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Chromatin-regulating proteins as targets for cancer therapy

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Chromatin-regulating proteins represent a large class of novel targets for cancer therapy. In the context of radiotherapy, acetylation and deacetylation of histones by histone acetyltransferases (HATs) and histone deacetylases (HDACs) play important roles in the repair of DNA double-strand breaks generated by ionizing irradiation, and are therefore attractive targets for radiosensitization. Small-molecule inhibitors of HATs (garcinol, anacardic acid and curcumin) and HDACs (vorinostat, sodium butyrate and valproic acid) have been shown to sensitize cancer cells to ionizing irradiation in preclinical models, and some of these molecules are being tested in clinical trials, either alone or in combination with radiotherapy. Meanwhile, recent large-scale genome analyses have identified frequent mutations in genes encoding chromatin-regulating proteins, especially in those encoding subunits of the SWI/SNF chromatin-remodeling complex, in various human cancers. These observations have driven researchers toward development of targeted therapies against cancers carrying these mutations. DOT1L inhibition in MLL-rearranged leukemia, EZH2 inhibition in EZH2-mutant or MLL-rearranged hematologic malignancies and SNF5-deficient tumors, BRD4 inhibition in various hematologic malignancies, and BRM inhibition in BRG1-deficient tumors have demonstrated promising anti-tumor effects in preclinical models, and these strategies are currently awaiting clinical application. Overall, the data collected so far suggest that targeting chromatin-regulating proteins is a promising strategy for tomorrow's cancer therapy, including radiotherapy and molecularly targeted chemotherapy.

Keywords: chromatin remodeling; histone modification; histone acetyltransferase; SWI/SNF complex; synthetic lethality; BRM

INTRODUCTION

Nucleosomes, which consist of chromosomal DNA and histone proteins, form a highly condensed structure known as chromatin. Recently, proteins involved in the regulation of nucleosome structure (i.e. chromatin-regulating proteins) have emerged as novel targets for cancer therapy. Recent studies have revealed that several chromatin-regulating proteins play pivotal roles in the repair of DNA double-strand breaks (DSBs) generated by ionizing irradiation (IR), and that inhibition of their activities leads to radiosensitization of cancer cells in preclinical models. Furthermore, recent large-scale genome analyses have identified frequent mutations in genes encoding chromatin-regulating proteins, especially those encoding subunits of the SWI/SNF chromatin-remodeling complex, in various human cancers. These findings have driven development of personalized treatment strategies based on the gene-mutation status of individual tumors. To date, several attractive treatment strategies targeting chromatin-regulating proteins have been proposed, and some of them are being tested in the clinic. In this article, we discuss the emerging strategies for targeting chromatin-regulating proteins in cancer therapy, including radiotherapy and molecularly targeted chemotherapy.

CHROMATIN STRUCTURE AND ITS ALTERATION BY CHROMATIN-REGULATING PROTEINS

Chromosomal DNA and histone proteins form a highly condensed structure known as chromatin (Fig. 1a). The basic unit of chromatin is the nucleosome, which consists of DNA wound around an octamer of histones H2A, H2B, H3 and H4. In human cells, many activities essential for cell survival, such as DNA transcription, synthesis and repair, are mediated by dynamic changes in nucleosome structure that facilitate access of DNA-binding proteins to double-stranded DNA [1]. Proteins that regulate the change in nucleosome structure are called chromatin-regulating proteins. These proteins can be classified into two groups that take part in distinct mechanisms: histone modification (Fig. 1b) and chromatin remodeling (Fig. 1c). Some histone modifiers attach substrates, such as phosphate, poly-ADP-ribosyl, acetyl, methyl, SUMOyl and ubiquityl groups to histone tails by covalent interaction, whereas other histone modifiers detach these groups from previously modified histones. Chromatin remodelers usually function as complexes that change nucleosome structure (e.g. by forming a DNA-loop or sliding a nucleosome) in an ATP-dependent manner. At present, several chromatin-remodeling complexes have been identified, including SWI/SNF, ISWI, INO80, SWR1, NURD/Mi2/CHD and NURF [1].

CHROMATIN-REGULATING PROTEINS AS TARGETS FOR RADIOSENSITIZATION

IR kills cancer cells by generating DNA damage. Among the types of DNA damage, DNA DSBs are considered to be most lethal. Repair of IR-generated DSBs requires alteration of chromatin structure at DSB sites by chromatin-regulating proteins [2]. Therefore, chromatin-regulating proteins involved in DSB repair are potential targets for radiosensitization. Recent findings regarding radiosensitization of cancer cells by targeting chromatin-regulating proteins are summarized below.

Histone acetyltransferases

Histone acetyltransferases (HATs) are a class of histone modifiers that attach acetyl units to specific lysine residues of nucleosomal histones. To date, several distinct families of HATs have been identified: the homologs CBP and p300, PCAF, GCN5, and the MYST family (which includes TIP60) [3]. Recent studies have demonstrated that histone acetylation by HATs at DSB sites facilitates alteration of chromatin structure to an 'open' state, allowing repair proteins to access the DNA ends [2]. We and others have revealed that CBP, p300 and TIP60 promote non-homologous end joining (NHEJ) and homologous recombination (HR), two major pathways for DSB repair, through histone acetylation at DSB

