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Chromatin remodeling and cancer, part II: ATP-dependent chromatin remodeling

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Connections between perturbations that lie outside of our genome, that is, epigenetic alternations, and tumorigenesis have become increasingly apparent. Dynamic chromatin remodeling of the fundamental nucleosomal structure (covered in this review) or the covalent marks residing in the histone proteins that make up this structure (covered previously in part I) underlie many fundamental cellular processes, including transcriptional regulation and DNA-damage repair. Dysregulation of these processes has been linked to cancer development. Mechanisms of chromatin remodeling include dynamic interplay between ATP-dependent complexes, covalent histone modifications, utilization of histone variants and DNA methylation. In part II of this series, we focus on connections between ATP-dependent chromatin-remodeling complexes and oncogenesis and discuss the potential clinical implications of chromatin remodeling and cancer.

Introduction

Eukaryotic genetic information is stored in chromatin, a string of repeating units of nucleosomal core particles where ~146 base pairs of DNA are wrapped approximately two times around a histone octamer that contains two copies each of H2A–H2B and H3–H4 dimer pairs. These nucleosomes are then further packed with linker histones and other architectural proteins into higher-order chromatin structures that remain poorly defined. During all DNA-templated cellular processes, chromatin structures undergo dynamic remodeling (opening and closing of higher-order structures) to allow access to associated DNA segments.

Over the last few decades, cancer research has delineated six essential pathways whose alterations collectively dictate malignant growth: self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis and tissue invasion and metastasis [1]. Traditional cancer research has focused on identification of genetic mutations, such as amplifications, deletions and point mutations, that target the molecular players involved in these pathways. It has revolutionized our understanding of the molecular mechanisms in cancer development and progression. Yet, recently, it has become increasingly apparent that epigenetic alternations (DNA-sequence-independent alterations, such as chromatin remodeling that alters the transcriptional regulation of tumor suppressors or proto-oncogenes) play equally important roles in tumorigenesis. Dynamic chromatin remodeling utilizes several basic mechanisms, including covalent histone modifications, ATP-dependent chromatin remodeling (Figure 1), utilization of histone variants (Figure 1) and DNA methylation to alter the accessibility of DNA. These mechanisms work either independently or in concert to allow optimal chromatin remodeling for efficient transcriptional regulation, DNA replication and DNA-damage repair. Because the oncogenic connections of DNA methylation have been extensively reviewed elsewhere [2–5] and those of covalent histone modifications have been reviewed in the previous article (see 'writers', 'erasers' and 'readers' of covalent marks in part I of this series [6]), here and for clarity we focus on the evidence and clinical implications of ATP-dependent chromatin remodeling in tumorigenesis (see Figure 1 for schematic on chromatin remodeling). However, we stress that all of these mechanisms are likely to work together to bring about functional chromatin states. For example, new evidence suggests that some 'readers' of certain covalent histone modifications are themselves subunits of ATP-dependent remodeling complexes, providing a direct link between covalent and non-covalent mechanisms (e.g. see NURF (nucleosomes remodeling factor) in Figure 2a). It also remains an intriguing possibility that certain chromatin-remodeling complexes exchange histone dimer pairs in nucleosomes as a means of introducing new epigenetic 'signatures' by altering the landscape of their post-translational modifications (see Figure 1 and part I of this series [6]).

ATP-dependent chromatin-remodeling enzymes and their functions

ATP-dependent chromatin-remodeling enzymes, which are highly conserved in organisms from yeast to humans, are similar to the SNF2 (sucrose non-fermenting 2) family of DNA translocases and all contain a catalytic ATPase subunit [7]. These ATPase machineries utilize the energy of ATP hydrolysis to mobilize nucleosomes along DNA, evict histones off DNA or promote the exchange of histone

