Taming the Master: SWI/SNF chromatin remodeller as a therapeutic target in cancer

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Eukaryotic cells use histone modifiers and chromatin remodellers to facilitate protein DNA interactions in the nucleus; an important requisite for regulating several cardinal nuclear processes including transcription, replication, DNA repair and recombination, etc. The SWI/SNF complex is the most well-studied chromatin remodeller and is conserved from yeast to mammals. The complex is recruited to specific DNA sites, where it uses energy from ATP hydrolysis to catalyse nucleosome sliding or histone eviction from DNA. Mutational inactivation of SWI/SNF components has been identified in neurological syndromes and in several cancers. Recent deep sequencing studies have revealed a SWI/SNF mutation frequency of 20% in cancer genomes. In addition to mutations in tumour samples, extensive studies on cell lines and animal models have revealed tumour suppressive features for many individual SWI/SNF components. Thus, components of the complex are classified as tumour suppressors. Interestingly, however, majority of mutations cause incomplete inactivation of the complex, leaving behind a 'residual' complex that can be targeted for therapy. In addition, characterization of multiple roles of SWI/SNF components has revealed several therapeutic options. The current review summarizes the multi-faceted therapeutic opportunities for tumour bearing mutations in genes, encoding SWI/SNF components.

Keywords: ARID1A, chromatin remodeller, SWI/SNF, therapeutic targeting.

Importance of the work

EFFORTS are ongoing for the past few decades to develop efficient therapeutic options for cancers, where conventional treatments including surgery and chemo/radiation have failed miserably. It is now well-understood that tumours harbour genetic lesions that are essential for their sustenance and therefore provide an attractive target for therapy. An alternative approach is to target specific weaknesses in tumour cells that arise due to mutations in specific genes. This review attempts to discuss several such vulnerabilities that may exist in tumour cells harbouring mutations in genes encoding components of the SWI/SNF chromatin remodelling complex.

The nucleus of a human cell harbours approximately 2 m long double-stranded genomic DNA containing 3.3 billion base pairs that are highly compacted to fit inside a 5–10 micron nuclear compartment. To achieve this density, DNA is condensed and tightly packed into a structure called chromatin. Nucleosome, the basic unit of condensed chromatin, includes 146 base pairs of DNA tightly wrapped around a specialized protein structure formed by an octamer of histone proteins. Each histone octamer is composed of two copies each of histones H4, H3, H2A and H2B. The wrapped DNA contacts the histone octamer through various types of noncovalent interactions. The nucleosomal structure provides stability and compactness besides facilitating protection of the genome. However, this arrangement places a huge constraint on proteins that depend on sequence-specific binding to DNA for regulating various nuclear processes such as replication, transcription, DNA repair, recombination, etc.[1,2]. Hence, the chromatin state has to be dynamic, with an inherent ability to switch between a tightly packed and a more 'loosened' state, the latter facilitating binding of proteins to DNA.

Eukaryotic cells employ several mechanisms to covalently modify histones and/or DNA to regulate spatio-temporal accessibility of proteins to DNA. In addition, energy-dependent processes are put in place to facilitate a 'loose' or 'open' chromatin structure, catalysed by chromatin-remodelling enzymes. Histone modifying enzymes catalyse an array of post translational modifications on the C-terminal tail of histones-enabling a change in the active state of chromatin by the recruitment of specific effector proteins. Chromatin remodelling enzymes, on the other hand, catalyse the uncoupling of DNA–histone contacts through nucleosome sliding, eviction, or replacement of canonical histones with histone 'variants' in an ATP dependent manner, thereby exposing underlying DNA sequences (enhancers, promoters, replication origins, sites of DNA recombination or damage) to sequence specific regulators[3]. The ATP-dependent chromatin remodelling enzymes are remarkably diverse and genome-wide binding studies have revealed about 20–40,000 sites[4], indicating the huge abundance of complexes in the cell. A typical chromatin remodeller is a multi-subunit

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Table 1. SWI/SNF proteins from human, drosophila and yeast

Human		Drosophila		Yeast	
BAF	pBAF	BAP	PBAP	SWI/SNF	RSC
BRG1/BRM	BRG1	BRM/Brahma		SWI2/SNF2	Sth1
ARID1A/ ARID1B	ARID2	OSA	BAP170	SWI1/Adr6	Rsc9
	BAF180/ PBRM1		Polybromo		Rsc1,2,4
	BRD7				
INI1/BAF47/SNF5		BAP45/SNR1		Snf5	Sfh1
BAF60a,b,c		BAP60		Swp73	Rsc6
BAF155/170		MMOIRA/BAP155		SWI3	Rsc8/Swh3
beta Actin		Actin		Arp 7,9	
BAF57		BAP111			
BAF53 a,b		BAP55/BAP47			
BAF45 a,b,c,d					

assembly consisting of a core ATPase subunit that uses ATP hydrolysis to mobilize nucleosomes. In addition, several (up to 20) non-catalytic protein subunits may be present to provide structural stability to the complex, as well as to impart specificity through interactions with other proteins or complexes. ATP-dependent chromatin remodellers identified till date are categorized into four families based on shared structural or functional domains: (1) SWI/SNF (switch/sucrose non-fermenting), (2) ISWI (imitation-switch), (3) NURD/CHD (chromodomain helicase DNA-binding) and (4) INO80 (inositol-requiring 80). The four complexes function in a non-redundant manner to influence discrete DNA dependent molecular functions. Dysfunction of chromatin remodellers is strongly associated with various developmental defects. More importantly, genetic alterations or aberrant expressions of chromatin remodellers have been identified as possible oncogenic driver in numerous cancer types.