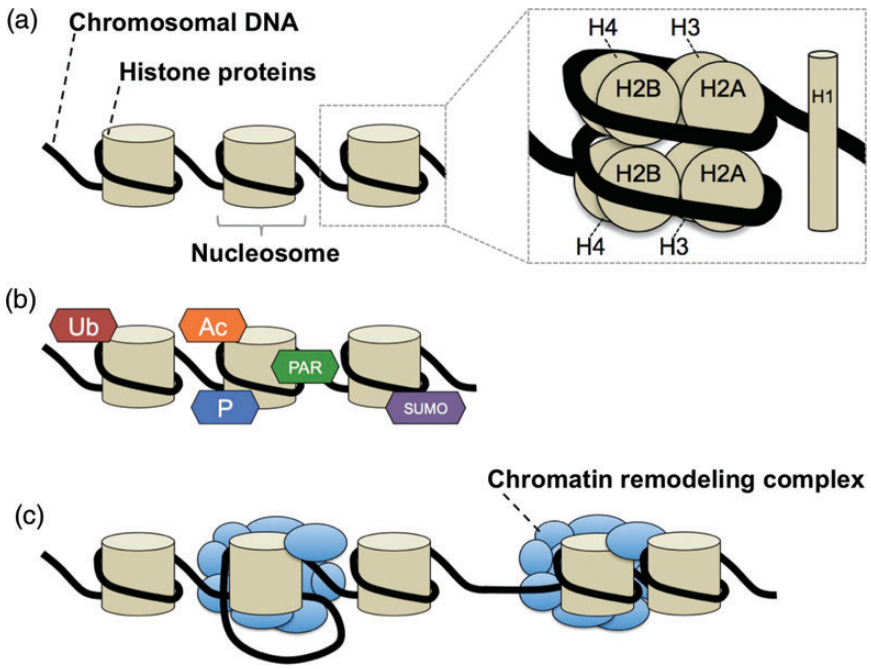

Fig. 1. Chromatin structure and its alteration by two distinct mechanisms: histone modification and chromatin remodeling. (**a**) Structure of chromatin and nucleosome. (**b**) Examples of histone modification. Ac = acetylation, PAR = poly-ADP-ribosylation, P = phosphorylation, SUMO = SUMOylation, Ub = ubiquitination. (**c**) Chromatin remodeling: DNA-loop formation (left) and nucleosome sliding (right).

Table 1. HAT inhibitors that suppress NHEJ in human cancer cells

Compound	MW	Target HATs	Other target proteins/pathways	References
Curcumin	368.38	CBP, p300	NF-kB pathway, AP-1, PI3K/Akt pathway	[22–24]
Anacardic acid	342.47	p300, PCAF, TIP60	NF-kB pathway, LOX-1, Xanthine oxidase	[10]
Garcinol	602.80	p300, PCAF	NF-kB pathway, Src, MAPK/ERK, PI3K/Akt pathways	[25, 26]

HAT = histone acetyltransferase, NHEJ = non-homologous end joining, MW = molecular weight.

sites in human cells [4–7]. Furthermore, we showed that acetylation of histone H3 and H4 by CBP and p300 at DSB sites is essential for the recruitment of KU70 and KU80, two key proteins involved in NHEJ [4]. Murr *et al.* demonstrated that acetylation of histone H4 by TIP60 is required for the accumulation of proteins involved in HR at DSB sites [5]. Bird *et al.* reported that ESA1, a HAT responsible for acetylation of histone H4 in budding yeast, is required for NHEJ and replication-coupled repair [6]. CBP and p300 also function in HR by regulating transcriptional activity of the *BRCA1* and *RAD51* genes [7]. These findings predict that inhibition of such HATs leads to radiosensitization.

Several known compounds derived from natural ingredients exhibit HAT-inhibitory activity (Table 1). Curcumin, a major curcuminoid found in the spice turmeric, is a specific inhibitor of CBP and p300 [8]. Anacardic acid, derived from the shell of *Anacardium occidentale* (cashew nut), inhibits p300, PCAF and TIP60 [9–11], whereas garcinol, found in the rind of *Garcinia indica* (mangosteen), inhibits p300 and PCAF [12]. Recently, curcumin, anacardic acid and garcinol were shown to suppress NHEJ in an assay system in which NHEJ activity against DSBs on chromosomal DNA generated by I-*Sce*I restriction enzyme can be evaluated in living human cancer cells by detecting expression of green fluorescent protein [4, 13]. Curcumin also suppresses HR in two ways: (i) by reducing expression of the *BRCA1* gene, which regulates HR, by impairing histone acetylation at the *BRCA1* promoter; and (ii) by inhibiting ataxia telangiectasia and Rad3-related protein (ATR) kinase, resulting in impaired activation of ATR-CHK1 signaling, which is necessary for HR and the DNA damage checkpoint pathway [14].

As predicted, curcumin, anacardic acid and garcinol sensitize cancer cells to IR (Table 2) [4, 9, 13, 15–19]. In our study, the radiosensitizing effect of garcinol was strongest, comparable with the effects of well-known radiosensitizers that target DNA repair proteins, e.g. olaparib, a poly (ADP-ribose) polymerase (PARP) inhibitor, and NU7026, a DNA-dependent protein kinase catalytic subunit (DNA-PKcs) inhibitor (Table 3) [13, 20, 21]. However, because curcumin, anacardic acid and garcinol may also affect many other proteins or pathways associated with cancer cell survival, it is not clear that the observed radiosensitizing effects of these compounds are entirely due to their HAT-inhibitory activity (Table 1) [10, 22–26].

Table 2. Radiosensitization by HAT inhibitors

Compound	Cells/mice	Cell lines	References
Curcumin	Cells, mice	SCC1	[15]
	Cells	H1299	[4]
	Cells	HCT116	[16]
	Cells	PC-3	[17]
	Cells	PC-3	[18]
Anacardic acid	Cells	H1299	[4]
	Cells	SQ20B, SCC35, HeLa	[9]
	Cells	U2OS	[19]
Garcinol	Cells	A549, HeLa	[13]

HAT = histone acetyltransferase.

Histone deacetylases

Histone deacetylases (HDACs) are another class of histone modifiers that remove acetyl units from lysine residues on histones. To date, 18 HDACs have been identified and classified into four groups (HDAC I, II, III and IV) according to their similarity, with reference to their analogues in yeast [27]. Several known compounds exhibit HDAC-inhibitory activity, and these compounds can be classified according to their structures and their inhibitory specificities relative to the HDAC classes. Hydroxamic acids, including suberoylanilide hydroxamic acid (SAHA or vorinostat) and panobinostat (LB589), inhibit class I and II HDACs. Short-chain fatty acids, such as sodium butyrate (NaB) and valproic acid (VPA), are active against class I and IIa HDACs. Cyclic peptides such as romidepsin mainly act against class I HDACs, but can also inhibit class II HDACs at higher concentrations. Benzamides, including entinostat and mocetinostat, inhibit class I HDACs.

