) or ATP-Dead (Group 3).
Figure 5.
Evaluation of PFI-3 in synovial sarcoma and rhabdoid tumor cells. A, viability of synovial sarcoma (Aska and Yamato) and HeLa cells treated with PFI-3 (96 hours) relative to DMSO-treated controls (SEM; n = 3). B, long-term (2-week) proliferation assay. Cells were split and replenished with fresh media/PFI-3 every 3 or 4 days counting viable cells (SEM; n = 3). C, PFI-3 treatment (3 days) does not repress SOX2 expression in Yamato cells. SOX2 transcript levels (RT-qPCR) normalized to GAPDH (SEM; n = 12). D, control (DMSO) and PFI-3-treated Yamato cells (day 3) subjected to anti-SMARCA4 ChIP followed by qPCR for SOX2 promoter regions (target gene) or MYOD1 exon1 locus (negative control). The decrease in occupancy at the SOX2 locus (10 μmol/L) is small but significant. *, P < 0.05 (SEM, n = 9). E–G, A-204 and G-401 rhabdoid cells transduced with SMARCA4-targeting (shS4-4, shS4-5) or control (shLuc) shRNAs and analyzed for protein knockdown (1 week after puromycin selection; E), colony formation (2–3 weeks after puromycin; F), and viability (CellTiter-Glo; 6 days after puromycin; G). Error bars, SD; n = 6. H, PFI-3 does not impair growth of G-401 cells (clonogenecity 1.5 weeks; similar data for A-204 not shown). Media/PFI-3 was replenished every 3 days.
Notably, expression/rescue using either SMARCA2 or SMARCA4 showed identical clustering behavior, and differences in mRNA levels within the three groups were nonsignificant. Hence, the transcriptional profiles reinforce the view that BRD-Mut is able to perform similar functions to the WT gene while ATP-Dead, despite retaining its ability to bind chromatin (Fig. 7A and B), cannot. Consistent with the phenotypic responses (Fig. 6), gene set enrichment analysis (GSEA) revealed upregulation of apoptosis and death pathways in Group 3 versus the rescued cell lines (Group 2) highlighting the observed context-specific synthetic lethality (Supplementary Fig. S8).

The ATPase activity common between SMARCA2 and SMARC4 shares a suppressive function on gene expression programs

Next, focusing on the requirement for ATPase activity, we compared the SMARCA2 and SMARCA4 rescue profiles to see whether similar gene expression programs may account for the observed functional complementation. To establish a framework for this analysis, we first derived gene expression signatures for SMARCA4 expression in A549 cells (in the context of SMARCA2 knockdown) comprising the top-100 upregulated and downregulated genes, respectively (Supplementary Table S4). Using GSEA, we then looked for enrichment of these signatures with gene lists from SMARCA2 rescue experiments as queries (Fig. 7E and F and Supplementary Table S5). When comparing ATP-Dead to WT, the enrichment profiles suggest that the ATPase enzymatic activity preferentially reverses expression of genes that are upregulated upon RNAi-mediated (synthetic lethal) knockdown of SMARCA2 (Fig. 7E). Genes downregulated upon SMARCA2 knockdown were not fully reversed by the rescue (Fig. 7F and Supplementary Table S5). Therefore, our microarray data clearly show that the ATPase
SMARCA2-KD signature (up)/SMARCA4 rescue (down)
SMARCA2-KD signature (down)/SMARCA4 rescue (up)

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domain of SMARCA2/4 complements each other at the transcriptional level exerting similar suppressive function on specific gene expression programs.

Next, using GSEA, we identified biologic pathways/states shared by both rescue experiments leveraging annotated pathways from the Molecular Signature Database (mSigDB, The Broad Institute). GSEA identified enrichment of cell proliferation (cell cycle, cyclin D1, G2–M checkpoint), chromatin remodeling (mitotic spindle, DNA replication and synthesis), and tumorigenesis (EMT, integrin, KRAS and P53 pathway signatures). These gene expression programs, which are enriched in rescued cells compared with cells lacking ATPase activity common to SMARCA2/4, have bonafide tumorigenic functions (Fig. 7G).

In conclusion, the SMARCA4 cDNA complementation (i.e., re-expression) and the SMARCA2 RNAi rescue experiments are consistent with the observed lack of pharmacologic activity of PFI-3 in SWI/SNF-mutant cancers, and we demonstrate for the first time that selective SMARCA2/4 bromodomain inhibition is not a feasible therapeutic strategy for targeting aberrant SWI/SNF activity in SWI/SNF-mutant cancers. Instead, drug discovery efforts should be focused on inhibiting the ATPase catalytic activity to deliver on the promise of robust, cancer-specific synthetic lethality treatment.

Discussion

A common theme has emerged from genetic studies where imbalances between various paralogous subunits within SWI/SNF (e.g., SMARCA2/4 and ARID1A/B) can render cells more tumorigenic and simultaneously hypersensitive to targeting of the residual complex (13, 20–23, 40, 41). However, despite the high prevalence of genomic lesions in SWI/SNF, studies have not addressed how this observation can be translated into effective, drug discovery endpoints.

