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Mutations in the SWI/SNF complex induce a targetable dependence on oxidative phosphorylation in lung cancer

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Contributions

Y.L. designed the studies, performed experiments, interpreted the data and wrote the manuscript; P.A.F. provided intellectual input and wrote manuscript, Y.S. performed Seahorse experiments and analysis; F.S.K. performed in vitro experiments and mouse genotyping, B.F. generated PDX model, R.M., T.K., J.G. and N.F. conducted in vivo pharmacology experiments, J. M. and C.W. performed bioinformatics analysis; C.T., V.K. and K.R. performed ChIP-Seq analysis, Y.H.L., F. M. and J.A. conducted metabolomics experiment; I.W., J.W. and V.P. provided BATTLE trial expression data, J.M. provided intellectual input; G.G., C.R., Q.P., F.R. provided technical support.

Competing financial interests

The authors declare no competing financial interests

Abstract

Lung cancer is a devastating disease that remains a top cause of cancer mortality. Despite improvements with targeted and immunotherapies, the majority of patients with lung cancer lack effective therapies underscoring the need for more therapeutics. Genomic studies have identified frequent alterations in components of the SWI/SNF chromatin remodeling complex including *SMARCA4* and *ARID1A*. To understand the mechanisms of tumorigenesis driven by mutations in this complex, we developed a genetically engineered mouse model of lung adenocarcinoma by ablating *Smarca4* in the lung epithelium. We demonstrate that *Smarca4* acts as a bona fide tumor suppressor and cooperates with *p53* loss and *Kras* activation. Gene expression analyses revealed signature of enhanced oxidative phosphorylation (OXPHOS) in *SMARCA4* mutant tumors. We further show that *SMARCA4* mutant cells have enhanced oxygen consumption and increased respiratory capacity. Importantly, *SMARCA4* mutant lung cancer cell lines and xenograft tumors have marked sensitivity to inhibition of OXPHOS by a novel small molecule, IACS-010759, that is under clinical development. Mechanistically, we show that *SMARCA4* deficient cells have a blunted transcriptional response to energy stress creating a therapeutically exploitable synthetic lethal interaction. These findings provide the mechanistic basis for further development of OXPHOS inhibitors as therapeutics against SWI/SNF mutant tumors.

Cancer genomic studies have demonstrated a high frequency of genetic alterations in multiple subunits of the SWI/SNF chromatin remodeling complex across numerous solid tumors¹. A meta-analysis of 44 studies revealed that 20% of all tumors have mutations in one or more subunits of this complex². Physiologically, SWI/SNF chromatin remodeling complex uses energy from the hydrolysis of ATP to move, eject or restructure nucleosomes. Thus remodelers dynamically regulate access to chromatin and facilitate gene transcription, DNA replication, DNA repair and recombination³. Recent studies have started to shed light on the biochemical consequences of mutations in the SWI/SNF complex including the functional antagonism between SWI/SNF and the Polycomb repressive complexes⁴, the inability of *SMARCA4* inactivating mutants to evict the PRC1 complex^{5,6} and impaired enhancer-mediated gene expression regulation by loss of *SMARCB1* or *ARID1A*^{7,8}. Genetic approaches including synthetic lethality screens have indicated the requirement of the residual SWI/SNF complex to drive oncogenic processes when one subunit is inactivated including the discovery that *SMARCA2* acts as a synthetic lethal genetic partner to *SMARCA4*⁹ and *ARID1B* to *ARID1A*¹⁰. Importantly, SWI/SNF complex has been shown to antagonize Myc activity and promote cell differentiation in human cancer¹¹. However, despite these highly informative studies and the prevalence of these mutations, our understanding of the mechanistic basis of tumorigenesis by mutations of the SWI/SNF complex at the cellular and tissue level remains rudimentary.

Clinically, there has been substantial success using targeted therapies against mutant EGFR and EML4-ALK fusion in lung cancer patients with the respective genetic alterations¹². However, most lung cancer patients do not harbor these genetic lesions and don't benefit from these therapies. Thus, lung cancer remains a top cause of cancer mortality. Recent TCGA (The Cancer Genome Atlas) studies have shown that lung adenocarcinoma, a major sub-type of lung cancer, harbors frequent mutations in SWI/SNF complex¹. In lung adenocarcinoma, *SMARCA4* and *ARID1A* are the most frequently inactivated subunits,

each at a frequency of 8%. These genetic alterations primarily consist of single nucleotide substitutions (frame-shift, nonsense and few recurrent missense mutations) with infrequent homozygous deletions¹ (compiled in Fig. 1a). TCGA's comprehensive study validates pioneering works that discovered mutations of SMARCA4 in lung cancer^{13–15}. In the present study, we sought to understand the mechanistic basis of tumorigenesis by mutations in the SWI/SNF complex, and decided to focus on SMARCA4. SMARCA4 is the core catalytic subunit of SWI/SNF complex and is frequently mutated across multiple cancer types^{1,2,16}.

Results

***Smarca4* deficient GEMM tumors and *SWI/SNF* deficient human lung adenocarcinomas have increased expression of OXPHOS genes**

To investigate the tumorigenesis process in a well-defined genetic context, we initially established a genetically engineered mouse (GEM) model by conditionally inactivating *Smarca4* alone or in combination with well-known lung cancer relevant genes, *p53* and *Kras*, selectively in the lung epithelium using adenovirus encoding Cre recombinase following previously established protocols¹⁷. Interestingly, conditional inactivation of *Smarca4* (*Smarca4*^{fl/fl}) alone did not result in lung adenocarcinoma development indicating the requirement of additional genetic alterations to drive tumor development (Fig. 1b). However, conditional inactivation of *Smarca4* together with loss of p53 and activation of oncogenic KRAS (*Kras*^{LSLG12D/WT}, *p53*^{fl/fl}, *Smarca4*^{fl/fl} - hereafter called KPS) resulted in robust development of highly penetrant lung adenocarcinomas as compared to p53 loss and activation of KRAS (*Kras*^{LSLG12D/WT}, *p53*^{fl/fl} - hereafter called KP) (Fig. 1b). For subsequent experiments, we routinely used tumors and cell lines derived from these two cohorts. Both KP and KPS tumors are highly pleomorphic, multifocal, show classic histopathologic features of adenocarcinoma, stain positive for pro-surfactant protein C (a marker of alveolar type II cells), and are negative for squamous carcinoma markers p63 and keratin 5, and can be readily detected by microCT imaging (Fig. 1c and Supplementary Fig. 1a–d and 2a–b).

Next, we characterized KP and KPS tumors by performing transcriptomic profiling using RNA-sequencing. Interestingly, gene set enrichment analysis (GSEA) revealed oxidative phosphorylation as the most prominently enriched pathway in KPS tumors compared to KP tumors (Fig. 1d and Supplementary Fig. 3a). Importantly, we performed a similar comparative analysis using RNA-Sequencing data from TCGA project of human lung adenocarcinoma tumors which again revealed the oxidative phosphorylation pathway as the most significantly enriched pathway in tumors with mutations in *SMARCA4* or *ARID1A* (Fig. 1e and Supplementary Fig. 3b). Prominent examples of genes in the OXPHOS pathway whose expression was increased in *SMARCA4* deficient tumors include the master mitochondrial biogenesis co-activator PGC1- α , mitochondrial ATP synthase F0 complex subunit *ATP5L*, and oxidative stress response genes like glutathione S-transferase *GSTO7* and *GSTO1* (Fig. 1f–g, Supplementary Fig. 3c). We further utilized gene expression data from an additional human lung cancer patient cohort (BATTLE trial¹⁸) to demonstrate the

increased expression of prominent OXPHOS genes in tumors with low expression of SMARCA4 (Supplementary Fig. 3d, Supplementary Table 1).

As PGC1- α is known to drive mitochondrial biogenesis and respiration^{19,20}, we sought to understand its role in SWI/SNF mutants in more detail. First, we confirmed by immunohistochemistry staining that PGC1- α protein level is increased in KPS tumors (Fig. 1h, Supplementary Fig. 3e). Furthermore, similar increases in PGC1- α mRNA and protein were observed in KPS tumor-derived cell lines compared to KP cell lines (Supplementary Fig. 3f).