Recent studies have implicated direct and indirect involvement of HDACs in both NHEJ and HR. Miller *et al.* showed that HDAC1 and HDAC2 are recruited to DNA DSB sites, where they reduce H3K56 acetylation and regulate recruitment of KU70 and Artemis, which are involved in NHEJ [28]. Meanwhile, several studies have shown that HDAC inhibitors suppress expression of DNA repair-related

proteins such as KU70, KU80, KU86, DNA-PKcs and RAD51 (Table 4) [29–33]. These observations predict that HDAC inhibitors have a radiosensitizing effect. Accordingly, several recent studies support this idea (Table 4). Vorinostat radiosensitizes prostate cancer, glioma, multiple myeloma, osteosarcoma and rhabdomyosarcoma cell lines [29–31]; NaB radiosensitizes melanoma cells [32]; and VPA radiosensitizes colon cancer cells [33].

Even though HATs and HDACs seemingly act in an opposing manner, both HAT and HDAC inhibitors exert radiosensitizing effects, at least *in vitro*. A clue toward resolving this apparent paradox has emerged from recent studies suggesting that HATs and HDACs act coordinately before DSB repair [4, 5, 28]. Further investigation will elucidate the detailed mechanisms of the coordination of HATs and HDACs in DSB repair following IR, and this knowledge will facilitate development of methods for radiosensitization.

Chromatin remodelers

Several studies have demonstrated the involvement of chromatin remodelers in DSB repair after IR, and the radiosensitizing effects of inhibiting the activities of these proteins. Lan *et al*. demonstrated in osteosarcoma cells that ACF1 and SNF2H, subunits of the CHRAC chromatin-remodeling complex, engage in an interaction with KU70, which is required for repair of DSBs generated by IR, and that knockdown of ACF1 or SHF2H results in radiosensitization [34]. Meanwhile, we showed that ablation of *BRM*, a catalytic subunit of the SWI/SNF chromatin-remodeling complex, leads to suppression of recruitment of KU70 to DSB sites after laser micro-irradiation in lung cancer cells [4], suggesting BRM as a target for radiosensitizing agents. Furthermore, our preliminary results indicate that heterozygous knockout of *BRG1*, another catalytic subunit of the SWI/SNF complex, leads to radiosensitization in lymphoma cells (unpublished data). These data indicate that not only histone modifiers, but also chromatin remodelers, are candidate targets for radiosensitization.

Table 3. Radiosensitizing effect of HAT, PARP and DNA-PKcs inhibitors

Compound	DER
Curcumin	1.23
Anacardic acid	1.51
Garcinol	1.61
Olaparib (PARP inhibitor)	1.67
NU7026 (DNA-PKcs inhibitor)	1.77

HAT = histone acetyltransferase, PARP = poly (ADP-ribose) polymerase, DNA-PKcs = DNA-dependent protein kinase catalytic subunit, DER = dose enhancement ratio. DER was evaluated using the Celltiter-Glo assay [13].

Toward clinical applications

Historically, extensive research has been carried out to develop radiosensitizers. Even though a large number of known compounds exert radiosensitizing effects in preclinical models, most of them have been judged inadequate for clinical application due to their toxicity in normal human tissues [35]. Therefore, it is essential to develop radiosensitizers with satisfactory therapeutic windows, i.e. sufficient anti-tumor effects coupled with limited toxicity to normal tissues. It is considered that cancer cells have defects in the DNA damage response (DDR), including DNA repair and cell-cycle arrest, that makes them more vulnerable to IR than normal cells [36, 37]. In this regard, targeting chromatin-regulating proteins involved in DDR after IR may be a reasonable strategy for radiosensitization of cancer cells, which may result in a large therapeutic window. Several results from basic research support this speculation: neither garcinol nor NaB exert radiosensitizing effects on fibroblasts at concentrations that sensitize cancer cells to IR [13, 32]. However, because the mechanisms underlying their relatively stronger radiosensitizing effect in cancer cells have not been elucidated, further studies are warranted.

Several clinical studies have administered HAT- or HDAC-inhibitory compounds to humans. The HAT inhibitors include curcumin, used either alone or combined with radiotherapy and chemotherapy to treat cancer patients, and garcinol, used for weight-loss therapy [38]. In both of those cases, the side effects of these compounds were tolerable, at least when the drugs were used alone. Regarding HDAC

Table 4. Radiosensitization by HDAC inhibitors *in vitro*

Compound	Cell lines	Target DNA repair proteins	References
Vorinostat	DU145, U373	DNA-PKcs, RAD51	[29]
	RPMI8226, U266B1, KMS-1, MM1.s	RAD51	[30]
	KHOS-242OS, SAOS2, A-204, RD, hFOB1.19	KU80, RAD51	[31]
NaB	A375, MeWo	KU70, KU80, DNA-PKcs	[32]
VPA	LS174T, HCT116		[33]

DNA-PKcs = DNA-dependent protein kinase catalytic subunit.

inhibitors, several retrospective studies have shown that administration of VPA, alone or with chemotherapeutic agents, to patients with thyroid or brain tumors caused no increase in adverse effects [39, 40]. Moreover, in the first published prospective clinical trial using an HDAC inhibitor and radiation, combined therapy with vorinostat (100–400 mg daily) and palliative X-ray irradiation (a total of 30 Gy in 10 fractions) was well tolerated in 16 gastrointestinal carcinoma patients [41]. Future clinical trials should explore the optimal way to improve the efficacy of radiation therapy by targeting HATs and HDACs.

MUTATIONS IN CHROMATIN-REGULATING GENES IN HUMAN CANCERS

Recent large-scale genome analyses have identified frequent mutations in genes encoding chromatin-regulating proteins in human cancers. Thus, aberrations in histone modification and chromatin remodeling play important roles in the genesis and development of malignant tumors. In this review, we summarize known mutations in chromatin-regulating genes (Table 5). We focus in particular on mutations in genes encoding the SWI/SNF chromatin-remodeling complex because the mutation rates in these genes are extremely high in human cancers.