The multi-subunit SWI/SNF chromatin remodelling complex

The SWI/SNF complex was the first ATP-dependent chromatin remodeller to be identified in the budding yeast *Saccharomyces cerevisiae* from two independent genetic screens and is the most well characterized till date. The screens revealed genes as transcription co-activators required for regulating mating type switching (SWI)[5] and sucrose fermentation (sucrose non-fermenting/SNF)[6]. Biochemical purification studies showed that the encoded proteins numbering about 9–12, physically associated to form a relatively large multi-subunit complex that exhibited ATP-dependent nucleosome remodelling activity *in vitro*[7,8]. Further studies led to identification of additional evolutionarily conserved homologous complexes in *S. cerevisiae* (the RSC complex)[9]. More importantly, a screen for identifying genes opposing the activation of homeobox genes by the polycomb complex in *Drosophila melanogaster* yielded similar complexes (BAP and PBAP)[10,11] and further studies identified counterparts in mammals (the BRG1/BRM – associated factor (BAF) complex[12] and the polybromo BRG1 – associated factor (pBAF) complex)[13] (Table 1).

The mammalian SWI/SNF complex is the primary chromatin remodeller for almost all stages of embryonic-development[14] as well as for differentiation of various cell types during adult life[15–17]. Various proteins that constitute the mammalian SWI/SNF complex can be classified into three categories, namely (a) enzymatic subunits having catalytic ATPase activity including the Brahma homologue (BRM, encoded by *SMARCA2*) and BRM-related gene 1 (BRG1, encoded by *SMARCA4*), (b) set of highly conserved 'core' subunits (SNF5 (also known as, INI1 or BAF47 encoded by *SMARCB1*), BAF155 and BAF170) and (c) accessory subunits that may contribute towards complex specificity (ARID1A/ARID1B, ARID2, etc.). Recently, a third SWI/SNF complex called non-canonical BAF (ncBAF, also termed GBAF) was identified, that contains GLTSCR1/GLTSCR1L instead of the ARID-domain containing proteins[18,19].

Only a set of four subunits (BRG1/SMARCA4,SNF5/SMARCB1, BAF155/SMARCC1 and BAF170/SMARCC2) in humans is necessary and sufficient to perform chromatin remodelling *in vitro*[20]. However, additional subunits may play a crucial role in binding of complex with chromatin (for example, recognition of acetylated-lysine by components harbouring the Bromo domain) or with transcription factors. Targeting of the complex to specific gene loci is facilitated by the heterogeneity in subunit composition that can manifest differently in different tissues or at different cell states in the same tissue.

Diversity in mammalian SWI/SNF complex composition ensures varied functions

The size of the BAF complex is estimated to be about 2 MDa making it one of the largest protein complexes in the mammalian cell. The mammalian complex is

substantially more diverse and multifaceted than the one initially discovered in yeast as it is encoded by at least 29 different genes, suggesting the possibility of assembling a multitude of complexes through combinatorial association. BRG1 or BRM, the two subunits that exhibit ATPase activity, are always present in different SWI/SNF complexes. In addition, the complex comprises three mutually exclusive DNA binding subunits, namely the AT-rich interaction domain 1A (ARID1A/BAF250a), ARID1B (BAF250b) or ARID2. ARID1A/ARID1B constitute disjoint components (paralogs) of the BAF (BRG1/BRM associated factor) complex[12] whereas ARID2 (BAF180) is a component of the pBAF complex[13], so named because of the presence of the exclusive PBRM1 subunit[11]. In addition, BRG1 can be present in both BAF and pBAF complexes while BRM is found only in the BAF complex[21]. The recently identified ncBAF complex harbours either GLTSCR1 or GLTSCR1L instead of ARID1A/ARID1B or ARID2 besides either of the two ATPase subunits BRG1 or BRM. The SWI/SNF complex can therefore be broadly classified into three types, namely BAF, pBAF and ncBAF. Several components of the SWI/SNF are encoded by gene families, whose protein products are known to assemble during complex formation in a mutually exclusive fashion despite exhibiting significant similarity in their respective amino acid composition. The general rule for differential complex formation therefore appears to be that, specific position of a subunit in the complex is occupied by any one of the family members (paralogs; position of BAF60 for example, can be occupied by any one of three alternatives BAF60a, b, or c). The mutual exclusivity in SWI/SNF subunit association is touted as a major mechanism that could in principle give rise to a variety of distinct complexes with differential activities. The alternating subunits cause differential activity as they perhaps project different interacting motifs. Indeed, the subunits of SWI/SNF are known to have various protein–protein and protein–DNA interaction domains including the LXXLL motif, the PHD domain, chromodomains, binding domains that recognize specific DNA structures or AT-rich sequences and domains important for recognizing histone marks (like the Bromodomain). The diversity in SWI/SNF subunit composition may mediate the interaction with specific transcription factors and/or modified histones allowing the recognition of specific target gene sets to regulate distinct functions. The ability of BRG1 and BRM for example, to have distinct transcriptional specificity despite an overall amino acid identity of ~70% is due to N terminal amino acid sequence variation which allows the interaction with distinct transcription factors[22]. Similarly, ARID1A and ARID1B are mutually exclusive paralogous subunits sharing 60% amino acid sequence identity but regulate distinct functions[23,24]. The recently identified ncBAF/GBAF complex harbours BRD9, a paralog of the bromodomain containing BRD7, that regulates gene sets distinct from that of other BAF and pBAF complexes[18,25]. Re-configuration of SWI/SNF composition has been shown to be important during developmental processes, especially for lineage specification. For example, the BAF53A and BAF45A subunits are replaced by BAF53B and BAF45B/BAF45C respectively, during differentiation of neural stem cells into mature neurons[26]. Similarly, SWI/SNF containing BAF60a (encoded by *SMARCD1*) promotes transcription of *Oct4* and *Sox2* (ref. 4), while the complex harbouring BAF60b (encoded by *SMARCD2*) regulates granulocyte differentiation[27] and the complex comprising BAF60c (encoded by *SMARCD3*) promotes cardiac and skeletal muscle development[28].