SMARCA4 deficient cells have increased mitochondrial respiration

In order to determine directly the role of PGC1- α in the growth of *SMARCA4* deficient cells, we performed a simultaneous shRNA mediated knockdown of PGC1- α in H1299, a *SMARCA4* deficient lung cancer cell line (Supplementary Fig. 3g). Importantly, we observed profoundly decreased colony formation in parental H1299 cells whereas re-expression of SMARCA4 in an inducible manner rescued the lack of colony formation demonstrating a selective dependence of *SMARCA4* deficient cells on PGC1- α (Fig. 2a).

Consistent with these observations, we identified an increase in the mitochondrial DNA content of *SMARCA4* deficient human lung adenocarcinoma tumors by analyzing publicly available mitochondrial DNA copy number of TCGA tumors²¹ (Fig. 2b). Additionally, we showed that KPS tumors have higher mitochondrial DNA content than KP tumors (Fig. 2c). It is important to note that with the exception of lung adenocarcinoma, mitochondrial DNA content and mitochondrial gene expression are decreased in other tumor types examined compared to their normal counterparts²¹. This is broadly consistent with the generally accepted Warburg phenomenon, whereby cancer cells primarily utilize glycolysis, and not mitochondrial OXPHOS, to generate energy and biomass^{22,23}. We next sought to investigate whether SWI/SNF mutant lung adenocarcinomas behave differently from other tumor types in their mode of energy generation.

First we asked whether the above observed gene expression changes in OXPHOS actually lead to a functional increase in mitochondrial respiration. To answer this, we utilized KP and KPS murine lung cancer cell lines and *SMARCA4* deficient lung cancer cell lines (H1299, H2023 and H2030) that were engineered to express control GFP or SMARCA4 inducibly. Importantly, re-expression of SMARCA4 in these cell lines did not affect rate of proliferation under regular cell culture media (Fig. 2d, Supplementary Fig. 3h). We then determined basal mitochondrial respiration and glycolysis using oxygen consumption rate (OCR) and extracellular media acidification rate (ECAR) as surrogates respectively. Interestingly, SMARCA4 re-expression significantly reduced the ratio of OCR to ECAR (Fig. 2e) indicating the presence of an increased rate of basal mitochondrial respiration in cancer cells lacking *SMARCA4*. Additionally, we observed a consistent increase in basal mitochondrial respiration, spare respiratory capacity, and ATP production in the *Smarca4* deficient KPS cell lines (Fig. 2f–h). Furthermore, using the engineered human cancer cell lines we showed a similar increase in basal mitochondrial respiration, spare respiratory capacity and ATP production in *SMARCA4* deficient cell lines (Fig. 2i–k and Supplementary Fig. 4a–d) confirming that the increase in OXPHOS gene expression

signature correlates with the increase in functional mitochondrial output both in murine and human cell systems. We also performed metabolomics studies to identify metabolites that are selectively altered in *SMARCA4* deficient cells. Interestingly, *SMARCA4* deficient cells show an increased amount of two prominent tricarboxylic acid (TCA) cycle inputs, glutamine and alpha-ketoglutarate (Supplementary Fig. 5a, b). Further, several metabolites that play key roles in the pentose-phosphate pathway (PPP) were elevated in *SMARCA4* deficient cells consistent with the known role of the PPP in generating NADPH, a potent antioxidant against oxidative stress and a reducing agent for fatty acid synthesis^{24,25} (Supplementary Fig. 5c). Suggesting a state of oxidative stress, *SMARCA4* deficient cell lines have elevated levels of reactive oxygen species (ROS) compared to *SMARCA4* reconstituted cells (Supplementary Fig. 6)

SWI/SNF mutant lung cancer cells and xenografts are sensitive to inhibition of OXPHOS

Next, we investigated whether *SMARCA4* deficient cells are more dependent on OXPHOS for growth than *SMARCA4* proficient cells. To answer this we used IACS-010759, a novel small molecule inhibitor of OXPHOS that is currently under clinical development (Fig. 3a). IACS-010759 is a clinical stage small molecule that inhibits complex I of the respiratory electron transport chain and has shown a promising safety profile (accompanying manuscript by Marszalek and others). We performed cell viability assays on 20 human lung cancer cell lines, half of which have inactivating mutations in either *SMARCA4* or *ARID1A* and half of which do not have detectable mutations in the core subunits of the SWI/SNF complex (Fig. 3b–c and Supplementary Fig. 7a–b). Notably, we observed that most of the responsive cell lines with GI50 values in the low single digit nM range have mutations in the SWI/SNF complex while most of the wild type cells are largely resistant to IACS-010759 with the difference being statistically significant ($p=0.0059$) (Fig. 3d and Supplementary Fig. 7b). Consistent with this, KPS tumor derived murine cells were more sensitive to IACS-010759 compared to KP tumor derived cell lines (Fig. 3e). To further confirm dependency of this phenotype on *SMARCA4*, we performed rescue experiments by doxycycline inducible restoration of *SMARCA4* in *SMARCA4* deficient lung cancer cell lines, A549, H1299 and H2030. Reconstitution with *SMARCA4* resulted in a robust rescue of cell viability when cells were grown to confluency (Fig. 3f). Next, we assessed whether IACS-010759 has differential antitumor *in vivo* efficacy by treating mice subcutaneously implanted with xenograft tumors derived from either KP or KPS murine cancer cells. IACS-010759 was able to inhibit growth of established KPS tumors but not KP tumors demonstrating its selective activity on *Smarca4* deficient tumors (Fig. 3g). Furthermore, we performed additional *in vivo* antitumor efficacy study by using a lung cancer patient derived xenograft (PDX) model, TC388, which is deficient in *ARID1A*. Inhibition of OXPHOS by IACS-10759 has a profound tumor growth inhibitory activity on PDX TC388 (Fig. 3h). Taken together, our findings demonstrate that cells and tumors with inactivating mutations in a subunit of SWI/SNF complex (either *SMARCA4* or *ARID1A*) have a profound dependency on OXPHOS compared to wild type cells revealing a valuable therapeutic window.

SMARCA4 is required for optimal expression of energy deprivation stress response genes

To decipher the inhibitory mechanism of action of IACS-010759 on growth, we focused on redox balance and energy supply as two prominent processes impacted by mitochondrial complex I²⁶. Importantly, administration of the anti-oxidant N-acetylcysteine had no impact on the growth inhibitory effect of IACS-010759 (Supplementary Fig. 8a). In contrast, removal of glucose from the growth media resulted in a profound increase in sensitivity of cells to IACS-010759, with GI50 values reaching picomolar range (Supplementary Fig. 8a). These data suggest that the most important mechanistic basis of efficacy of IACS-010759 is its capacity to deprive cells of energy and not necessarily massive generation of reactive oxygen species (ROS). Finally, to begin to understand why SWI/SNF mutants in particular are highly dependent on OXPHOS we focused our investigations on cellular energy supply and demand balance. In principle, SWI/SNF mutants could have either defective glycolysis as an alternate pathway of energy generation, or elevated demand through biosynthetic pathways, or a combination of the two.

First, we measured the rate of glycolysis using ECAR and lactate secretion, two well accepted surrogates. We observed a modest reduction in glycolysis and glycolytic capacity in *SMARCA4* deficient cells compared to *SMARCA4* reconstituted counterparts (Fig. 4a, b and Supplementary Fig. 8b). Then we sought to understand the interplay between glycolysis and *SMARCA4*. Interestingly, we observed that re-expression of *SMARCA4* was able to rescue cell death induced by deprivation of glucose from growth media of *SMARCA4* deficient A549 and H1299 cells (Supplementary Fig. 8c). This points to a broader role of *SMARCA4* in response to energy deprivation induced stress. To understand this further, we performed transcriptomic analysis of parental H1299 cells or H1299 cells reconstituted with *SMARCA4* under normal growth conditions, IACS-010759 treatment or glucose deprivation (to induce severe energy stress). Remarkably, one of the top most consistently upregulated genes under both IACS-010759 treatment and glucose deprivation only when *SMARCA4* is re-expressed was HIF2a, a master regulator of survival pathways activated by various cellular stresses such as hypoxia^{27,28} (Fig. 4c). Consistent with this observation, gene set enrichment analysis revealed genes in the hypoxia response and glycolysis pathways to be highly enriched when *SMARCA4* expressing cells are exposed to energy stress (Fig. 4d).