SWI/SNF chromatin-remodeling complex

The SWI/SNF complex is a chromatin remodeler that utilizes the energy of ATP to disrupt contact between nucleosomal DNA and histones, leading to sliding, ejection and/or insertion of histone proteins [42, 43]. Although the mechanisms by which the SWI/SNF complex elicits biological effects are still not fully understood, previous studies have shown that the SWI/SNF complex regulates the expression of numerous genes. For example, Medina *et al*. showed that exogenous expression of BRG1, a catalytic subunit of the SWI/SNF complex, resulted in altered gene expression of ~1% of the entire genome in a BRG1-null cancer cell line [44]. Recent studies identified several transcriptional target genes of the SWI/SNF complex, including *RB*, *MYC* and *RHOA*, all of which are involved in carcinogenesis and tumor progression. The SWI/SNF complex directly interacts with RB, a tumor suppressor, to repress RB target genes including *E2F* and *CCND*, leading to cell-cycle arrest at the G1/S transition [45]. The expression of E2F target genes in mouse embryonic fibroblasts is upregulated following inactivation of Snf5 [46]. These data suggest that the SWI/SNF complex negatively regulates cell-cycle progression. The SWI/SNF complex also interacts with MYC, an oncogenic transcription factor that regulates gene expression during cell-cycle progression, apoptosis and differentiation. The SWI/SNF complex mediates the transcription activity of MYC through a direct interaction between SNF5 and MYC [47]. Meanwhile, RHOA regulates cell migration by stimulating stress-fiber formation and contractility. Knockdown of SNF5 increases RHOA enzyme activity and stimulates cell migration, suggesting that the SWI/SNF complex suppresses the migration and metastasis of cancer cells [48].

There are two distinct SWI/SNF complexes, BAF and PBAF, both of which consist of various subunits categorized as catalytic ATPase subunits, core subunits, or variant subunits (Fig. 2) [49–51]. The catalytic ATPase subunits include BRG1 and BRM, whose presence in a given SWI/SNF complex is mutually exclusive. Core subunits include SNF5, BAF155 and BAF170. Variant subunits include ARID1A and ARID1B, mutually exclusive components of the BAF complex, and BAF180, BAF200 and BRD7, which are specific to the PBAF complex. In mammals, different types of the SWI/SNF complex with distinct subunit compositions contribute to the regulation of gene expression in a tissue-specific manner [52].

Accumulating evidence suggests that the SWI/SNF genes are frequently mutated and inactivated in various human cancers. Shain *et al*. analyzed whole-exome sequencing data from 24 published studies encompassing 669 cases with 18 neoplastic diagnoses; their results demonstrated that mutations in the SWI/SNF genes are widespread across various cancers, with an overall frequency approaching that of *TP53* mutations (19% in the SWI/SNF complex, 26% in TP53) [53]. Mutations in the SWI/SNF genes were significantly skewed toward deleterious forms (e.g. frameshift, nonsense or splice-site mutations), consistent with the inactivated nature of mutated SWI/SNF subunits. Together, these data suggest that the SWI/SNF complex is a *bona fide* tumor suppressor and may have a significant impact on the properties of cancer cells, as in the case of TP53. Below, mutations identified in the SWI/SNF genes are summarized with respect to each subunit.

BRG1/SMARCA4

BRG1 encodes a catalytic ATPase subunit of the SWI/SNF complex. Sequencing of the *BRG1* gene from various cancer cell lines has demonstrated that *BRG1* is mutated in a variety of human cancers, including >30% of non-small-cell lung carcinoma (NSCLC) [54]. Notably, most of the mutations identified are homozygous mutations and deletions, indicating that BRG1 plays a role as a tumor suppressor. A recent large-scale genome analysis confirmed that *BRG1* mutations play a prominent role in aberrant chromatin remodeling in lung adenocarcinoma [55]. *BRG1* mutations were also identified recently in Burkitt lymphoma [56], medulloblastoma [57–59], and esophageal cancer [60]. Furthermore, loss of BRG1 protein expression is observed in 10–50% of surgical lung cancer specimens [61–63]. A recent report showed that both genetic and epigenetic alterations are involved in the loss of BRG1 expression [55].

Patients with rhabdoid tumor (RT) predisposition syndrome, in which RTs occur on a familial basis, harbor a

Table 5. Mutation in chromatin-modifying genes in human cancers

Gene	Function of gene product	Cancer type	Frequency (%)	References
Inactivating mutation				
BRG1/SMARCA4	SWI/SNF catalytic subunit	Non-small-cell lung carcinoma	35	[54]
		Lung adenocarcinoma	10	[55]
		Burkitt lymphoma	15	[56]
		Medulloblastoma	4–13	[57–59]
		Esophageal cancer	6	[60]
		Lung cancer (reduced expression)	15–50	[61–63]
BRM/SMARCA2	SWI/SNF catalytic subunit	Lung cancer (reduced expression)	38–75	[61–63]
		Gastric cancer (reduced expression)	42	[66]
		Prostate cancer (reduced expression)		[67]
SNF5/SMARCB1/ BAF47/INI1	SWI/SNF core regulatory subunit	Rhabdoid tumor	98	[68, 69]
		Meningioma		[70, 71]
		Shwannoma		[70, 72]
ARID1A/BAF250A	SWI/SNF variant regulatory subunit	Ovarian clear cell carcinoma	46–57	[75, 76]
		Endometriosis-associated ovarian carcinoma	30	[75, 76]
		Renal clear cell carcinoma	34	[77]
		Burkitt lymphoma	15	[56]
		Hepatocellular carcinoma	13	[78]
		Transitional cell carcinoma of the bladder	13	[79]
		Gastric cancer	10	[80]
		Esophageal cancer	8	[60]
		Serous endometrial cancer of uterine	6	[81]
		Neuroblastoma	6	[82]
		Pancreatic cancer	4	[83]
		Malignant melanoma	3	[84]
		Medulloblastoma		[85]
		Lung adenocarcinoma		[55]
ARID1B/BAF250B	SWI/SNF variant regulatory subunit	Hepatocellular carcinoma	11	[78]
		Neuroblastoma	7	[82]
		Breast cancer	4	[86]
		Malignant melanoma	2	[84]
ARID2/BAF200	SWI/SNF variant regulatory subunit	Hepatocellular carcinoma	6–11	[78, 87]
		Malignant melanoma	7	[84]
		Esophageal cancer	5	[60]