SWI/SNF in disease

The SWI/SNF complex plays an essential role in epigenetic regulatory mechanisms that impact several developmental processes[1]. Given the fundamental nature of SWI/SNF function, it is but natural to expect the malfunctioning or loss of its subunits to be associated with disease conditions. Of the four distinct mammalian chromatin remodellers (SWI/SNF, ISWI, CHD, INO80), inactivation of SWI/SNF subunits is strongly implicated in various diseases. Indeed, mutations and other forms of genetic lesions in genes encoding SWI/SNF components cause various familial syndromes, multi-system developmental disorders especially related to neuro-development and several types of cancers.

Neuro-developmental disorders

The SWI/SNF orchestrates various pre- and post-natal neural developmental events besides performing a cardinal role in the proliferation of neuronal stem cells[29]. More importantly, conditioned ablation of BAF155 and BAF170 core subunits causes severe defects in the development of forebrain and related structures[30], as well as defects in cerebral cortical size and structure[31] in mice. Based on these observations, alterations and mutations in its components can be expected to cause neuro-developmental defects. Indeed, mutations in several components of the SWI/SNF complex have been reported in intellectual disability syndromes[8,9]. Inactivating mutations in ARID1A, ARID1B, ARID2, SMARCA2, SMARCA4, SMARCB1 and SMARCE1 were reported in Coffin–Siris syndrome[10]. Similarly, missense mutations in SMARCE1, SMARCC1 and SMARCC2 were identified in autism spectrum disorders[11,12] and mutations in SMARCA2 were detected in patients with Nicolaides-Baraitser syndrome[32]. During the past decade, mutations in several genes encoding SWI/SNF components have been described to be causal to neurological syndromes; the information is summarized in Table 2.

Table 2. Role of SWI/SNF components in neurological disorders

Gene name	Mutation type	Syndrome/symptoms	Reference
ARID1A	Nonsense, frameshift, indel	Coffin–Siris syndrome	127
ARID1B	Translocation, frameshift, indel, nonsense, missense, microdeletion	Coffin–Siris syndrome, autism, Nicolaides–Baraitser syndrome, schizophrenia, Hirschsprung's disease, Intellectual disability	128–131
ARID2	Frameshift, indel	Coffin-Siris syndrome	132
SMARCA2	Partial deletion, missense, intronic alteration, duplication	Coffin-Siris syndrome, Nicolaides–Baraitser syndrome schizophrenia	133–135
SMARCA4	Partial deletion, missense	Coffin–Siris syndrome, autism	127, 136
SMARCB1	In-frame deletion, missense	Coffin-Siris syndrome, Kleefstra syndrome phenotypic spectrum	127, 137, 138
SMARCE1	Missense	Coffin-Siris syndrome	139–141
SMARCC1	Missense	Autism, social deficits and communication difficulties, restricted and repetitive behaviours	142
SMARCC2	Splice site mutation	Autism, social deficits and communication difficulties, restricted and repetitive behaviours	142
PBRM1	Missense	Autism, social dficits and communication difficulties, restricted and repetitive behaviours	143
BCL11A	Micro deletion, missense and frameshift mutations	2p15–16.1 microdeletion syndrome; autism; schizophrenia	136, 144
ATRX	Missense	ATRX syndrome	145
SMARCAL1	Deletion, missense, nonsense and frameshift mutations	Schimke immuno osseus dysplasia	146

Human cancers

More than eleven genes coding various components of the SWI/SNF have been reported to be altered in various cancers (Table 3). The alterations include missense mutations, gene fusions, copy number variations, epigenetic silencing and even overexpression in some cases. The association of SWI/SNF complexes with cancer was first reported in the late 1990s; bi-allelic inactivation of *SMARCB1* was identified in the lethal childhood sporadic cancer called Rhabdoid tumours (RT)[33–35]. Germline inactivation of *SMARCB1* predisposed patients to RTs[36] and a second somatic mutation resulted in full-blown disease. The cardinal role of *SMARCB1* in causing RT was confirmed in mice studies; mono-allelic inactivation or conditional ablation causes aggressive rhabdoid-like tumours and T-cell lymphomas[17,18], whereas a bi-allelic loss resulted in rapid embryonic lethality (as early as 3.5 to 5.5 days post-coitum)[16]. Various types of genetic lesions that inactivate genes encoding various SWI/SNF components including ARID1A, BRG1, ARID1B, BRM, etc. in a wide variety of cancers are described below.

Mutations

Deep sequencing studies performed over the past decade or so have revealed frequency of mutations in SWI/SNF components in human cancers to be about 20% (refs 37 and 38). We analysed the mutation frequency of SWI/SNF in various cancers from the MSK-IMPACT Clinical Sequencing Cohort[39]; the complex was found to be altered in 2150 of 10336 (19.6%) tumour samples, accessed through the cBioPortal (Figure 1), inferring that proper functioning of the polymorphic BAF and pBAF complexes could be a major mechanism of tumour suppression. The various components of SWI/SNF complex reported to be mutated or altered in human cancers are listed in Table 3. Of the many components, *ARID1A*, *SMARCA4* and *SMARCB1* exhibit a significantly higher frequency of mutation in cancers. In addition, several subunits exhibit high frequency of mutational inactivation in specific cancer types while harbouring very low or nil mutations in other cancers. For example, *SMARCB1* and *PBRM1* exhibit a mutation frequency of 95% and 40% in Rhabdoid tumours and renal clear cell carcinoma respectively, but are rarely mutated in other cancers[40]. Unlike classical homozygous tumour suppressor events, most SWI/SNF somatic mutational events are mono-allelic indicating a dosage sensitivity or a dominant effect. Only exception is the bi-allelic inactivation of *SMARCB1* in RTs, wherein the effect is akin to a classical tumour suppressor.