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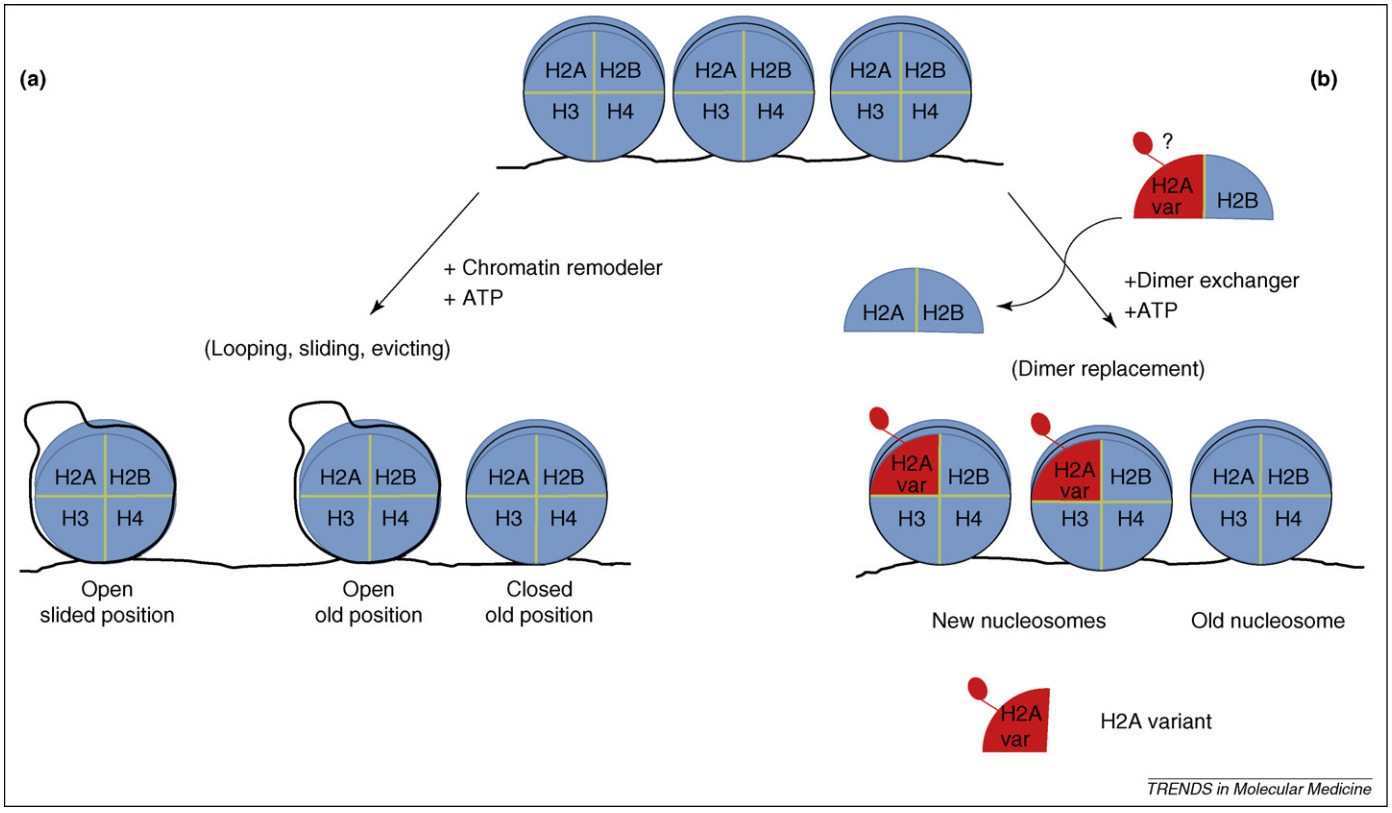