Continued

Table 5. *Continued*

Gene	Function of gene product	Cancer type	Frequency (%)	References
PBRM1/BAF180	SWI/SNF variant regulatory subunit	Renal clear cell carcinoma	41	[77]
		Esophageal cancer	3	[60]
		Breast cancer		[88]
CPB/CREBBP	H3H4 acetyltransferase	B-cell lymphoma	40	[89]
		Acute lymphoblastic leukemia	18	[90]
		Small-cell lung carcinoma	17	[91]
		Transitional cell carcinoma of the bladder	13	[79]
		Lung cancer	10	[92]
p300	H3H4 acetyltransferase	Transitional cell carcinoma of the bladder	13	[79]
		Small-cell lung carcinoma	10	[91]
		Colorectal, breast and pancreatic cancer		[93]
MLL	H3K4 methyltransferase	Acute leukemia	5–10	[94]
		Small-cell lung carcinoma	10	[91]
		Transitional cell carcinoma of the bladder	7	[79]
SETD2	H3K36 methyltransferase	Lung adenocarcinoma	5	[55]
UTX/KDM6A	H3K27 demethylase	Transitional cell carcinoma of the bladder	21	[79]
		Various types of cancer	3	[99]
JARID1C/KDM5C Activating mutation	H3K4 demethylase	Renal carcinoma	3	[98]
EZH2	H3K27 methyltransferase	Diffuse large B-cell lymphoma (germinal center type)	22	[95]
		Diffuse large B-cell lymphoma, other lymphomas	15	[96]
		Follicular lymphoma	7	[95]
		Myelodysplastic syndrome	12	[97]
		Esophageal cancer	3	[60]

heterozygous germline *BRG1* mutation that truncates the encoded protein, and their RTs are homozygous for this mutation [64]. Mutations in *SNF5*, which are present in the germline of most cases of RT predisposition syndrome, were not detected in the patients analyzed in that study. These results suggest that deficiencies of the SWI/SNF complex may play a role in the predisposition of RT, and support the idea that BRG1 is a tumor suppressor.

BRM/SMARCA2

BRM encodes another catalytic ATPase subunit of the SWI/SNF complex. In contrast to *BRG1*, somatic mutations in *BRM* are rarely identified in human cancers. However, in a mouse model in which lung tumors are induced by exposure to carbamate ethyl, inactivation of one or both *Brm* alleles led to a significant increase in the number of tumors, indicating that Brm plays a role as a tumor suppressor [65]. In fact, BRM protein expression is absent in human lung, gastric and prostate cancers [61–63, 66, 67]. Furthermore, low BRM expression in NSCLC correlates with a worse prognosis [62, 63]. These findings suggest that epigenetic silencing of *BRM* may be critical for the development of a subset of cancers.

SNF5/SMARCB1/BAF47/INI1

The *SNF5* gene encodes a core regulatory subunit of the SWI/SNF complex and is inactivated via biallelic genetic alterations, including deletions and nonsense, missense and frameshift mutations, in nearly all RTs [68]. Thus, *SNF5* is considered a major driver gene of RTs. Consistent with this, a significant proportion of RT predisposition syndrome

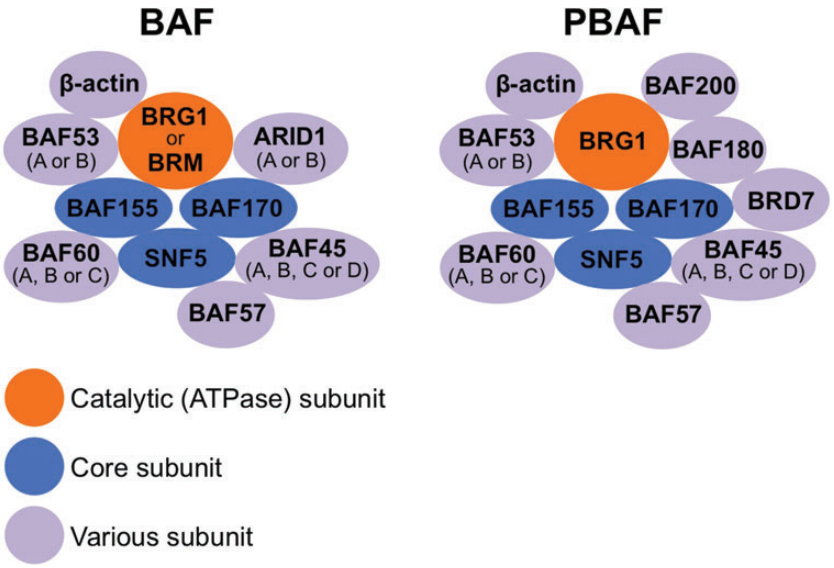

Fig. 2. Composition of the SWI/SNF chromatin remodeling complex.

patients harbor heterozygous *SNF5* deleterious germline mutations, and RTs arise via loss of the remaining wild-type *SNF5* allele [68, 69]. Germline *SNF5* mutations also result in predisposition to meningioma and schwannoma [70–72].

Genetically engineered *Snf5*-heterozygous mice develop sarcomas that closely resemble human RTs [73]. Furthermore, biallelic inactivation of *Snf5* results in the development of sarcoma and lymphoma with a median onset of only 11 weeks, which is a remarkably rapid rate considering that the median onset of tumors induced by biallelic inactivation of *p53* and *Rb* is 16 weeks in the same experimental model. These data strongly suggest that *SNF5* acts as a tumor suppressor. However, somatic mutation of *SNF5* is rarely detected in common cancers [49, 51, 74], indicating that, in contrast to mutations in other SWI/SNF genes detected in wide range of cancers, the *SNF5* mutation is specific to a subset of non-epithelial malignancies.

ARID1A/BAF250A and ARID1B/BAF250B

Recent genome-wide sequencing studies identified *ARID1A*, which encodes a subunit of the SWI/SNF complex, as one of the most frequently mutated genes in a variety of cancers (Table 5). *ARID1A* is mutated in 46–57% of ovarian clear cell carcinomas, one of the most lethal subtypes of ovarian cancer, and in 30% of endometriosis-associated ovarian carcinomas [75, 76]. *ARID1A* mutations have also been detected in a variety of cancers including renal clear cell carcinoma [77], Burkitt lymphoma [56], hepatocellular carcinoma [78], transient cell carcinoma of the bladder [79], gastric adenocarcinoma [80], esophageal cancer [60], uterine serous endometrial cancer [81], neuroblastoma [82], pancreatic cancer [83], malignant melanoma [84], and medulloblastoma [85]. *ARID1A* is also mutated in a subset of lung adenocarcinomas, although at a lower frequency than *BRG1* [55]. Most *ARID1A* mutations detected in cancer cells to date are inactivating mutations, indicating that *ARID1A* has a tumor-suppressive function.

ARID1B is mutated in a small subset of patients with hepatocellular carcinoma [78], neuroblastoma [82], breast cancer [86], and malignant melanoma [84].