Epigenetic silencing

RTs are characterized by a remarkable genetic stability as the tumours exhibit a very low mutation rate; the primary mechanism of carcinogenesis appears to be loss of epigenetic control mediated by the SWI/SNF on the *CDKN2A* tumour suppressor loci. Thus, RT is perhaps one of the first examples of a cancer arising purely due to a genetic defect that manifests at the epigenetic level. In addition to inactivating mutations, other underlying mechanisms, such as epigenetic silencing through nucleosome remodelling, methylation of histones and DNA, also contribute to the inactivation or loss of function of chromatin remodellers in human cancer. For example, the BRM gene is inactivated in adult lung[41], breast, ovary, bladder and esophagus cancers, and pediatric rhabdoid tumours

Table 3. Role of SWI/SNF components in cancers

Gene name	SWI/SNF component	Cancer
SMARCB1	BAF47	Rhabdoid tumours, epitheloid tumours, schwnnomatosis multiple meningiomas
SMARCE1	BAF57	Clear cell meningioma
BRD9	BRD9	Acute myeloid leukemia
ARID1A	BAF250A	Clear cell ovarian, endometrial, colorectal, stomach, and bladder cancers
ARID1B	BAF250B	Colorectal, pancreatic and breast cancer and neuroblastoma
BRG1	SMARCA4	Non-small-cell lung carcinoma, Burkitt's lymphoma, childhood medulloblastoma, pancreatic adenocarcinoma, ovarian clear cell carcinoma and melanoma
BRM	SMARCA2	Lung, breast, ovary, bladder and esophagus cancers and pediatric rhabdoid tumours
BAF60a	SMARCD1	Breast
BRD7	BRD7	Breast cancer, Nasopharyngeal carcinoma
ARID2	BAF200	Melanoma, hepatocellular carcinoma
PBRM1	BAF180	Clear cell renal cell carcinoma, pancreatic cancer
SMARCAL1	SMAL1	Schimke immuno osseus dysplasia
SS18:SSX	SS18	Synovial sarcoma

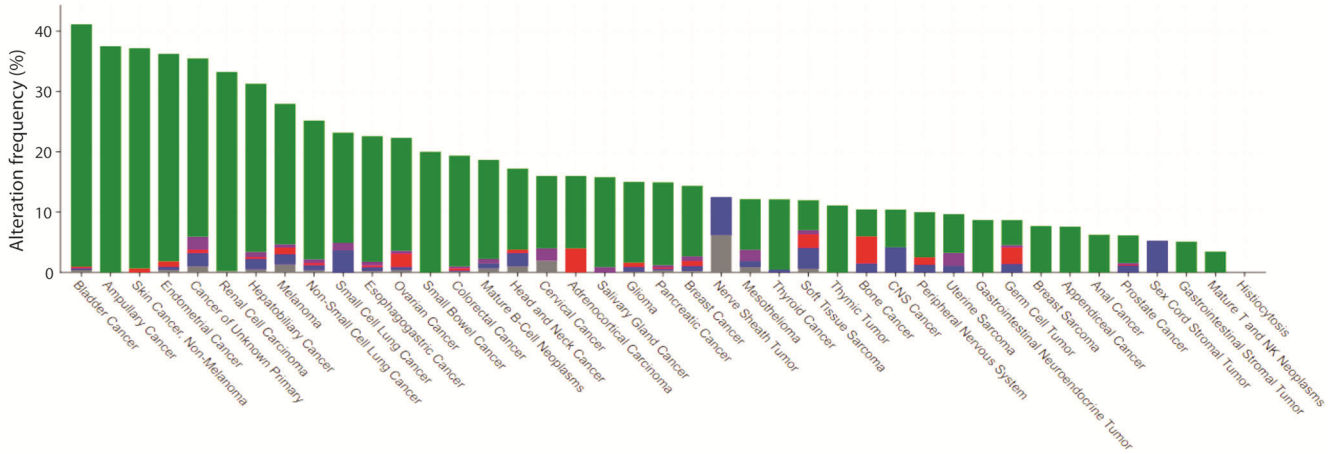

Figure 1. Frequency of SWI/SNF inactivation in various cancers. The mutation frequency was determined from cBioPortal as described in the text. Colour code: green, mutation; red, amplification; blue, deep deletion; purple, fusion; grey, multiple alterations.

through epigenetic silencing by DNA methylation, promoter mutations and other non-mutation modes[42]. In addition, BAF250B exhibits significantly reduced or loss of expression in pancreatic cancer cell lines[43] because of promoter hyper methylation.

How does SWI/SNF perturbation drive oncogenesis?