Figure 1. Schematic illustration of two major mechanisms of ATP-dependent chromatin remodeling. **(a)** The first mechanism involves utilization of the energy from ATP hydrolysis to bring about 'chromatin remodeling', generally defined as nucleosomal structural changes that involve dissociation of DNA-histone contacts (looping), translocation of the nucleosome along DNA (sliding) or eviction of nucleosomes; these changes create more-open or -exposed chromatin regions with increased DNA accessibility. **(b)** The second mechanism involves utilization of the energy from ATP hydrolysis to bring about 'exchange' of nucleosomal subunits, such as H2A–H2B or H2A variants (H2Avar)–H2B dimers, that can be either unmodified or pre-modified with specific post-translational modifications (shown by question mark, see part I). Structural features harbored in histone variants impart context-dependent biological consequences.

variants (Figure 1), which in turn modulate DNA accessibility and alter nucleosomal structures (Figure 1 and Figure 2) [8]. Although mechanistic details of exactly how nucleosomal 'sliding', 'looping' and 'twisting' occur are still unclear in all cases, most evidence suggests that critical histone-DNA contacts are being disrupted in an energy-dependent process, leading to models of 'regulated nucleosome mobility' [9,10] that might contribute to chromatin dynamics by mechanisms distinct from modifications directed toward the histone tail (see part I of this series [6]).

Based on distinct domain structures, there are four well-characterized families of mammalian chromatin-remodeling ATPases: the SWI/SNF (switching defective/sucrose non-fermenting) family, the ISWI (imitation SWI) family, the NuRD (nucleosome remodeling and deacetylation)/Mi-2/CHD (chromodomain, helicase, DNA binding) family and the INO80 (inositol requiring 80) family [8,11] (Table 1). Both members of the SWI/SNF family of ATPases, BRM (homologue of *Drosophila* protein 'brahma') and BRG1 (BRM/SWI2-related gene 1), contain a C-terminal bromodomain that binds to acetylated histone tails [12]. ISWI family members, SNF2H and SNF2L, have a SANT ('SWI3, ADA2, NCOR and TFIIIB' DNA-binding domains) and a SLIDE (SANT-like ISWI) domain that mediate interaction with unmodified histone tails and linker DNA [13]. NuRD/Mi-2/CHD family members, CHDs 1–5, have unique tandem chromodomains that specifically recognize methylated histone tails [14,15]. INO80 family members, INO80, SNF2-related CREB-activator protein (SRCAP) and p400, are characterized by split ATPase domains [16]. Although the ATPase domains are highly similar, the presence of distinct chromatin-interacting domains (bromo, chromo and SANT domains) in different ATPase remodelers suggest that they can be selectively targeted to chromatin regions with distinct modification patterns to carry out specialized roles. Moreover, these ATPase-dependent remodeling enzymes all act in the context of multisubunit complexes (Table 1), which adds an additional layer of fine-tuned specificity in ATP-dependent chromatin remodeling.

One fundamental role of chromatin remodeling is transcriptional regulation. SWI/SNF remodeling complexes primarily disorganize and reorganize nucleosome positioning to promote accessibility for transcription-factor binding and gene activation [17]; however, they also promote transcriptional-repressor binding and gene repression under certain conditions [18]. ISWI remodeling complexes primarily organize and order nucleosome positioning to induce repression [19], although they also mediate transcriptional activation [19,20] and transcriptional elongation [19,21]. NuRD/Mi-2/CHD remodeling complexes primarily mediate transcriptional repression in the nucleus [8]; however, they are also involved in transcriptional activation of rRNA in the nucleolus [22]. Similarly, the INO80 remodeling complexes appear to have both activating and repressive effects for a