ARID2/BAF200

Genome sequencing studies conducted by Li *et al.* and Fujimoto *et al.* found that *ARID2*, which encodes a SWI/SNF regulatory subunit, was mutated in ~10% of surgical specimens of hepatocellular carcinoma [78, 87]. Large-scale exome sequencing in malignant melanoma also revealed that 7.4% of the cases harbored mutations in *ARID2*, most of which were inactivating mutations. In that study, *ARID2* mutation was identified as one of the driver mutations resulting from C-to-T transitions caused by exposure to UV light; this is in contrast to other driver mutations in malignant melanoma, such as those in *BRAF* and *NRAS*, that are only weakly associated with UV-induced damage [84]. *ARID2* is also mutated in a small subset of esophageal cancers [60].

PBRM1/BAF180

Most recently, mutations in the *PBRM1* gene, which encodes a regulatory subunit protein, were identified in 41% of renal clear cell carcinomas [77], making *PBRM1* the second most frequently mutated gene in renal cell carcinoma after *VHL*. *PBRM1* mutations have also been detected in esophageal cancer patients [60] and in several breast cancer cell lines [88].

Mutations in histone modifier genes

Recently, mutations in several histone modifier genes have been identified. The gene encoding CBP HAT is mutated in B-cell lymphoma, acute lymphoblastic leukemia, and

carcinomas of the lung and bladder [79, 89–92]. Meanwhile, the gene encoding p300 HAT is mutated in bladder carcinoma, small-cell lung carcinoma, colorectal cancer, breast cancer and pancreas cancer [79, 91, 93], although at a lower frequency than CBP.

Histone methytransferases (HMTs) are a class of enzymes that catalyze methylation of histones at lysine and arginine residues. In acute leukemia, MLL, an HMT specific for lysine 4 of histone H3 (H3K4), is rearranged to generate fusion proteins with diverse partners. In most cases, the rearrangement results in the loss of the H3K4 HMT enzymatic activity [94]. MLL is also mutated in small-cell lung carcinoma and bladder carcinoma [79, 91]. SETD2, another HMT specific for H3K36, is mutated in a small fraction of lung adenocarcinomas [55]. On the other hand, activating mutations in the gene encoding EZH2, an HMT directed against H3K27 that is the catalytic subunit of the polycomb repressive complex 2 (PRC2), have been identified in diffuse large B-cell lymphoma (DLBCL), follicular lymphoma, myelodysplastic syndrome, and esophageal cancer [60, 95–97].

In contrast to HMTs, histone demethylases (HDMs) are enzymes that remove methyl units from histones. Some tumors contain alterations in HDM-encoding genes. The gene encoding JARID1C, a HDM specific for H3K4, is mutated in renal carcinoma [98], and the gene encoding UTX, a HDM specific for H3K27, is mutated in multiple cancers [79, 99].

To date, the mechanisms by which mutations in histone modifiers are involved in carcinogenesis and tumor growth have not been fully elucidated. Nevertheless, in several cases, recent research has uncovered new leads for the development of treatment strategies targeting these mutations. These investigations will be discussed in the next chapter.

PERSONALIZED TREATMENT STRATEGIES THAT TARGET MUTATIONS IN CHROMATIN-REGULATING GENES

In parallel with the elucidation of the genetic landscape of various human cancers by genome-wide sequencing analyses, large numbers of chemotherapeutic agents targeting gene mutations (i.e. molecularly targeted drugs) have been developed, driving the advancement and spread of personalized cancer therapy based on gene-mutation profiles. Cancers harboring activating gene mutations can be treated with specific inhibitors of the mutated gene products, thereby suppressing their abnormally high activity. For example, selective cell killing by the tyrosine kinase inhibitors gefitinib, crizotinib, and vandetanib in cancer cells harboring EGFR mutation, ALK fusion, and RET fusion, respectively, has been demonstrated by our group and others, and these treatment strategies have been applied in the clinic [100–104]. Meanwhile, for the treatment of cancers in which certain proteins or pathways are inactivated by genetic and/or epigenetic causes, strategies based on synthetic lethality have attracted a great deal of attention [105]. Furthermore, chromatin-regulating proteins involved in the transcriptional regulation of genes essential for cancer cell survival can be also targeted. The emerging strategies for personalized cancer therapy targeting chromatin-regulating proteins are summarized below.

Drugs targeting activating mutations in chromatin-regulating genes

MLL-rearranged leukemia is a subset of acute myeloid leukemia (AML) with poor prognosis. Recently, inhibition of DOT1L, a HMT specific for H3K79, has emerged as a promising strategy for the treatment of MLL-rearranged leukemia. MLL regulates the transcription of various genes involved in cellular development. The carboxyl-terminal portion of the MLL protein contains a domain with HMT activity specific for H3K4 [94]. Rearrangement of MLL results in the loss of the H3K4 HMT domain and in-frame fusion of the amino-terminal region of MLL to diverse partner proteins. In most cases, these fusions lead to more efficient recruitment of DOT1L to MLL target genes. The resulting hypermethylation at H3K79 by DOT1L leads to aberrant expression of a characteristic set of genes, including *HOXA9* and *MEIS1*, which drive leukemogenesis. In MLL-rearranged leukemia, DOT1L is also required for the development and maintenance of leukemia cells. Daigle *et al*. developed a compound, EPZ00477, which selectively inhibits DOT1L HMT [106]. They reported that EPZ00477 selectively killed MLL-rearranged leukemia cells in culture and prolonged survival in a mouse xenograft model. Moreover, they further identified another selective inhibitor of DOT1L HMT, EPZ-5676, which possesses superior pharmacokinetic properties [107]. Continuous intravenous infusion of EPZ-5676 in a rat xenograft model of MLL-rearranged leukemia caused complete tumor regressions. The state of complete tumor response was sustained beyond the period of compound infusion with no significant toxicity. EPZ-5676 is now the subject of a clinical trial for MLL-rearranged leukemia patients, as the first inhibitor of an HMT to be tested in humans.