In order to show direct regulation of hypoxia and metabolic genes by *SMARCA4* we determined the genomic localization of *SMARCA4* using ChIP-Seq in H1299 cells after inducible induction of *SMARCA4* expression. As expected from its chromatin remodeling activity to facilitate gene expression, *SMARCA4* is primarily localized around gene promoters and 5' regulatory regions (Supplementary Fig. 9a, b). Gene set enrichment analysis of genomic regions occupied by *SMARCA4* reveals that several key pathways including hypoxia response regulation by HIF network is significantly enriched in *SMARCA4* occupancy (Fig. 4e). We observed that *SMARCA4* occupies the promoter region of HIF2a transcription factor and key glycolytic enzyme HK2 among many other key metabolic regulators (Fig. 4f, g and Supplementary Fig. 9b). We further validated these observations using ENCODE *SMARCA4* ChIP-Seq data and analyzing the pattern of genomic regions directly occupied by *SMARCA4*. Gene set enrichment analysis identified the HIF network as the top pathway whose constituent genes are occupied directly by

SMARCA4 (Supplementary Fig. 9c). Examples of such genes include HIF2a itself and critical glycolytic enzymes such as hexokinase 2 (HK2) whose promoters were occupied by SMARCA4 (Supplementary Fig. 9d, e). Taken together, these data strongly suggest that SMARCA4 by directly occupying the promoters of energy stress response genes such as HIF2A facilitates the transcriptional network necessary for effective response and cellular survival. By contrast, in the absence of SMARCA4 cells have a blunted response to energy stress and compromised viability (Fig. 3f, 4c–g and Supplementary Fig. 8–9).

SMARCA4 mutant cells have elevated energy needs for biosynthetic processes

Next, we asked whether *SMARCA4* deficient cells have elevated demand for energy due to increased biosynthetic needs. To answer this we focused on two of the most energy intensive anabolic processes in cells, i.e. protein synthesis and fatty acid synthesis pathways, which can account for up to 75% of the total cellular energy budget²⁹. With the aim of determining the activation status of various signaling pathways that regulate metabolism, we first analyzed TCGA human lung adenocarcinoma protein expression data generated using the reverse phase protein array (RPPA) platform. Amongst the 160 proteins/post-translational modifications assayed, the most significantly downregulated protein in *SMARCA4* and *ARID1A* deficient human tumors was Serine 79 phosphorylated Acetyl-CoA carboxylase (pACC) (Fig. 5a). Acetyl-CoA carboxylase (ACC) catalyzes the rate limiting step in long chain fatty acid synthesis and phosphorylation at Serine 79 is known to inactivate the enzyme and inhibit cellular fatty acid synthesis³⁰. Hence, the reduction in pACC in SWI/SNF deficient tumors is expected to result in increased fatty acid synthesis. Consistent with this observation, we were also able to show markedly reduced pACC by western blot in multiple *SMARCA4* deficient cancer cell lines compared to SMARCA4 expressing cells (Fig. 5b). Next we hypothesized that inhibition of elevated fatty acid synthesis would reduce energy expenditure and might rescue the growth inhibitory effect of IACS-010759. We treated *SMARCA4* deficient H1299 cells using increasing concentration of IACS-010759 alone or in combination with well characterized fatty acid synthesis inhibitors, Cpd-10v or IPI-9119^{31,32}. We noted a modest but significant rescue of the growth inhibitory effect of IACS-010759 by co-inhibition of fatty acid synthesis (Fig. 5c and Supplementary Fig. 10) supporting the notion that reducing energy expenditure helps cells to survive the energy stress introduced by OXPHOS inhibition. Finally, we investigated whether inhibition of protein synthesis also alleviates energy stress caused by IACS-010759. We treated H1299 cells with IACS-010759 alone or in combination with different doses of rapamycin, a well-characterized mTOR inhibitor that reduces overall protein translation. While rapamycin alone, up to a concentration of 10nM, did not have any discernible effect on growth of H1299 cells, it was potently able to rescue the growth inhibitory effect of IACS-010759 on these cells (Fig. 5d). Taken together, these data support our proposed model of the critical importance of energy supply and demand imbalance in the selective sensitivity of SWI/SNF mutants to OXPHOS inhibition (Fig. 5e).

In summary, our work demonstrates the presence of a complex metabolic rewiring in SWI/SNF mutant lung adenocarcinoma tumors that results in a heightened reliance on OXPHOS. We observed primary defects in energy generation with reduced glycolytic capacity as well as increased energy consumption for biosynthetic pathways in these cells

contributing to a state of energy stress (Fig. 5e). Importantly, this work identified OXPHOS as a therapeutically viable vulnerability of SWI/SNF mutant lung tumors and demonstrated that the small molecule IACS-010759 has a potent and differential antitumor efficacy in such tumors.

Discussion

The similarity between the transcriptomic changes observed in our KPS GEM model and human lung adenocarcinomas with SWI/SNF mutation indicates that the KPS lung cancer model recapitulates, at least in part, relevant human disease processes and could be a valuable experimental model system for future mechanistic and therapeutic investigations. In our GEM model both heterozygous and homozygous loss of *SMARCA4* have similar effect on tumor development indicating *SMARCA4* acts as a haplo-insufficient tumor suppressor. This is in contrast to human lung cancer where *SMARCA4* loss of heterozygosity is common^{13,33}. This observation could be attributed to differences in the tumor initiating event (i.e. targeted manipulation of select genes in GEM model versus smoking in humans), differences in the cell of origin of tumors as well as status of co-occurring mutations. As a caveat, our GEM models are on mixed genetic background which could act as a confounder in some experiments and would benefit from backcrossing to create a pure genetic background.

Previous work has identified lung adenocarcinoma as the only tumor type among the 12 most common carcinomas to have increased mitochondrial DNA content compared to adjacent normal tissue²¹. Our work provides additional data that shows SWI/SNF mutant lung tumors have elevated mitochondrial DNA content compared to wild type tumors. While mutations in SWI/SNF are widespread among solid tumors, the existence of similar dependencies on OXPHOS in other tumor types with SWI/SNF mutation is still unclear and needs further investigation. It is also interesting that lung cancer has frequent mutations in *LKB1* and KEAP1/NRF2 pathways, which are major integrators of cellular response to energy and oxidative stress. While glucose and aerobic glycolysis (the Warburg effect) have received the most attention in cancer metabolism, several other sources of energy and biomass have been elegantly demonstrated recently including glutamine³⁴, autophagy-derived alanine in pancreatic ductal adenocarcinoma (PDAC)³⁵, branched-chain amino acids in lung cancer³⁶, OXPHOS in oncogene-ablation resistant PDAC cells³⁷ and serine in *LKB1* mutant PDAC³⁸. Similarly, our work further expands on the central role of dysregulated metabolism in lung cancer pathogenesis pointing to potential organ/tissue specific processes that are still not fully understood. Further research including metabolic flux analysis is needed to characterize the dynamic changes in metabolism in SWI/SNF mutants.

It is interesting to note that SWI/SNF chromatin remodeling complex was discovered in screens to identify genes that regulate mating-type and sucrose non-fermenting phenotypes in the yeast *S.cerevisiae* whereby *snf2* (the yeast homologue of SWI/SNF) mutants lacked the ability to generate energy from sucrose due to their inability to de-repress and express invertase³⁹. Interestingly, the same screens identified *snf1*, whose mammalian homologue is AMPK, a master sensor of cellular energy status that is regulated primarily by LKB1 tumor suppressor⁴⁰.

Our study highlights that the fundamental role of the SWI/SNF complex in facilitating efficient expression of critical genes for energy generation is evolutionarily conserved. It is known that peroxisome proliferator-activated receptor gamma (PPAR γ), a master metabolic regulator, requires SWI/SNF complex for its regulation of adipogenesis, lipid metabolism, thermogenesis, and glucose homeostasis⁴¹. Additionally, Myc activity has been shown to be antagonized by SWI/SNF complex and is well known to play a major role in metabolic dysregulation of tumors^{11,42}. While we did not observe changes in gene expression of major nuclear hormone receptors, metabolic regulators such as PPAR γ and Myc in our model systems, we cannot rule out their role in the metabolic reprogramming of SWI/SNF mutant tumors due to alteration in the function of these genes at post-transcriptional and post-translational levels. SWI/SNF complex is also known to regulate several aspects of metabolism under physiological conditions including fatty acid β -oxidation. In particular the BAF60a subunit induces the expression of genes involved in peroxisomal and mitochondrial fatty acid β -oxidation, including hydroxyacyl-CoA dehydrogenase (Hadha) and acetyl-CoA acyltransferase 2 (Acaa2). Furthermore, BAF60a has been shown to link the SWI/SNF complexes to the transcriptional coactivator PGC1 α , indicating there is a role for SWI/SNF in hepatic lipid metabolism⁴³.