In this study, we first demonstrated context-specific antiproliferative phenotype of SMARCA2 depletion in SMARCA4-deficient lung cancer using multiple, non-overlapping hairpins, as well as independent siRNAs, validating the synthetic–lethal relationship between SMARCA2 and SMARCA4 (20–22). We further showed that expression of either SMARCA2 or SMARCA4 completely rescued the effects of SMARCA2 knockdown in SMARCA4-deficient cells, indicating paralog dependence and reciprocal role of these two subunits in tumorigenesis. The functional complementation of SMARCA2/4 was also evident at the transcriptional level where the ATPase activity appears to control gene programs related to proliferation, cell cycle, and chromatin remodeling. Furthermore, genomic analysis revealed mutual exclusivity of SMARCA2 and SMARCA4 mutations in LUSC and LIJAD carcinoma and expression-based biomarker analysis outlined a SMARCA4-deficient patient population predicted to depend exclusively on SMARCA2 activity (Fig. 1). Because SMARCA2-deficient mice are viable showing no overt phenotype (42), while SMARCA4 inactivation is embryonic lethal (43), one might anticipate a significant therapeutic window, if selective small-molecule SMARCA2 inhibitors can be developed that mimic the RNAi knockdown phenotype.

SMARCA2 contains an ATPase and bromodomain, suggesting at least two tractable avenues for inhibitor development. Targeting the acetyl-lysine recognition function of SWI/SNF bromodomains (e.g., the SMARCA2/4, PBRM1, BRD7, and BRD9 subunits) represents an unexplored opportunity for perturbation of the SWI/SNF complex. Recently, the anticancer activity of BET bromodomains inhibitors has fueled the development of novel chemical scaffolds that selectively target other bromodomains (6, 24, 25), and the PFI-3 inhibitor exemplifies one such novel chemical probe. However, despite being broadly available from SGC, no phenotypic data have yet been reported. Hence, we subjected PFI-3 to rigorous biochemical and cellular characterization confirming its exquisite selectivity, potency and cell permeability (Fig. 3). Such pharmacodynamics studies are a critical component of drug target validation studies as they provided confidence that a compound-induced phenotype (or lack thereof) correlate with biochemical target engagement in cells.

Surprisingly, in contrast with SMARCA2 knockdown, PFI-3 did not display any antiproliferative phenotype in SMARCA4-deficient lung cell lines across a variety of biologic assays. Likewise, in models harboring defined SWI/SNF alterations, including synovial sarcoma (SSX-fusion), rhabdoid tumors (SMARCB1-null), and leukemia (SMARCA4-dependent), PFI-3 did not mimic the anticancer phenotype observed upon RNAi-mediated knockdown of SMARCA2/4 (31, 38, 44). Mechanistically, and consistent with the lack of cellular phenotype, we discovered that PFI-3 cannot displace endogenous SMARCA2 (i.e., lung) or SMARCA4 (i.e., synovial sarcoma) from chromatin potentially due to the activity of other chromatin-interacting SWI/SNF subunits highlighting challenges in targeting large protein complexes. This result is in sharp contrast with efficient chromatin displacement of endogenous BRD4 by JQ1 (Fig. 4C), and we note the contrasting feature of the BET family of tandem bromodomains, which are not flanked by other known regulatory or conserved domains.

Recent studies have highlighted the role of residual SWI/SNF complex along with paralog dependence, indicating a potential combinatorial role of chromatin-interacting domains in SWI/SNF recruitment (5). Our data further highlight the need to conduct similar target identification/validation studies of other paralog subunits like ARID1A and ARID1B that form mutually exclusive SWI/SNF complexes and display a synthetic lethal relationship (40). Additional vulnerabilities like antagonism between SMARCB1 and EZH2, which renders rhabdoid tumors dependent.

Figure 7.

Chromatin binding and gene set enrichment highlights the importance of ATPase catalytic activity for cancer-specific vulnerability. A, immunofluorescent images (H1299 cells) expressing either vector control or HA-tagged SMARCA2 wild-type, bromodomain-mutant or ATPase-dead constructs (red), and Hoechst counterstain (blue). B, quantification of chromatin binding (normalized to non-extracted immunofluorescent signal). C and D, clustering of 1,000 most variable genes for SMARCA2 (C) and SMARCA4 rescue experiments (D). E and F, SMARCA2 knockdown signatures (derived from A549 cells reconstituted with SMARCA4) comprising upregulated (up; E) and downregulated (down; F) genes. The ranked gene list (x-axis) was derived from the SMARCA2 rescue experiments comparing ATM-Dead with WT as query. Genes responding differently (interaction contrast, Group 3 vs. Group 2) were ranked according to their P values with direction provided by the fold change. G, significantly enriched gene sets (mSigDB) shared between SMARCA2 and SMARCA4 focusing on their ATPase activity (i.e., WT vs. ATP-Dead cDNA expression).
on EZH2 for disease maintenance (45), present another promising approach to target SWI/SNF-mutant cancers. The antiproliferative response to SMARCA4 knockdown and EZH2 inhibitor treatment highlights clear dependencies on SWI/SNF activity (Fig. 5E–Hand Supplementary Fig. S7A). However, it remains to be investigated whether other SWI/SNF-mutant cancers are sensitive to EZH2 inhibition since we did not see activity of the EZH2 inhibitor in SMARCA4-deficient lung cancer cells (Supplementary Fig. S7B).