Activating mutations of EZH2 in non-Hodgkin lymphoma have provoked interest in this protein as a therapeutic target. Y641 and A677 are hot spots for lymphoma-associated EZH2 mutations [95, 96]. These mutations increase the activity of the encoded proteins, leading to elevated levels of trimethylated H3K27, thereby promoting proliferation of lymphoma cells [96, 108, 109]. Several groups have developed selective small-molecule inhibitors of EZH2 HMT and demonstrated promising preclinical results. McCabe *et al*. showed that the compound GSK126 decreased global methylation at H3K27 and reactivated silenced PRC2 target genes in EZH2-mutant DLBCL cell lines [110]. Furthermore, this compound effectively inhibited the proliferation of the EZH2-mutant DLBCL cells, and suppressed tumor growth in a mouse xenograft model. Qi *et al*. developed another

compound, El1 [111], which when administered to DLBCL cells carrying the Y641 mutation also decreased the H3K27 methylation level genome-wide, activated PRC2 target genes, and decreased cell proliferation. Knutson *et al*. reported that the compound EPZ005687 induced apoptotic cell death in lymphoma cells with heterozygous mutations in Y641 or A677, with minimal effect on the proliferation of wild-type cells [112]. On the other hand, several studies have demonstrated that AML cells harboring MLL-AF9 fusion require EZH2 for survival, suggesting that EZH2 inhibition is also a candidate strategy to treat this disease [113, 114]. Most recently, Kim *et al*. developed a peptide called stabilized α-helix of EZH2 (SAH-EZH2), which inhibits EZH2 inhibition by a different mechanism from GSK126, El1, and EPZ005687 [115]. SAH-EZH2 selectively disrupts the contact between EZH2 and EED, another subunit in the PRC2 complex, whereas the other EZH2 inhibitors target the HMT catalytic domain. As in the case of GSK126, SAH-EZH2 decreases the H3K27 trimethylation level, resulting in growth arrest of PRC2-dependent MLL-AF9 leukemia cells. Together, these studies provide hope for the establishment of novel treatment strategies against EZH2-mutant hematologic malignancies. Moreover, recent studies have revealed the overexpression of various HMTs in human cancers [116]. It is speculated that the resulting histone hypermethylation patterns are associated with the malignant features of cancer cells in a cancer-type specific manner. Further studies of these cancer-specific patterns and the roles of histone hypermethylation should lead to the development of novel targeted therapies.

Synthetic lethality therapy targeting inactivation of chromatin-regulating proteins

As discussed, cancers with gain-of-function gene mutations can be treated by inhibition of the mutated gene products. However, in many cases, tumor growth is also driven by loss-of-function mutations in tumor-suppressor genes. Previous studies have demonstrated that the restoration of the lost function of tumor-suppressor proteins is not a simple process [117–119]. Therefore, it is necessary to develop alternative strategies to treat cancers harboring inactivating mutations in tumor-suppressor genes. Synthetic lethality holds great promise as a strategy for specific killing of cancer cells possessing inactivating mutations that are not present in normal cells [105]. Synthetic lethality is based on genetic interactions between two mutations in which the presence of either mutation alone has little or no effect on cell viability, but the combination of mutations in both genes becomes lethal. The presence of one of these mutations in cancer cells, but not in normal cells, presents opportunities to selectively kill cancer cells by mimicking the effect of the second genetic mutation with targeted therapy. The synthetic-lethal relationship between *PARP1* and *BRCA1* or *BRCA2*, genes involved in DNA repair, was demonstrated in preclinical models in 2005 [120, 121] and translated to the clinic in 2009 through the application of PARP inhibitors to *BRCA1/BRCA2*-deficient tumors [122]. These advances have driven researchers to explore novel combinations of genes to identify additional synthetic-lethal relationships [123–125].

Most recently, synthetic-lethal relationships in chromatin-regulating genes have been identified, e.g. between BRG1 and BRM [61] and between SNF5 and EZH2 [126]. BRG1 or BRM are present in a mutually exclusive manner as catalytic subunits in the SWI/SNF chromatin-remodeling complex. Previous studies of the embryos of genetically engineered mice have indicated that BRG1 and BRM play complementary roles in development [127]. Together, these data suggest that BRG1 and BRM have a synthetic-lethal relationship. Accordingly, silencing of *BRM* suppresses the growth of BRG1-deficient cancer cells relative to BRG1-proficient cancer cells (Fig. 3), by inducing senescence via activation of p21/CDKN1A. Moreover, in a conditional RNAi experiment using a mouse xenograft model, BRM depletion suppressed the growth of BRG1-deficient tumors [61]. These results offer a rationale for treating BRG1-deficient cancers, including subsets of NSCLCs,

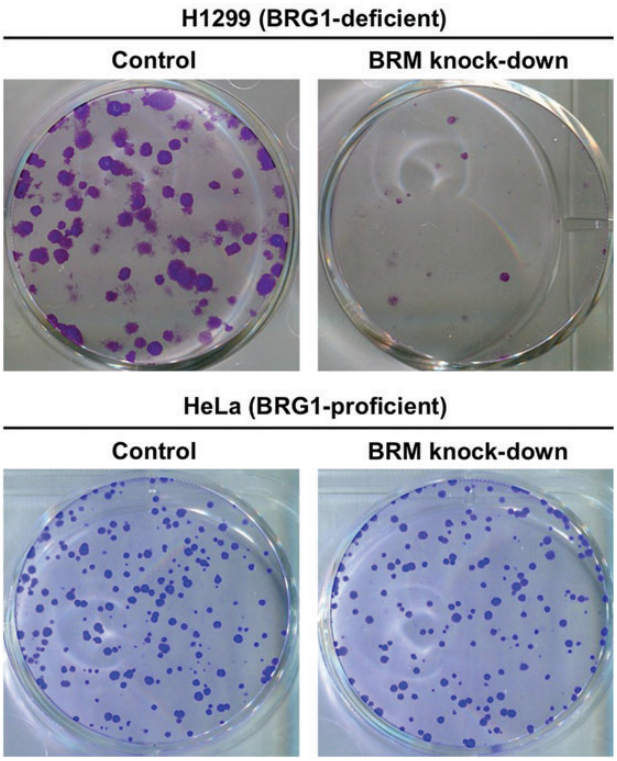

Fig. 3. Specific cell killing of BRG1-deficient H1299 cells, but not BRG-proficient HeLa cells, by BRM knockdown based on synthetic lethality. Cells were treated with BRM-siRNA (BRM knockdown) or non-targeting siRNA (control) (Dharmacon). After 48 h, the cells were subjected to clonogenic survival assays. Colonies fixed and stained after incubation for an additional 10 d are shown.

medulloblastoma, and Burkitt lymphoma, by inhibition of the BRM ATPase. To this end, screening for small-molecule compounds with selective BRM ATPase inhibitory activity is currently underway.