Mechanistically, our observations suggest that SMARCA4 facilitates expression of energy stress response genes such as HIF2A that in turn promote a pro-survival transcriptional program. Lack of SMARCA4 resulted in a blunted response to both glucose deprivation and OXPHOS inhibition pointing to a broader role of SMARCA4 in metabolic regulation as well as revealing a potentially attractive vulnerability of these mutants. It has been previously shown that cells that lack *TSC2* or *LKB1* were exquisitely sensitive to glucose deprivation⁴⁴. This study adds SMARCA4 to this category of genes. Notably, one of the cell lines used in our study, A549, has an inactivating mutation in *LKB1* and is known to be exquisitely sensitive to glucose deprivation⁴⁵. Remarkably, re-expression of SMARCA4 in A549 cells, and also in *LKB1* proficient H1299 cells, was able to partially rescue the cell death phenotype pointing to a robust contribution of SMARCA4 to survival response independent of the status of LKB1. Our study indicates that the primary therapeutic mechanism of action of IACS-010759 in SWI/SNF mutants is through energy deprivation. This was corroborated by rescue experiments that showed reduction of cellular energy expenditure by inhibiting fatty acid synthesis or protein translation was able to successfully counter the effect of IACS-010759.

The work reported here complements recent studies that have indicated potential strategies to target SWI/SNF mutants. Inhibition of the histone methyltransferase EZH2 has shown growth inhibitory effect in a subset of SWI/SNF mutants⁴⁶. A recent siRNA screen has indicated that *SMARCA4* mutant non-small cell lung cancer cell lines are sensitive to Aurora kinase A inhibitor⁴⁷. PARP inhibition has also shown some inhibitory effect in *ARID1A* mutant ovarian cancer cell lines⁴⁸. In summary, our study identified a fundamental role for SMARCA4 in energy stress response which creates vulnerability of *SMARCA4* mutant tumors to energy depriving therapeutic strategies such as OXPHOS inhibition.

Methods

Genetically engineered mouse model (GEMM) of lung adenocarcinoma

Cryopreserved mixed background B6:129S2-*Smarca4*^{flox/flox} sperm with *loxP* sites flanking Exons 2 and 3 of mouse *Smarca4* gene were received from the mutant mouse resource and research centers supported by NIH (MMRRC). The original targeting of this allele was performed by the laboratory of Pierre-Chambon⁵² who generously deposited the allele at MMRRC. Live mouse re-derivation was conducted by the genetically engineered mouse (GEM) facility of the University of Texas MD Anderson Cancer Center. The resulting *Smarca4*^{flox/flox} mice were crossed with previously described *K-ras*^{LSL-G12D/WT}; *p53*^{flox/flox} mice¹⁷. *K-ras*^{G12D/WT}; *p53*^{-/-} and *K-ras*^{G12D/WT}; *p53*^{-/-}; *Smarca4*^{-/-} lung tumors were initiated via intranasal inhalation of Adenovirus encoding for Cre in six weeks old *K-ras*^{LSL-G12D/WT}; *p53*^{flox/flox}, *K-ras*^{LSL-G12D/WT}; *p53*^{flox/flox}; *Smarca4*^{flox/flox} or *Smarca4*^{flox/flox} mice as described previously¹⁷. Adeno5-CMV-Cre virus was purchased from Viral Vector Core facility of Baylor College of Medicine at a viral titre of 10¹² particles/ml. All mice were kept in specific pathogen free vivarium at the MD Anderson Cancer Center mouse facilities. Mice were fed commercial rodent diet (PicoLab Rodent diet 5053 from Labdiet) and water *ad libitum*. The University of Texas MD Anderson Cancer Center Institutional Animal Care and Use Committee (IACUC) approved all animal studies and procedures.

Immunohistochemistry

Tumors were fixed in formalin for 24 hours, paraffin embedded, sectioned and stained according to standard procedures. Briefly, endogenous peroxidases were inactivated by 3% hydrogen peroxide. Non-specific signals were blocked using 3% BSA, 10% goat serum in 0.1% Triton X-100. After antigen retrieval in citrate buffer, slides were stained using respective antibodies overnight at 4°C, [PGC1α (Abcam #54481, 1:200), SMARCA4 (Abcam #110641, 1:75), pro-Surfactant protein C (Abcam, 40879, 1:1000), p63 (Biocare Medical #PM366AAK, ready to use dilution), cytokeratin 5 (Abcam, 52635 1: 200)]. After overnight incubation, the slides were washed and incubated with secondary antibody (HRP-polymers, Biocare Medical) for 30 min at room temperature. The slides were washed three times and stained with DAB substrate (ThermoFisher Scientific). The slides were then counterstained with haematoxylin and mounted with mounting medium.

Cell culture

Human lung cancer cell lines H1299, A549, H2030 and H2023 were obtained from ATCC. These cells were routinely cultured in RPMI 1640 (Life Technologies 11965) supplemented with 10% heat inactivated FBS. The cell lines were authenticated by short tandem repeat (STR) profiling at the Institute for Applied Cancer Sciences, MD Anderson Cancer Center. Murine tumor derived cell lines (KP and KPS) were generated by mechanically mincing lung tumors, dissociation with a combination of collagenase I, IV and dispase. These cells were grown in Dulbecco's modified Eagle's medium (Gibco) supplemented with 10% FBS and 1% Pen/Strep (Life Technologies 15140). All cells were routinely tested for mycoplasma by PCR. For metabolic and metabolomic assays, 10% dialysed FBS (Atlanta Biologicals Inc.) was used.

Inducible ectopic expression of SMARCA4

eGFP as control or SMARCA4 were cloned into the pInducer20 doxycycline inducible lentiviral vector (Addgene 44012). Lentivirus was produced using standard virus production methods by co-transfecting target and packaging plasmids (psPAX2 – Addgene12260 and pMD2.G- Addgene 12259) into HEK293T cells. Cell lines were then transduced with 0.45uM filtered and ultracentrifuge concentrated viral particles with Polybrene (8µg/ml). After 16 hours of transduction, media was changed into fresh regular growth media, and 48 hours later selection started using G418 (0.2–0.6µg/ml). After selection was complete in 72 hours, cells were termed stably transduced. GFP or SMARCA4 expression was induced within 24 hours of induction with doxycycline 0.5µg/ml.

Cell viability Assays

Human and murine cancer cell lines were plated at a density of 2000 cells per well in a 96-well plate and cell viability over 72–96 hours was assessed in clear bottom 96-well plates (Costar 3603, Corning Incorporated) by CellTiter-Glo (Promega G7572). Compounds were routinely added 24 hours after plating by substituting media with drug containing media. Assay was usually done 72–96 hours post treatment with various compounds and determined by the mean of at least four wells per condition and compared to DMSO treated wells as control. Luminescence was measured on a POLARstar Omega plate reader and results plotted by GraphPad.

Proliferation and colony-formation assays

Cell growth of SMARCA4 reconstituted and parental cell lines was assayed either through crystal violet staining or by live-cell confluence measurement with IncuCyte (Essen BioScience). Growth curves using IncuCyte were generated by confluence imaging every 2 h with quadruplicate replicates of an initial seeding of 2000 cells per well in 96-well plates. For colony-formation assays, 500–1000 cells were seeded in 6-well plates. At the indicated time point (usually 10–14 days), cells were fixed with 80% methanol and stained with crystal violet solution overnight. All experiments were performed in triplicates.