For SWI/SNF-mutant cancers, our target validation approach has focused on dissecting the functional contribution of an impaired SMARCA2/4 bromodomain or ATPase domain to cellular phenotype through parallel cDNA complementation and rescue experiments. The observation that expression of BRD-Mut, but not ATP-Dead, can rescue the SMARCA2 knockdown phenotype is consistent with the pharmacologic PFI-3 inhibitor data. Thus, our genetic and chemical findings converge and we unequivocally conclude that small-molecule inhibition of the bromodomain is dispensable for the ability of the SWI/SNF complex in controlling tumor growth. As such, the present study is the first to deprioritize SMARCA2/4 bromodomain inhibition as a tractable target in genetically defined lung, synovial sarcoma, leukemia, and rhabdoid tumors. However, we cannot exclude that compounds with a selectivity profile that simultaneously inhibits additional bromodomains in SWI/SNF (e.g., PBRM1, BRD7, and BRD9 subunits) could be an efficacious strategy (although pleiotropic bromodomain inhibition in normal cells could be a potential concern). Although the importance of ATPase activity has previously been shown for chromatin remodeling (20), our studies pinpoint the ATPase activity as the molecular synthetic-lethal target providing a genetically validated strategy for targeting SWI/SNF-mutant mutant lung cancer.

ATPases represents a large and diverse family of proteins, many of which perform chaperone-like functions assisting in the assembly, operation, and disassembly of protein complexes (46). Not surprisingly, because numerous cellular processes are driven by energy-dependent conformational changes in multisubunit complexes, ATPases have been implicated in various human diseases with several inhibitors in clinical use. However, most of these do not directly engage/bind the nucleotide-binding site (47). Developing potent inhibitors that must compete with intracellular concentrations of ATP (2–10 mmol/L) have been challenging. Phosphate groups contribute significantly to the nucleotide-binding affinity, but to overcome poor cell-permeability of negatively-charged phosphate groups, the majority of synthetic ATP analogues are devoid of highly charged phospho-mimetic groups (47). Moreover, the high sequence homology between ATP-binding sites (among ATPases and other ATP-binding proteins) represents a profound selectivity challenge. Overall, there is a need for the development of novel, potent, and bioavailable ATPase inhibitors, and the successful design of ATP-competitive kinase inhibitors, yet another class of ATP-binding enzymes, supports at least in principal, the feasibility of targeting the ATP-binding site of SMARCA2.

SMARCA2 belongs to the SF2 family of chromatin remodeling ATPases and contains most of the conserved motifs found in SF2 helicases (48). However, SMARCA2/4 share little overall sequence homology with other helicases and even less homology with other ATPases (46, 48). Moreover, the sequence homology of the SMARCA2 ATPase is limited to only two SF2 clusters in the human genome—related SMARCA proteins and a class of DNA helicases, suggesting possibility for achieving exquisite selectivity over other ATPases. An obvious challenge would be to obtain selectivity over SMARCA4, as these enzymes are highly homogenous in their active site and dual inhibition in normal cells could limit the therapeutic window (29). Structural insights often guide the design of selective inhibitors, however, very few X-ray crystal structures for ATPase domains are currently available for SMARCA2/4-related proteins with most being in open inactive conformation, like the yeast Chd1 ATPase domain, highlighting the need for furthering structural biology. Another barrier for pharmaceutical development of selective ATPase inhibitors is the current lack of commercial high-throughput screening assays and selectivity panels against the large family of ATP-binding proteins. Nevertheless, structural diversity in the vicinity of the nucleotide-binding sites, including possible allosteric sites, should enable SMARCA2 ATPase drug discovery supported by prior identification of potent and selective inhibitors of the ATPase activity of KIF11, Hsp90, and VCP (49, 50). The recent development of selective and cell-potent covalent inhibitors that block ATP binding, as well as allosteric inhibitors that impair nucleotide turnover for the VCP ATPase (50), is also an encouraging avenue for the development of inhibitors targeting the SMARCA2 ATPase catalytic activity.

Taken together, our target validation studies identify the SMARCA2 ATPase domain, but not the bromodomain, as a tractable, albeit more challenging therapeutic target for a well-defined SMARCA-deficient patient population representing more than 20,000 patients a year in the United States alone (i.e., 10%–20% of NSCLC cases). Moreover, the SMARCA4-deficient patient population generally lacks targetable oncoproteins (such as known EGFR or ALK translocations; ref. 20), which further emphasize the potential medical impact of developing inhibitors of the ATPase domain of SMARCA2/4.

Disclosure of Potential Conflicts of Interest
T.A. Paul reports receiving commercial research grant from Pfizer, Inc. No potential conflicts of interest were disclosed by the other authors.

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