SNF5 is a core subunit in the SWI/SNF complex. *SNF5*-heterozygous mice develop sarcomas that closely resemble human malignant RTs in which the second allele of *SNF5* is spontaneously lost. The genesis of the sarcoma in *SNF5*-heterozygous mice can be completely suppressed by deletion of *EZH2*. These data suggest a synthetic-lethal relationship between *SNF5* and *EZH2*. In line with this concept, Alimova *et al*. showed that disruption of EZH2 by RNAi and/or 3-deazaneplanocin A, an indirect and general inhibitor of methyltransferases, impaired growth of *SNF5*-mutant atypical teratoid RT cells [128]. Furthermore, Knutson *et al*. developed EPZ-6438, a selective small-molecule inhibitor of EZH2, and demonstrated that EPZ-6438 specifically killed *SNF5*-mutant malignant RT cells both *in vitro* and *in vivo* [126]. EPZ-6438 decreased cellular H3K27 methylation levels and activated the PRC2 target gene *CDKN2A* in *SNF5*-mutant malignant RT cells, but not in wild-type cells. Because the PRC2 complex regulates gene expression in several cancer-associated pathways such as RB, Cyclin D1, MYC [129] and Hedgehog [130] in a manner that antagonizes the function of the SWI/SNF complex, EZH2 inhibition by EPZ-6438 might contribute to restoration of the balance between the PRC2 and SWI/SNF complexes, leading to an anti-tumor effect. SNF5 is inactivated through biallelic mutation in nearly all cases of malignant RTs and atypical teratoid RTs [131]. Synthetic lethality therapy using EZH2 inhibitors in such tumors is awaiting clinical application.

Targeting BRD4, which is involved in transcriptional regulation of cancer-related genes

BRD4 is a member of the bromodomain and extraterminal (BET) subfamily. These proteins recognize acetylated lysine residues on chromatin and bind to them via bromodomains, facilitating activation of transcription along surrounding DNA. JQ1, a small-molecule inhibitor of BRD4, competitively binds to the bromodomain in BRD4, leading to displacement of BRD4 from chromatin [132]. Recent studies have revealed the promising anti-tumor effects of JQ1 in various hematologic malignancies [133–135]. In an AML mouse model, Zuber *et al*. performed a screen for epigenetic vulnerability using short-hairpin RNAs targeting known chromatin-regulating proteins and identified BRD4 as a key hit [133]. Consistent with this, they showed that JQ1 inhibits proliferation of diverse subtypes of AML cells. Merz *et al*. demonstrated that cell lines derived from various hematologic malignancies were highly susceptible to JQ1 [134]. Delmore *et al*. showed that JQ1 prolongs survival of mice bearing multiple myeloma [135]. Furthermore, Dawson *et al*. developed another BRD4 inhibitor, I-BET151, and showed that this compound prolonged the lifespan of mice with mixed-lineage fusion leukemia treated with I-BET151 [136]. On the other hand, JQ1 also exerts an anti-tumor effect in nuclear protein in testis (NUT) midline carcinoma, a rare subtype of squamous cell carcinoma with an aggressive nature, in which t(15;19) chromosomal translocation results in a fusion between BRD4 and NUT [137].

Previous studies have suggested that the anti-tumor effect of BRD4 inhibition depends on suppression of *MYC* transcription, leading to genome-wide downregulation of *MYC*-dependent target genes [134, 135]. In addition to *MYC*, two other well-known cancer-related genes, *BCL2* and *CDK6*, have recently been identified as targets of transcriptional regulation by BRD4 [136]. The preclinical results are encouraging with regard to potential clinical applications of BRD4 inhibitors. However, because BRD4 is broadly expressed in almost all normal human cells and regulates gene expression throughout the genome, the off-target effects of BRD4 inhibition in humans should be carefully investigated. Most recently, the mechanism underlying selective activation of *MYC* transcription by BRD4, which has been termed a ‘super-enhancer’, was revealed in multiple myeloma cells [138]. Further studies are needed to establish the optimal way to treat cancer by targeting BRD4.

PERSPECTIVES

The growing incidence of cancer worldwide indicates that radiation therapy will become increasingly significant in cancer treatment [139]. Enhancing the efficacy of IR against cancer cells is paramount if we are to improve local control of tumors. Recent studies have elucidated the involvement of various chromatin-regulating proteins in the DDR after irradiation, and these proteins represent promising targets for radiosensitization. As we discussed, several inhibitors of HATs or HDACs exert radiosensitizing effects in preclinical models. However, the detailed mechanisms by which these compounds sensitize cancer cells to IR are still largely unknown. Moreover, it remains unknown whether these compounds can achieve clinically significant radiosensitization in humans at a dose that causes no (or at least low) toxicity; this issue warrants further investigation.

Large-scale genome sequencing techniques have elucidated the genetic landscape of various cancers, accelerating the development of molecularly targeted drugs. High frequencies of mutation in chromatin-regulating genes, especially in the SWI/SNF genes, highlight the pivotal roles of chromatin-regulating proteins in cancer cells, and thus offer rationales for targeting these mutations in cancer therapy. DOT1L inhibition in MLL-rearranged leukemia, EZH2 inhibition in EZH2-mutant or MLL-rearranged hematologic malignancies and SNF5-deficient tumors, BRD4 inhibition in hematologic malignancies, and BRM inhibition in BRG1-deficient tumors all hold great promise and are

awaiting clinical applications. Moreover, considering the role of chromatin-regulating proteins in the DDR [4], elucidation of the association between mutation status in chromatin-regulating genes and the clinical outcome of radiation therapy and/or chemotherapy will facilitate the selection of patients who will (or will not) respond to such therapies. Accordingly, we recently reported that single-nucleotide polymorphisms in the *BPTF* gene, which encodes a bromodomain PHD finger transcription factor contained in the NURF chromatin-remodeling complex, affect the risk of lung adenocarcinoma in the Japanese population [140]. Future studies will elucidate the molecular mechanisms underlying the effect of aberrant chromatin-regulating proteins on carcinogenesis and cancer progression, and will contribute to the establishment of personalized treatment based on the genetic profiles of tumors.

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