Mitochondrial respiration and glycolysis measurements

OCR and ECAR experiments were performed using the XF-96 Analyzer apparatus from Seahorse Bioscience. Cells were plated (10,000 cells per well) in quadruplicates for each condition the day before the experiment. The next day, medium was replaced with reconstituted DMEM with 25 mM glucose and 2 mM glutamine (no sodium bicarbonate) adjusted to pH~7.4 and incubated for 30 min at 37% in a CO₂-free incubator. For the mitochondrial stress test (Seahorse 101706–100), oligomycin, FCCP, antimycin and rotenone were injected to a final concentration of 2 µM, 0.5 µM and 4 µM, respectively. For the glycolysis stress test (Seahorse 102194–100), glucose, oligomycin and 2-deoxyglucose were injected to a final concentration of 10 mM, 2 µM and 100 mM, respectively. OCR and ECAR were normalized to cell number as determined by CellTiter-Glo analysis at the end of the experiments. OCR and ECAR values were used to compute basal respiration, spare capacity, proton leak, ATP production, glycolysis and glycolytic capacity. Calculations were done by ExcelMacro Report Generator Version 3.0.3 provided by Seahorse Biosciences and

two-sided Student's t-test computed using GraphPad taking the mean and s.d values provided by Report Generator 3.0.3 and n=8 (4 values from 2 independent experiments). Formulas are described here. Basal Respiration= (Last rate measurement before first injection)- (Non-mitochondrial Respiration Rate), Spare Respiratory Capacity = (Maximal Respiration)-(Basal Respiration). Proton leak= (minimum rate measurement after Oligomycin injection)-(non-mitochondrial respiration). ATP production = (Last rate measurement before oligomycin injection)-(Minimum rate measurement after Oligomycin injection). For Glycolysis = (Maximum rate measurement before oligomycin injection)-(Last rate measurement before glucose injection) and for glycolytic capacity = Maximum rate measurement after oligomycin injection)-(Last rate measurement before glucose injection).

Metabolomics via Selected Reaction Monitoring Tandem Mass Spectrometry

One 15 cm² plate of cells (~10–15 million) per sample was extracted with 80% methanol (–80 °C) for 15 min. Dried metabolite pellets were resuspended in 20 µL of LC/MS-grade water, and 5 µL aliquots were injected for targeted LC/MS/MS on a 5500 QTRAP hybrid triple-quadrupole mass spectrometer coupled to a Prominence ultrafast liquid chromatography (UFLC) system from 287 selected reaction monitoring (SRM) transitions with positive/negative polarity switching. Samples were separated on a 4.6 mm i.d. × 100 mm Amide XBridge hydrophilic interaction liquid chromatography (HILIC) column at 360 µL/min starting from 85% buffer B (100% ACN) and moving to 0% B over 16 min. Buffer A was 20 mM NH₄OH/20 mM CH₃COONH₄ (pH = 9.0) in 95:5 water/ACN. Q3 peak areas were integrated by use of MultiQuant 2.1 software (AB/SCIEX). MetaboAnalyst 2.0 (<http://www.metaboanalyst.ca>) was used to normalize data, calculate heat maps, and perform pathway analysis. All metabolite samples were prepared as biological triplicates.

Transcriptomic profiling by RNA-seq

RNA was isolated from tumor tissues using Trizol extraction followed by purification with the Qiagen RNeasy kit as described by the manufacturer. RNA-seq was performed by the Sequencing and Microarray Facility (SMF) core at MD Anderson. Libraries were generated using Illumina's TruSeq kit and were sequenced using the Illumina HiSeq2000 Sequencer. RNA sequencing reads of the mouse samples were mapped to mm10 reference genomes respectively using the STAR aligner. For calculation of gene expression, raw count data of each gene were first obtained using with HTSeq and are normalized by scaling the raw library size using calcNormFactors in edgeR package in R. Then, we applied Voom transformation to normalized counts and fit a linear model for differential expression analysis using the Limma package. Significantly deregulated genes between any two sample groups were selected (p < 0.05 and fold change > 1.5). Pathway analyses of differentially expressed genes were performed using Gene Set Enrichment Analysis analysis and the web-accessible program DAVID (Database for Annotation, Visualization and Integrated Discovery).

Transcriptomic profiling by microarray

Total RNA isolation from H1299 cells was performed with the Qiagen RNeasy kit, according to the manufacturer's instructions. Human Genome Clarion S chip containing

probe sets for >20,000 characterized genes was used. Sample labeling and processing, hybridization, and scanning were performed according to Affymetrix protocols. Briefly, doublestranded cDNA was synthesized from total RNA with GeneChip 3' IVT express kit (Affymetrix), with a T7 RNA polymerase promoter site added to its 3' end (Genset, La Jolla, CA). Biotinylated cRNAs were generated from cDNAs in vitro and amplified by using GeneChip 3' IVT express kit. After purification of cRNAs by the RNeasy mini kit (Qiagen, Hilden, Germany), 15 µg of cRNA was fragmented at 94°C for 35 min. Approximately 12.5 µg of fragmented cRNA was used in a 250-µl hybridization mixture containing herring-sperm DNA (0.1 mg/ml; Promega), plus bacterial and phage cRNA controls (1.5 pM BioB, 5 pM BioC, 25 pM BioD, and 100 pM Cre) to serve as internal controls for hybridization efficiency. Aliquots (200 µl) of the mixture were hybridized to arrays for 16 h at 45°C in a GeneChip Hybridization Oven 640 (Affymetrix). Each array was washed and stained with streptavidin–phycoerythrin (Life Technologies) and amplified with biotinylated anti-streptavidin antibody (Vector Laboratories) on the GeneChip Fluidics Station 450 (Affymetrix). Arrays were scanned with the GeneArray G7 scanner (Affymetrix) to obtain image and signal intensities. CHP files were then imported into Affymetrix Expression console and Transcriptome Analysis Console softwares where normalization and comparison between different groups was performed.

TCGA Data analysis

Lung Adenocarcinoma (LUAD) TCGA data was retrieved from Firehose using the `getFirehoseData` from the `RTCGAToolbox` R package⁵³. We downloaded RNAseq, Mutation, Methylation, Copy Number (GISTIC output), and RPPA data. Mutation data was filtered to consider only frame shift insertions and deletions, in-frame deletions, missense, nonsense, and splice site mutations.

Gene-deficient samples were defined as samples presenting a mutation, deep deletion (GISTIC value = -2), or scaled-expression ≤ -2 . Samples were grouped as deficient or non-deficient to compare different data. P-values were calculated using a two-sided Wilcoxon rank-sum test for continuous data, while for discrete data a Fisher's exact test was used.

We obtained mitochondrial DNA content from TCGA LUAD samples from the supplementary materials of a recent report²¹. The log2 ratio (Tumor mtDNA / Normal mtDNA) was used to compare deficient and non-deficient samples. We used blood or normal tissue mtDNA depending on data availability. P-values were calculated using a two-sided Wilcoxon rank-sum test or Student t-Test.

BATTLE trial data set analysis

mRNA gene expression data for SMARCA4 and several OXPHOS genes was collected from existing human lung cancer cohort, the BATTLE clinical trial⁵⁴.

Data Processing: Affymetrix GeneChip HuGene-1.0 ST array was used for gene expression profiling. The platform consists of 33252 probesets, representing 20254 unique genes. After receiving all CEL files, QC analysis was performed first. In this step, we only kept arrays with the RNA integrity number (RIN) ≥ 4 , RIN is used for assigning integrity values to RNA

measurements, and malignant cell percentage 10. Next, for these array past QC, the CEL files were processed using *aroma.affymatrix*, an R package that commonly used for HuGene-1.0 ST array data processing. In the package, Robust Multiarray Analysis (RMA) algorithm was used for background correction. Then quantile normalization approach was utilized on the background-corrected expression data. The normalized data was logarithm transformed (base2) for future analysis. We performed Pearson correlation analysis to determine whether there is a significant association between expression level of SMARCA4 and PGC1alpha, ATP5L, COX7A2 and GSTO1.

ENCODE SMARCA4 ChIP-Seq data analysis

Bed files from SMARCA4 ChIP-seq conducted in HeLa cells was downloaded from the ENCODE project website <https://www.encodeproject.org/experiments/ENCSTR000EZC/> which contains the detailed experimental setup and conditions similar to other ENCODE projects. Bed files were inputted into GREAT and regulator domains identified using GREAT default parameters. <http://bejerano.stanford.edu/great/public/html/>. FDR corrected significant enrichment in MSigDB Pathways were retrieved from GREAT output. For selected individual genes, ENCODE link to UCSC Genome browser was used to plot SMARCA4 ChIP-Seq tracks.