As mentioned above, the SWI/SNF family of chromatin remodellers regulate several cardinal cell processes including transcription, DNA repair and recombination, cell cycle, cell death or survival, differentiation and genomic stability. These could be a direct effect of faulty remodelling of chromatin or an indirect effect of deregulated expression of target genes. Since these processes are expected to be cardinal for tumour suppression and given that SWI/SNF appears to have an extensive footprint across the mammalian genome, it is likely to be involved directly or indirectly in several tumorigenic processes. Earlier studies have elucidated SWI/SNF-directed gene expression programs regulating specific pathways and how mutation in SWI/SNF components promotes cancer. SWI/SNF was shown to interact with both Rb (a tumour suppressor)[44] and MYC (an oncogene)[24,45] leading to perturbations of genes controlled by these master regulators. The complex also binds to and regulates transcriptional program controlled by p53 (ref. 46). In fact, p53 appears to depend on interaction with SWI/SNF components BAF60a/SMARCD1 (ref. 47), SNF5/INI1 (ref. 48), ARID1A/SMARCF1 (ref. 49) and BRG1/SMARCA4 (ref. 50) to activate its target genes. Thus, SWI/SNF appears to be an important component of the tumour suppressor function performed by p53. Several reports revealed multi-faceted effect of SWI/SNF on cell cycle progression. Through its interaction with Rb, SWI/SNF regulates transcription of p16 and E2F targets[51]. SWI/SNF induces expression of additional cell cycle regulating and senescence inducing genes such as p21 (refs 43, 52, 53) and p53 (ref. 53) besides being an important mediator of the cell cycle regulation performed by TGF-β signaling[54,55].

A major role of SWI/SNF in regulating tumorigenesis appears to emanate from its antagonistic action on the PRC2/EZH2 (Polycomb group) complex[56]. One of the strongest associations of a SWI/SNF component with tumorigenesis is that of SMARCB1/INI1/SNF5 with RTs. Interestingly, the mechanistic link of SMARCB1 inactivation with tumorigenesis appears to act primarily through antagonism with PRC2/EZH2 mediated histone H3K27 tri methylation[57]. Several additional mechanisms including activation of cyclin D1, perturbation of sonic hedgehog and Wnt signalling have also been proposed[57,58]. Loss of BRG1 promotes tumour aggressiveness in primary and established lung cancer cell lines through perturbations in global chromatin architecture especially in gene loci pertinent for the disease[59].

Though initial studies focused on evaluating the role of SWI/SNF at or near promoters to facilitate transcription initiation by RNA polymerase II[60], work during the past few years has provided evidence for their requirement at enhancers especially pertaining to their role in cancers. SWI/SNF appears to bind to and recruit CBP/p300 histone acetyl transferase at regular (not super) enhancers especially related to genes important for lineage specification[61,62] and the complex also regulates enhancers that function to establish and maintain cell identity[63,64]. The enhancer regulation property of SWI/SNF appears to play a major role in tumorigenesis as well. Genetic knockout of ARID1A in the mouse colon causes colon cancers through loss of ARID1A containing SWI/SNF activity on enhancers. ARID1B containing complexes, however, maintain activity of a distinct set of enhancers[65], probably required for tumour sustenance. Similarly, RTs caused by SMARCB1 also emanate from a loss of function of enhancers related to lineage-specific function but not of super-enhancers whose activity is essential for survival of the tumour[66]. Thus, loss of SWI/SNF leads to de-activation of enhancers driving lineage and differentiation-related genes, perhaps resulting in de-differentiation and a proliferative phenotype.

The SWI/SNF pBAF complex is known to play a role in regulating repair of DNA double-strand breaks by promoting transcription repression and this activity is dependent on Ataxia telangiectasia mutated (ATM)[67]. More recently, it was shown that inhibition of pBAF mediated transcription repression led to increased chromosomal re-arrangements suggesting a possible role in promoting tumorigenesis[68].

Therapeutic options in tumours harbouring genetic lesions in SWI/SNF components

About two decades ago, several laboratories began to work towards developing targeted therapies given the poor success achieved with conventional therapies like radiation and chemotherapy. Years of research has yielded targeted therapies with significant success against oncogenic events in specific cancers such as the HER2 amplification in breast cancer (Herceptin/Trastuzumab)[69], oncogenic gain of function mutation in *EGFR* (lung cancer)[70–72], *ALK* gene rearrangements (also in lung cancer)[73], and a few others. Despite the initial success, most targeted therapies encountered resistance in a significant proportion of patients and addressing this issue is a major research focus in laboratories the world-over. In addition, efforts were also initiated towards other modes of targeted therapies. One such approach focused on determining vulnerabilities in tumour cells arising out of specific genetic lesions.

Targeting vulnerabilities arising from inactivation of tumour suppressive SWI/SNF

As already described, inactivating mutations in a SWI/SNF component appear to occur in about 20% of all cancers. It was initially thought that genetic lesions in different SWI/SNF components in different cancers had a similar effect on tumorigenesis emanating from inactivation of SWI/SNF function. However, additional observations provided evidence against identical action of various mutations. First, ablation of each component is usually associated with one or a few unique cancer types. For example, loss of *ARID1A* is common in ovarian cancer[74,75], that of *SMARCA4* in lung cancer[76] and of *SMARCB1* in RTs[77]; this specificity of individual SWI/SNF subunits has also been validated in mouse models[35,78]. Secondly, mouse knockout studies revealed a distinct phenotype for each SWI/SNF component tested. For example, *SMARCB1* and *SMARCA4* knockouts were embryonic lethal[79,80], whereas that of *SMARCD3* resulted in abnormal cardiac development[81].

Inactivation of one SWI/SNF component/complex generates synthetic lethality in another

Wang and colleagues mooted the idea whether the differential behaviour emanating from inactivation of each component could result from differential activity of the residual complex which in turn could be crucial for tumour cell survival[82]. They tested this hypothesis by ablating *SMARCA4* (which encodes the ATPase subunit BRG1) in the background of *SMARCB1* loss and the results indeed revealed a dependency of *SMARCB1* ablated cells on *SMARCA4*. Thus, *SMARCA4* loss is synthetic lethal with *SMARCB1* ablation and can be utilized as a therapeutic target. Though the authors proposed that the residual BAF complex could be important for oncogenic potential of the tumour cell, it is possible that this dependency was due to action of the pBAF complex that incorporates BRG1 as the only ATPase subunit; a possibility not considered by the authors. Nevertheless, this observation plus the fact that: (a) previous studies showed

that the complex could be assembled even in the absence of one component[83,84] and (b) the SWI/SNF complex is the most important chromatin remodeller during ontogeny and adult life, led to several initiatives directed towards identifying genes that could be synthetic lethal with other inactivated SWI/SNF components. In such a screen, a cancer cell line harbouring inactivation of one SWI/SNF component is used to generate a library of clones each harbouring inactivation of one additional gene; the clones are evaluated for any one of several tumorigenic characteristics (such as survival, growth rate, viability, cell motility/migration, etc.). These screens interestingly revealed a synthetic lethal dependency of *ARID1A* mutant cells on *ARID1B* (loss of one ARID domain BAF subunit makes the cells dependent on the remaining one)[85] as well as of *SMARCA4* inactivated cells on *SMARCA2* (loss of one ATPase subunit makes the cells dependent on the remaining one)[84,86]. These results point to the fact that ablation of one SWI/SNF subunit does not de-activate the whole complex as thought earlier, but the remaining complex appears to perform some function crucial for sustenance of the tumour cell. Interestingly, a large-scale proteomic analysis following individual subunit deletion in yeast did reveal presence of active residual SWI/SNF complexes[87], further supporting the results obtained in humans.

A more recent study revealed cancers with perturbation in BAF components to be dependent on ncBAF[19], thus highlighting a synthetic lethal relationship between two distinct SWI/SNF complexes as against paralogs of the same complex. Perhaps perturbation of a core component (for which no paralog is available) makes the cell dependent on a distinct SWI/SNF complex. One common theme that has emerged from these studies, however, is that SWI/SNF complex is essential for tumour maintenance and thus the residual complex offers an excellent therapeutic option. Of course, the therapeutic target in the residual complex will differ depending on the tumour type as well as identification of the inactivated SWI/SNF component.

Vulnerabilities in PRC1/2

Another approach to identify therapeutic targets in tumours harbouring SWI/SNF inactivation is based on the Polycomb Repressive Complexes (PRC1/PRC2), categorized under Polycomb Group (PcG) of proteins, that exhibit histone methyl transferase enzymatic activity which is antagonistic to the SWI/SNF and can be expected to exhibit augmented oncogenic activation in the background of SWI/SNF inactivation. Initial studies performed in Drosophila provided the first evidence for an antagonistic relationship between the SWI/SNF and PRC2 complexes. During initial stages of Drosophila development, the rapid burst of cell proliferation is primarily driven by the activation of PcG proteins that includes the PRC1/PRC2 complex. These complexes use their inherent histone methyl transferase activity to transcriptionally silence genes belonging to the Homeobox (HOX) family. During the later stages of development, a reduction in proliferation and initiation of differentiation is triggered by the activation of the Trithorax group of proteins (including members of the SWI/SNF complex) which inhibit PRC2 inducing thereby the expression of HOX genes[88]. The antagonistic relationship between the two complexes has been validated in mammals as well[56,89]. Based on this knowledge, it was hypothesized that RTs that arise due to inactivation of *SMARCB1* could be critically dependent on activity of the PRC2 complex; the hypothesis was tested and successfully validated by applying an EZH2 synthetic inhibitor in a mouse xenograft model for malignant rhabdoid tumours[90]. Similar efforts were undertaken for tumours harbouring inactivation of other SWI/SNF components. For example, ARID1A mutated ovarian cancer cells were found to be synthetic lethal for EZH2 inhibition in cell lines and mouse models[91,92]. More importantly, it seems fair to assume that tumours developing due to inactivation of a SWI/SNF component might be dependent on PRC2 proteins for their sustenance. However, whether this can be applicable to a broad range of cancer types is an open question. Of note, there are intrinsic differences in the PRC2-SWI/SNF antagonism between Drosophila and humans; the Drosophila–BAF complex inhibits action of Polycomb proteins at the HOX locus[93] whereas in humans, both complexes work synergistically to place the suppressive histone H3K27 trimethylation mark on the HOX loci[94].

An interesting link between BRG1 and EZH2 was revealed in non-small cell lung cancer. Tumours harbouring genetic inactivation of BRG1 were sensitive to EZH2 inhibition that resulted in increased susceptibility to TopoII inhibitors[95]. However, there is a complete reversal of the response in BRG1 wild type tumours which become resistant to TopoII inhibition upon EZH2 ablation[95]. Therefore, BRG1 status appears to be an important biomarker to predict response to EZH2 and TopoII inhibition in NSCLC.

Vulnerabilities in additional non-SWI/SNF proteins/complexes

Additional screens revealed a dependency on other non-SWI/SNF genes/complexes. In an siRNA-based screen for essential genes in lung cancer cells exhibiting *SMARCA4* loss, Aurora kinase A was identified suggesting importance of SWI/SNF function in centrosome independent mode of mitotic spindle assembly which is probably upregulated in lung cancer[96]. Thus, SWI/SNF functions not directly related to transcriptional regulation could also be evaluated for targeted therapies in tumours exhibiting loss of one SWI/SNF component.