SMARCA4 Chromatin immunoprecipitation-sequencing (ChIP-Seq) and analysis

H1299 cells stably transduced with pINDUCER20-SMARCA4 vector were treated with doxycycline to induce expression of SMARCA4 for 5 days or left untreated. Cells were then treated with IACS-10759 (100nM) for 24 hours, cells were then harvested and prepared for ChIP-Seq. ChIP assays were performed as described in⁵⁵ with minor modifications. Briefly, $\sim 8 \times 10^7$ cells were harvested by cross-linking with 1% (wt/vol) formaldehyde for 10min at 37°C with shaking. After quenching with 150mM glycine for 10min at 37°C with shaking, cells were washed twice with ice-cold PBS and frozen at -80°C for further processing. Cross-linked pellets were thawed and lysed on ice for 30min in ChIP harvest buffer (12mM Tris-Cl, .1x PBS, 6mM EDTA, .5% SDS) with protease inhibitors (Sigma). Lysed cells were sonicated with a Bioruptor (Diagnode) to obtain chromatin fragments ~ 200 to 500bp and centrifuged at 15,000g for 15min to obtain a soluble chromatin fraction. In parallel with cellular lysis and sonication, SMARCA4 antibody (Abcam: ab110641) at $5\mu\text{g}/3 \times 10^6$ cells was coupled to 30ul of magnetic protein G beads in binding/blocking buffer (PBS+, 1%TWEEN+.2% BSA) for 2h at 4°C with rotation. Soluble chromatin was diluted 5x using ChIP dilution buffer (10mM Tris-Cl, 140mM NaCl, .1% DOC, 1% Triton-X, 1mM EDTA) with protease inhibitors and added to the antibody- coupled beads with rotation at 4°C overnight. After washing, samples were treated with elution buffer (10mM Tris-Cl pH 8.0, 5mM EDTA, 300mM NaCl, 0.5% SDS), RNAase A and Proteinase K and cross-links were reversed overnight. ChIP DNA was purified using AMPure XP beads (Agencourt) and quantified using the Qubit 2000 (Invitrogen) and Bioanalyzer 1000 (Agilent).

Library Preparation and Illumina Sequencing:

Libraries for Illumina sequencing were generated following the New England BioLabsNEBNext Ultra DNA Library Prep Kit protocol. A total of 10x cycles were used during PCR amplification for the generation of all ChIP-seq libraries. Amplified ChIP DNA

was purified using double-sided AMPure XP to retain fragments ~200 to 500bp and quantified using the Qubit 2000 and Bioanalyzer 1000 before multiplexing.

ChIP-seq Data Processing:

Raw fastq reads ChIP-seq experiments were processed using FastQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) and quality reads were aligned to the hg19 reference genome using Bowtie version 1.1.2⁵⁶ with the following criteria: -m 1 --best. Duplicate reads were removed using SAMBLASTER⁵⁷. To directly compare Control and SMARCA4-induced ChIP-seq samples, uniquely mapped reads for each mark were normalized by total reads per condition to ~10 million, sorted and indexed using samtools version 1.5⁵⁸.

Identifying ChIP-seq enriched regions:

We used Model based analysis of ChIP-seq (MACS) version 1.4.2⁵⁹ peak calling algorithm with a p-value threshold of 1e-7 to identify SMARCA4 enrichment over “input” background.

Identification and Visualization of Unique SMARCA4 binding sites:

Unique SMARCA4 binding sites were identified using the concatenate, cluster and subtract tools from the Galaxy/Cistrome web based platform⁶⁰. Briefly, a shared peak set was first generated by clustering intervals of SMARCA4 -DOX peaks that directly overlapped SMARCA4 +DOX peaks by a minimum of 1bp. Unique peaks were then identified by subtracting the total number of SMARCA4 peaks in each condition by the shared peak set. To visualize ChIP-seq libraries on the IGV viewer deepTools version 2.4.0⁶¹ was used to generate bigWig files by scaling the bam files to reads per kilobase per million (RPKM).

Quantitative PCR

Reverse transcription from total RNA was performed from 2 µg of total RNA using Superscript III first-strand synthesis system (Invitrogen-Life Technologies) according to the manufacturer's instructions. Quantitative RT-PCR was performed with SYBR Green dye using an Mx3000PTM instrument (Stratagene). PCR reactions were performed in triplicate and the relative amount of cDNA was calculated by the comparative deltaCT method using the 18S ribosomal RNA sequences as a control.

Mitochondrial DNA content measurement

Total genomic DNA including mitochondrial DNA was extracted from KPS and KP tumors using Qiagen tissue genomic DNA isolation kit. Mitochondrial DNA copy number was determined using qPCR method using mouse mitochondrial DNA quantification kit from Detroit Biosciences (Cat. No. MCN3) following manufacturer's guidelines.

Immunoblotting

Cells were washed twice in ice-cold phosphate-buffered saline (PBS), scraped and collected as pellets after centrifugation at 4,000 r.p.m. for 5 min. The pelleted cells were incubated in RIPA buffer with proteinase and phosphatase inhibitors for 15 min. Lysates were then

collected and centrifuged at 14,000 r.p.m. for 15 min at 4 °C. Protein concentrations were measured using the DC Protein Assay Kit (Biorad, Cat. No. 5000111). SDS–PAGE and immunoblotting were performed as described previously in pre-cast bis-Tris 4–20% gradient gels (Invitrogen). The following antibodies were used for immunoblotting, PGC1 α (Abcam #54481, 1:1000), SMARCA4 (Abcam #110641, 1:1000), GAPDH (Cell Signaling 5174, 1:1000), Phosphoser79-Acetyl-coA Carboxylase (Cell signaling 3661, 1:1000), phospho-AMPK Thr172 (Cell signaling 2535, 1:1000), AMPK (Cell Signaling 5831, 1:1000), β -actin (Sigma A5441), and secondary HRP conjugated anti-rabbit (Thermo-Fisher, 31460) and anti-mouse (Thermo-Fisher 31430).

In vivo anti-tumor efficacy studies

1×10^5 KP or KPS tumor-derived cells were either injected into the flanks of nude female mice at 6 weeks of age (Taconic NcrNu-F). For PDX model TC388, 2mm tumor fragments were transplanted subcutaneously in NSG mice. Tumor take was monitored visually and by palpation bi-weekly. Tumor diameter and volume were calculated based on caliper measurements of tumour length and height using the formula tumor volume = (length $\times$ width²)/2. When tumors reached approximately 200mm³, mice were randomized into control and experimental groups. Control mice were treated with vehicle (0.5% methylcellulose) orally every morning on a 5-on 2-off schedule for a total of three weeks. Experimental mice were treated with IACS-010759 (7.5mg/kg) by oral gavage daily for a total of three weeks. Tumor volume and body weight were measured three times a week.

microCT tumor imaging

The mice were anesthetized using ~3% isoflurane for induction and 2% isoflurane for maintenance. The mice were quickly intubated using a 22gauge x 1inch IV catheter and secured to an animal holder. They were then transferred on the holder to the CT scanner (Precision XRay XRAD 225Cx). The mice were mechanically ventilated during the CT acquisition to allow for a breathe hold. A 20 second 3D CT image was acquired at 60KVp and 4mA with a gantry rotation of 3 rpm. During the acquisition a breathe hold of the mice was performed for 20 seconds at 20cm H₂O to fully inflate the lung and minimize motion artifacts. After the imaging procedure the mice were extubated and allowed to recover in a clean warm cage.

Lactate production

Cells grown in RPMI1640 with 10% dialyzed FBS. Media was collected and lactate secretion were measured using Trinity Lactate kit. Briefly, 200 μ l of lactate reagent were added to a 96-well plate and mixed with 10- μ l samples (media supernatant) that were diluted 1:10 in PBS. For lactate measurement, the plate was incubated for 1 h at 37 °C and absorbance was read at 540 nm. Raw data were normalized to total protein per well.

Measurement of total cellular ROS

A DCFDA cellular ROS detection assay (ThermoFisher Scientific) was used to measure total ROS activity within the cells. A total of 2.5×10^4 cells per well were seeded on a 96-well plate and allowed to attach overnight. The cells were then stained with 25 μ M DCFDA

for 45 min at 37 °C. After staining, the cells were washed and measured using flow cytometry.