Screening for a gene which provides a vulnerability target for SWI/SNF mutant cancers is not the only approach employed by researchers for exploring treatment options. In a more standard approach, Deribe *et al.*[97] decided to determine transcriptome signature to identify differential pathways in SWI/SNF mutant lung cancer. They performed a comparative transcriptome analysis between KRAS/p53 ablated tumours generated in mice and tumours generated from KRAS/P53/SMARCA4 ablation and identified genes regulating mitochondrial oxidative phosphorylation (OXPHOS) to be enriched in tumours arising out of the triple genetic event compared to KRAS/P53 ablated tumours[97]. These tumours were increasingly dependent on this important mitochondrial function for their energy requirement and vulnerable to small molecular inhibitors of OXPHOS[97].

The continuing research on SWI/SNF components has revealed several (non-transcriptional) roles which may provide alternative strategies for therapeutics. A study focused on identifying binding partners of ATR, a protein involved in initiation of DNA damage response, revealed ARID1A as a binding partner. Further evaluation revealed essentiality of ARID1A for double-strand break repair as well as for regulating the G2/M DNA damage checkpoint. More importantly, loss of ARID1A makes cells partially deficient for double-strand break repair (similar to the situation arising due to *BRCA1* mutations) and makes them vulnerable to PARP inhibitors[98]. Given the earlier studies that ascribed an important role for SWI/SNF in DNA repair, the current discovery of importance of ARID1A in double-strand break repair and synthetic lethality with PARP is not surprising.

Recently, another interesting link between DNA damage repair and SWI/SNF was elucidated. It was shown that SWI/SNF promotes double-strand break and nucleotide excision repair not only by facilitating access of DNA repair proteins by nucleosomal remodelling but also by activating expression of GTF2H1, a component of the TFIIH complex. Interestingly, however, GTF2H1 cannot be used as a synthetic lethal target under ablation of BRG1 or BRM as the cells somehow manage to retain adequate levels of GTF2H1 (ref. 99). Though BRG1 levels were proposed as a marker to predict response to DNA damage-based therapies such as 'Cisplatin', it appears that GTF2H1 levels are a better predictive biomarker. More importantly, it may be important to delineate mechanism of compensation in GTF2H1 levels upon BRG1/BRM ablation which could become a vulnerability that can be exploited for therapy.

Screening for synthetic lethality associated with ARID1A inactivation

Since ARID1A is the most frequently mutated SWI/SNF component in ovarian clear cell carcinoma (OCCC), several studies performed genetic screens to identify synthetic lethality with ARID1A mutations. An shRNA-based synthetic lethal 'kinome' screen was performed in a large panel of OCCC cell lines which identified BRD2 as a synthetic lethal target in cell lines having mutant ARID1A which was validated in mouse xenografts derived from OCCC cell lines as well as from patient tumours[100]. Interestingly, using chromatin immunoprecipitation, BRD2 was shown to bind to the promoter regions of several SWI/SNF genes including *ARID1A*, *ARID1B*, *SMARCE1*/BAF57 and *SMARCC2*/BAF155, suggesting perhaps that BRD2 might be involved in transcriptional activation of SWI/SNF genes. A separate functional genomics screen using anti-cancer drugs already in use, identified dasatinib as synthetic lethal with ARID1A mutation in OCCCs; dasatinib probably acts by removing the ablation of p21/Rb1 caused due to ARID1A mutation[101]. A third synthetic lethality screen using shRNAs against eleven histone deacetylases (HDACs) identified HDAC6 to be synthetic lethal with ARID1A mutation[102]. Interestingly, ARID1A appears to be a transcriptional repressor of HDAC6 and mutation induced ARIDIA loss of expression alleviates repression of HDAC6 which in turn acts by inhibiting the apoptosis promoting function of wild type p53 through deacetylation[102]. A separate study based on a proteome screen to detect ARID1A binding partners revealed ARID1A binding to MSH2 (ref. 103). Further, ARID1A was shown to participate in mismatch repair and ARID1A mutations were shown to increase the tumour mutation burden and causing generation of several 'neo-antigens' similar to tumours arising from mismatch repair inactivation resulting in microsatellite instability. More importantly, ARID1A mutant tumours generated in syngeneic mice were sensitive to immune checkpoint therapy[103]. Thus, ARID1A mutations can be used as a biomarker to predict response to immune checkpoint inhibitors.

The repertoire of cell processes where SWI/SNF (particularly the ARID1A subunit) plays a cardinal role seems to be expanding with more and more laboratories reporting new findings. A recent study revealed a unique link between SWI/SNF and the tumour suppressive cytoplasmic Hippo signalling cascade, which represses activity of the YAP/TAZ transcription factors that control cell proliferation in relation to organ size. ARID1A binds to and represses activity of YAP/TAZ. Mechanical stress induced by attainment of optimum organ size and relayed through F-actin dynamics perturbs the interaction between ARID1A and YAP/TAZ allowing functional activation of the latter through binding with TEAD[104]. Thus, loss of SWI/SNF could potentially activate YAP/TAZ function revealing yet another therapeutic vulnerability.

Given the multiple nuclear functions of the SWI/SNF (such as DNA recombination, repair, YAP/TAZ regulation, etc.), one would expect several vulnerabilities arising from its inactivation, some of which are already being evaluated for possible targeted therapies as enumerated

above. In addition, the possibility of SWI/SNF components exhibiting non-SWI/SNF dependent functions(s) (such as the E3 ubiquitin ligase activity of ARID1B that targets histone H2B[105]), provides further opportunities for possible therapeutic targeting. Indeed, we have not yet understood the complete repertoire of SWI/SNF function; work in several laboratories is ongoing in this direction and one can expect additional breakthroughs in the near future.

Direct targeting of oncogenic SWI/SNF?

Though the SWI/SNF complex is classified as a tumour suppressor, the fact that nucleosome repositioning is likely to be used by several (possibly including oncogenic) cellular processes, it should not be surprising to detect possible oncogenic roles for protein components of the complex. In addition, given the importance of SWI/SNF role in functioning of enhancers, one would expect the complex to perhaps regulate the activation of oncogenes as well. Many studies have reported components of the SWI/SNF to be widely overexpressed in human cancers, such as BRG1 and BRM in acute leukemia[106], colorectal cancer[107], prostate cancer[108] and primary breast cancers[109]; BAF53a in rhabdomyosarcoma[110]and glioma[111]; BAF57 in prostate cancer[112]; BAF60a in gastric cancer[113] and BAF155 in prostate[114] and colorectal cancer. Leukemia cells appear to rely on BRG1 containing SWI/SNF to maintain their oncogenic status[115] by activating the *MYC* oncogene[106]. Another study revealed requirement of BRG1 for proliferation of breast cancer cells[116,117] as well as for the maintenance of neuroblastoma cell viability[118]. The importance of SWI/SNF in activation of oncogenic target genes due to action of the EWSR1-FLI fusion was highlighted recently in Ewing's sarcoma[119]. The EWSR1-FLI fusion recruits SWI/SNF to specific enhancers to facilitate expression of oncogenic target genes[119]. A more recent meta-analysis study revealed BRG1 to be routinely overexpressed in several tumour types[120].

A more direct evidence for oncogenic mode of action of a mutant SWI/SNF was revealed in a study on synovial sarcomas. Synovial sarcoma is defined at the molecular level by a recurrent driver chromosomal translocation t(X; 18) (p11.2; q11.2) resulting in the fusion of SS18, a recently described component of SWI/SNF, with the related protein SSX. Interestingly, the fusion protein incorporates itself into and evicts both SS18 and the core subunit BAF47 from the SWI/SNF complex. The altered complex containing the fusion protein binds to the *SOX2* locus relieving the repressive histone H3K27 trimethylation mark thereby inducing *SOX2* expression and cell proliferation. Since the altered complex is recruited to an oncogene (here *SOX2*), the effect is a propagation of tumorigenic features[121,122]. Interestingly, the action of the altered complex at the *SOX2* locus is identical to that of the wild type, i.e. to displace proteins of the polycomb group (here EZH2) and reduce or remove the transcriptionally repressive H3K27 trimethylation mark. These observations provide renewed evidence for the antagonism between EZH2 and SWI/SNF on many gene loci. Thus, the SS18-SSX fusion protein becomes a possible direct target for therapy in synovial sarcomas.

As described above, wild type p53 is known to utilize various SWI/SNF components including SNF5 (ref. 48), BRG1 (ref. 50), ARID1A (ref. 49) and BAF60a (ref. 47) to facilitate its transcriptional activation and tumour suppressor function. *TP53* is the most frequently mutated gene in human cancers and specific recurrent mutations (affecting 'hotspot' amino acids located in the region encoding its DNA binding domain) have been shown to exhibit an oncogenic gain of function. Though these mutant p53 forms lose their respective DNA binding activities, they are able to co-operate with other transcription factors to activate expression of oncogenes. Interestingly, several mutant p53 appear to hijack SWI/SNF components to facilitate their oncogenic functions. For example, mutant p53 was shown to cooperate with SWI/SNF to induce the expression of pro-angiogenic factor VEGFR2 in breast cancer cells[123]. Similarly, knockdown of BRG1 was shown to induce senescence in CRC cells through a p21 and p53 dependent mechanism[124].

Classical tumour suppressor genes (TSGs) and oncogenes harbour distinct mutation pattern in tumours. TSGs often exhibit loss of function (inactivation) mutations such as nonsense or frame shift, whereas mutations in oncogenes cause a gain of function and therefore are mostly of the missense category. While loss-of-function mutations in SWI/SNF subunits seems to be highly prevalent in cancer, missense mutations have also been described[125,126]. It is unclear whether these point mutations result in complete or partial loss of function or potentially an oncogenic gain of function. The preliminary observations enumerated above highlight a possible oncogenic action of SWI/SNF components and could be a prelude to additional discoveries. Future work in this direction may thus reveal whether an oncogenic function of SWI/SNF components could be targeted for therapy in specific tumours.

Concluding remarks

Decades of research has revealed SWI/SNF to be a master regulator of chromatin dynamics impinging on several cardinal nuclear processes. More importantly, it is one of the most frequently mutated protein complexes in cancer. Despite this, we still know very little about the mechanism of the multi-protein complex in regulating fundamental nuclear processes or tumorigenesis. It is unclear why unlike classical tumour suppressors, SWI/SNF components do not exhibit bi-allelic mutations (with *SMARCB1*

being the only exception). It is possible that genes encoding SWI/SNF components are essential and a bi-allelic mutation is lethal (as shown with mice knockout studies) whereas a mono-allelic mutation disrupts the complex due to a dosage effect.

Of note, the susceptibility of a tumour cell harbouring inactivating mutation in a SWI/SNF subunit could emanate from activity of the residual complex or vulnerability of an unrelated function or from the activation of an oncogenic function originally suppressed by SWI/SNF. In contrast, there is also evidence of some cancer types hijacking a possible oncogenic function of SWI/SNF components. It will be important to design therapies based on these contrasting features differing from tumour to tumour and patient to patient, paving the way for therapeutic regimes tailor-made for each cancer type and each patient, what is popularly known as 'precision medicine'. Recent findings have increased our basic knowledge of these mechanisms and revealed unique therapeutic options arising from inactivation of individual SWI/SNF components. Moving forward, furthering our understanding of the basic roles of wild type and mutated SWI/SNF will likely reveal additional therapeutic options for cancer types critically dependent on SWI/SNF function.

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