Lentiviral mRNA targets

shRNA vectors were obtained from the Center for co-clinical trials (CCCT) facility at UT MD Anderson Cancer Center. The RNAi Consortium clone IDs for each shRNA are as follows: shPGC1 α #2: TRCN0000001166AAA15 A 7 TRC_1, shPGC1 α #4: TRCN0000001166AAA15 A 9 TRC_1

Compounds

IACS-010759 was synthesized by the Institute for Applied Cancer Science (IACS) of UT MD Anderson Cancer Center. IPI-9119 was also synthesized at IACS based on information from the patent WO 2011140190, A1 20111110. All other compounds were purchased from commercial vendors including DMSO (Sigma-Aldrich), rapamycin (Selleckchem), cpd-10v (Sigma-Aldrich) and n-Acetylcysteine (Sigma-Aldrich).

Primer sequences

Sequences for qPCR primers are as follows: human PGC1 α _Fw, TCTGAGTCTGTATGGAGTGACAT, PGC1 α _Rv: CCAAGTCGTTACATCTAGTTCA, 18S_Fw: cagccacccgagattgagca, 18S_Rv: tagtagcgacggcggtgtg, HIF2A_Fw: CGGAGGTGTTCTATGAGCTGG, HIF2A_Rv: AGCTTGTGTGTTTCGAGGAA.

Kits

Mitochondrial stress test (Seahorse 101706–100) and glycolysis stress test (Seahorse 102194–100) kits, lactate measurement kit (Trinity Biotech) were purchased from Seahorse Bioscience and used according to the manufacturer's instructions.

Statistical analysis

Graphpad Prism version 7.0 software was used to conduct the statistical analysis for most of the data unless expressed otherwise. For in vivo anti-tumor efficacy studies a sample size of at least n=10 mice per group was used. Differences in GEM model animal survival (Kaplan-Meier survival curves) were analyzed by Log-rank (Mantel-Cox) test. Comparisons in large scale data from public sources (TCGA) were done using two-sided Wilcoxon rank-sum test and adjusting for multiple hypothesis testing. Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) measurements were done by Seahorse XF96 and data was generated from independent experiments. Derivative values like respiration rate were computed using a Microsoft Excel Macro (Report Generator) provided by the company. Data are presented as mean $\pm$ s.d. except when stated otherwise. Comparisons between two groups were assessed by unpaired two-sided Student's *t*-test after confirming that the data met appropriate assumptions (normality and independent sampling). Comparisons between more than two groups were performed by one-way ANOVA. Unless otherwise indicated, for all *in vitro* experiments, three biological replicates were analyzed. Global gene expression profiling from GEM tumors by RNA-seq were conducted from 4 independent tumors and gene set enrichment analysis was conducted using GSEA online tool (<http://>

software.broadinstitute.org/gsea/msigdb/annotate.jsp) which generates enrichment values based on false discovery rate (fdr) corrected p-values.

Life Sciences Reporting Summary

Further information on experimental design and reagents is available in the Life Sciences Reporting Summary

Data availability

The RNA-seq, microarray and ChIP-seq data have been submitted to the Gene Expression Omnibus under accession number GSE10911 and GSE109020 respectively. Other raw data generated or analyzed during this study are included in this published article (and its Supplementary Information files). Uncut western blots are displayed in Supplementary Figures 11-13.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. *Smarca4* deficient GEMM tumors and SWI/SNF deficient human lung adenocarcinoma have enrichment of OXPHOS pathway.

a, Oncoprint plot showing the genomic profiles of *SMARCA4*, *ARID1A* and other lung cancer relevant genes, data compiled from the cBioportal^{49,50} based on original data from TCGA lung adenocarcinoma project¹. **b**, Kaplan-Meier survival curve of GEM models showing increased penetrance of KPS model (*Kras*^{LSLG12D/WT}, *p53*^{fl/fl}, *Smarca4*^{fl/fl}) compared to KP model (*Kras*^{LSLG12D/WT}, *p53*^{fl/fl}), 95% CI 1.03 to 7.33 and the non-tumorigenic *Smarca4*^{fl/fl} cohort, 95% CI 0.0028 to 0.0638. *p*-values calculated using Log-

rank (Mantel-Cox test). Both male and female mice are used. **c**, Immunohistochemistry of KP and KPS tumors indicating positive staining for pro-surfactant protein C (pro-Spc) in both KP and KPS. While heterogenous, SMARCA4 signal is primarily lost in KPS tumors. Scale bar represents 500µM in primary images and 100 µM in insets. Experiments repeated four times on independent tumor tissues. **d**, Top enriched pathways in KPS tumors compared to KP tumors based on gene-set enrichment analysis (GSEA) on RNA-seq data generated (n=4 independent tumors for each cohort). **e**, Top enriched pathways in ARID1A and SMARCA4 mutated human lung adenocarcinoma tumors revealed by GSEA analysis of TCGA RNA-seq data. n=445 for “WT” and n=70 for *ARID1A* and *SMARCA4* mutated tumors. **f**, log2 normalized expression data showing representative OXPHOS genes, ATP5L, GSTO7 and PGC1α elevated in KPS tumors. Box-plots representation: from top to bottom: maximum value, 75th percentile, median, 25th percentile and minimum values. n=4 independent tumors. **g**, log2 normalized RNA-Seq data show ATP5L and GSTO1 are elevated in SWI/SNF mutant human lung adenocarcinoma tumors. WT, n= 445, *SWI/SNF* mutated n= 70 independent tumors. *p*-values computed using two-sided Wilcoxon rank-sum test and adjusted for multiple hypothesis testing. Box-plots representation: from top to bottom: maximum value, 75th percentile, median, 25th percentile and minimum values. **h**, Immunohistochemical staining on tumor tissues using PGC1α antibody indicates elevated levels in KPS tumors. Scale bar represents 100µM in primary images and 20µM in insets. Experiments repeated three times on independent tumor tissues.

Figure 2: *SMARCA4* deficient cells have increased mitochondrial respiration.

a, PGC1α is selectively required for growth of *SMARCA4* deficient cells (left panel) as demonstrated by knockdown using two shRNAs (right panel) and rescue by *SMARCA4* expression but not by GFP. Experiment repeated three times. **b**, Plot of mitochondrial DNA content comparison between SWI/SNF deficient and proficient TCGA lung adenocarcinoma tumors based on mitochondrial DNA content across 12 tumor types²¹. *p*-values computed using two-sided Wilcoxon rank-sum test. Box-plots representation: from top to bottom: maximum value, 75th percentile, median, 25th percentile and minimum values. **c**,

mitochondrial DNA content in KPS and KP tumors (each n=5 independent tumors). Genomic DNA qRT-PCR based quantification of mitochondrial genome using beta-actin as a control for nuclear genome abundance. Bar graphs show mean and error bars denote s.d, *p*-value computed using a two-tailed Student's t-test. ** denotes significant *p*-value (*p*=0.0036). **d**, Cropped immunoblot showing inducible expression of SMARCA4 in *SMARCA4* deficient human lung cancer cell lines by administration of doxycycline (0.5µg/ml) with GFP as control. Experiment repeated three times and uncropped images available in Supplementary Fig. 11–1. **e**, Oxygen consumption rate (OCR) and extracellular media acidification rate (ECAR) were measured for each of the cell lines using the XF-96 analyzer and ratio computed. Plots show mean values from 8-wells (from 2 experiments) compared by two-sided Student's t-test. *** indicate *p*-values <0.0001. **f**, A representative trace of OCR values (mean ± s.d) from a mitochondrial stress test using XF-96 analyzer showing KP-derived cell line (red) and KPS-derived cell line (blue) from which **g**, basal respiration and spare respiratory capacity were computed. A two-sided Student's t-test computed. *** indicate *p*-values <0.0001. Graphs indicate mean and error bars denote s.d from 8 wells (from 2 experiments). **h**, proton leak and ATP production were computed from **f** and two-sided Student's t-test computed. *** indicate *p*-values <0.0001, ** indicates *p*-value=0.0073. Graphs indicate mean and error bars denote s.d from 8 wells (from 2 experiments). **i**, A representative trace of OCR values (mean ± s.d) of mitochondrial stress test using XF-96 analyzer showing H1299 parental (blue) and H1299 cell line reconstituted with SMARCA4 (red) from which **j**, basal respiration and spare respiratory capacity were computed. A two-sided Student's t-test computed. *** indicate *p*-values <0.0001 and ** indicates *p*=0.02 Graphs indicate mean and error bars denote s.d from 8 wells (from 2 experiments). **k**, proton leak and ATP production were computed from **i**. Graphs indicate mean and error bars denote s.d. from 8 wells (from 2 experiments). A two-sided Student's t-test computed. *** indicate *p*-values <0.0001

Figure 3. SWI/SNF mutant lung cancer cells are sensitive to inhibition of OXPHOS

a, Chemical structure of IACS-010759. **b & c**, CellTiter-Glo viability assays 3 days after administration of DMSO control or the indicated 8 doses of IACS-010759 across two different cohorts of 20 lung cancer cell lines repeated twice. Plots show mean and s.d. of three technical replicates. **d**, Waterfall plot of scaled GI50 values of 20 lung cancer cell lines to inhibition by IACS-010759 showing significantly more SWI/SNF mutants (blue) are sensitive than SWI/SNF wild type cells (gray), two-sided Student's t-test for difference of means $p=0.0059$ **e**, CellTiter-Glo 4-day viability assay after administration of indicated 8

doses of IACS-010759 and DMSO control in KPS and KP-derived murine cell lines showing dramatic sensitivity of KPS cells ($n=3$ independent experiments for each). Plots represent mean and s.d at each data point. **f**, Cell viability rescue experiment with A549, H1299 and H2030 that were reconstituted with SMARCA4 in an inducible manner by addition of doxycycline in growth media ($0.5\mu\text{g/ml}$). IACS-010759 (10 or 100nM) or control DMSO was added to confluent plates, cells were incubated for 24–48 hours, fixed and were stained with crystal violet. Experiment was repeated two times. **g**, In vivo anti-tumor efficacy of IACS-010759 was assessed by treating Ncr-nude mice ($n=15$ mice per cohort) subcutaneously implanted with 100,000 cells derived from KPS or KP tumors. IACS-010759 was administered by oral gavage at 7.5mg/kg in methylcellulose carrier once daily for a total of three weeks. Tumor volume was monitored three times per week and plotted as mean $\pm$ s.e.m. Vehicle is methylcellulose carrier alone. **h**, In vivo anti-tumor efficacy of IACS-010759 was assessed by treating NSG mice ($n=10$ mice per cohort) subcutaneously implanted with PDX model TC388. IACS-010759 was administered by oral gavage at 7.5mg/kg in methylcellulose carrier once daily for 7 days for a total of three weeks. Tumor volume was monitored three times per week and plotted as mean $\pm$ s.e.m. Vehicle is methylcellulose carrier alone.

Figure 4. SMARCA4 is required for efficient transcriptional response to energy stress.
a, A representative trace of ECAR values (mean+/- s.d) from a glycolytic stress test using XF-96 analyzer and quantification showing reduced glycolysis and glycolytic capacity in *SMARCA4* deficient H1299 cells. Bar graphs indicate mean and error bars denote s.d. from 8 wells (from 2 experiments). A two-sided Student's t-test computed. *** indicate p-values <0.0001 **b**, A representative trace of ECAR values (mean+/- s.d) from a glycolytic stress test using XF-96 analyzer and quantification showing reduced glycolysis and glycolytic capacity in *SMARCA4* deficient A549 cells. Bar graphs indicate mean and error bars denote

s.d. from 8 wells (from 2 experiments). A two-sided Student's t-test computed. *** indicate p-values <0.0001 and ** indicates p=0.0011 **c**, Left panel: mRNA expression of Hif2a by qPCR in H1299 parental and SMARCA4 reconstituted cell lines grown under regular growth media (RPMI-1640 with 1500mg/ml glucose, 10% FBS) or no glucose added media (RPMI-1640 -Glucose, 10% FBS). Results show significantly higher basal as well as induced Hif2a by glucose deprivation upon SMARCA4 expression. Graph shows mean $\pm$ s.d. of HIF2A mRNA first normalized to GAPDH and then compared between the SMARCA4 deficient and proficient groups. ** *P*-value < 0.0001 by two-sided Student's t-test, n=3 independent experiments. Right panel: mRNA expression of Hif2a by qPCR in H1299 parental and SMARCA4 reconstituted cell lines treated with DMSO control or 100nM IACS-010759 for 24 hours. Results show significantly higher basal as well as induced Hif2a by IACS-010759 upon SMARCA4 expression. ** *P*-value < 0.0001 by two-sided Student's t-test. Graph shows mean $\pm$ s.d. of HIF2A mRNA normalized to GAPDH. n=3 independent experiments **d**, GSEA analysis of microarray transcriptomic profiling revealed response to hypoxia and glycolysis as top pathways enriched in response to IACS-010759 treatment in SMARCA4 reconstituted cells, n=2 independently cell culture grown cells were profiled by microarray for each treatment condition (-Dox, +Dox each with DMSO control or IACS-010759 treatment) **e**, GREAT (Genomic Regions Enrichment of Annotations Tool)⁵¹ was used to perform gene set enrichment analysis on genomic regions occupied by SMARCA4 in H1299 cells stably expressing doxycycline inducible SMARCA4 vector. Cells were treated (+Dox) or not (-Dox) with doxycycline (0.5 μ g/ml) for 5 days to induce SMARCA4 expression and with 100nM IACS-10759 for 24 hours to induce energy stress, n=6,072 genomic regions were used to generate enrichment plot. **f**, Representative integrative genome viewer tracks of views of SMARCA4 ChIP-Seq peak on the HIF2A genomic locus and **g**, hexokinase 2 genomic locus in H1299 cells re-expressing SMARCA4. ChIP-Seq experiment was done once for the -Dox and +Dox pair independently.

Figure 5. SMARCA4 deficient cells and tumors have elevated bioenergetic requirement
a, Enrichment plot of reverse phase protein array (RPPA) data showing significantly reduced expression of pACC Ser79 (red arrow) in SWI/SNF mutant lung adenocarcinoma tumors (TCGA). Data compiled by cBioportal, n=181 independent tumors. Two-side t-test performed to derive the p-value. **b**, Cropped immunoblot showing markedly reduced phospho-ACC S79 signal from total cell lysates of control and SMARCA4 inducibly expressing H1299, H2030 and H2023 cells (induction of SMARCA4 expression by 0.5μg/ml of doxycycline). Experiment repeated three times and uncropped images available

in Supplementary Fig.11–2. **c**, Viability assay of H1299 parental and SMARCA4 reconstituted cells treated with different doses of IACS-010759 (100, 30 or 10nM) alone or in combination with the fatty acid synthesis inhibitor Cpd-10V (10μM). Results were compared to DMSO control treated cells (set at 100%) and show partial rescue of growth inhibition at each dose by Cpd-10v. Graph represents mean $\pm$ s.d, of n=4 independent cell culture grown and treated cells, Two-sided Student's t-test used to derive p values. **p=0.0007, **p=0.0004 and **p=0.0011 for 100, 30 and 10nM groups respectively **d**, Viability assay of H1299 parental and SMARCA4 reconstituted cells treated with different doses of IACS-010759 (100, 30 or 10nM) alone or in combination with the mTOR inhibitor rapamycin (0.1, 1 or 10nM). Results were compared to DMSO treated cells (first black bar, set at 100%) and show significant rescue of growth inhibition in a dose dependent manner by rapamycin. Rapamycin alone did not affect cell growth compared to DMSO. Graph represents mean $\pm$ s.d, of n=3 independent cell culture grown and treated cells, Two-sided Student's t-test used to derive p values. ** for 10nM IACS-10759 group p<0.0001, p=0.007 and p<0.0001 for 0.1, 1 and 10nM rapamycin treated cells respectively. ** for 30nM IACS-10759 group p=0.06, p=0.0002 and p=0.0045 for 0.1, 1 and 10nM rapamycin treated cells respectively. ** for 100nM IACS-10759 group p<0.0001, p<0.0001 and p=0.0005 for 0.1, 1 and 10nM rapamycin treated cells respectively. **e**, A working model: *SMARCA4* deficient tumors have an energy imbalance resulting from increased OXPHOS, decreased glycolysis and increased anabolic processes. The cumulative result is energy stress that creates an attractive therapeutic window to target OXPHOS.