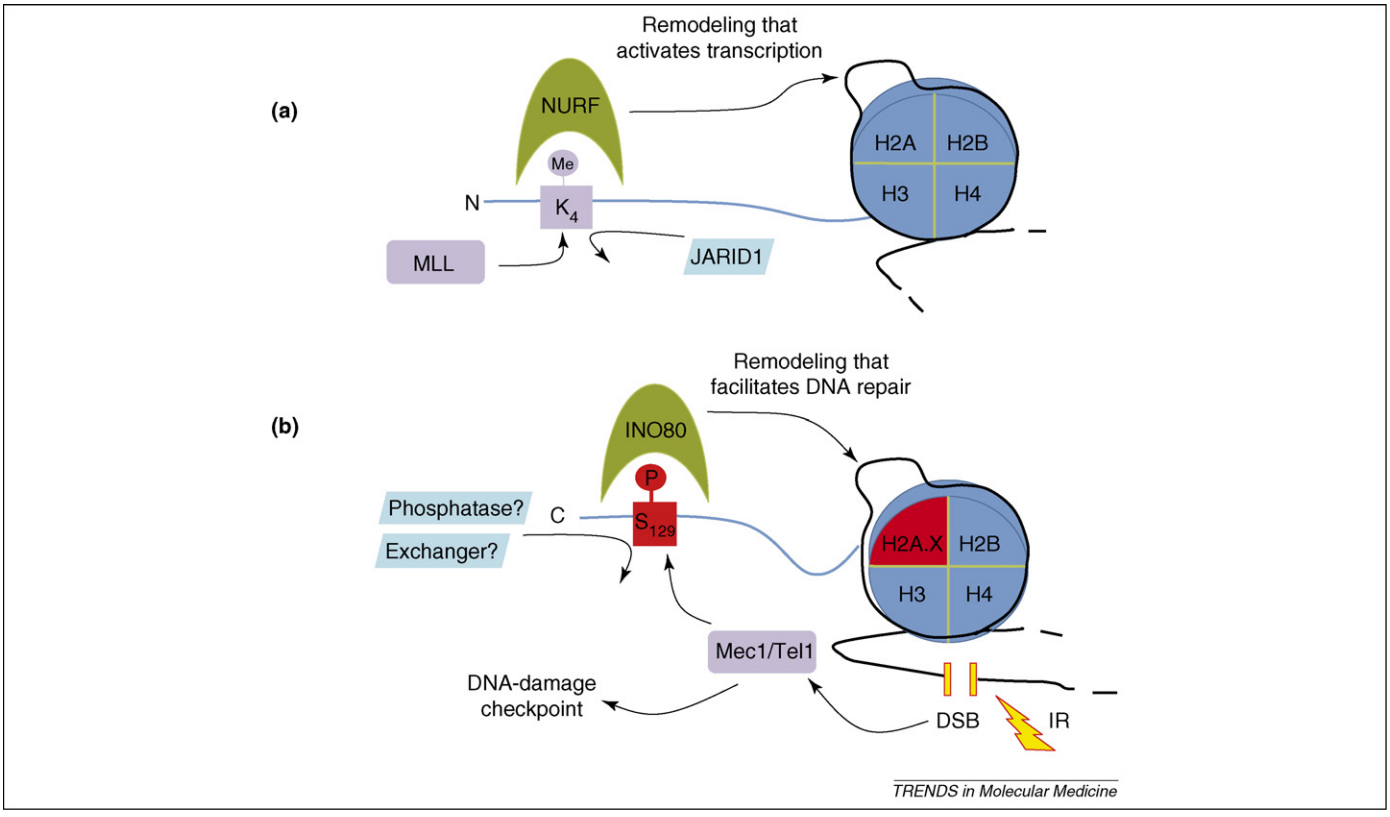

Figure 2. Schematic representation of interaction between ATP-dependent chromatin remodeling and covalent histone modifications during transcriptional regulation and the DNA-repair response. **(a)** Cross-talk between H3K4 trimethylation and nucleosome remodeling factor (NURF) complex during gene activation. Dynamics of H3K4 trimethylation, a prominent covalent histone modification enriched in active chromatin regions, is maintained by its specific 'writers', (e.g. the MLL-family histone methyltransferases) and antagonizing 'erasers' (e.g. JARID1-family demethylases). Incorporation of the trimethyl-H3K4 mark into an 'aromatic cage' formed by the PHD finger within BPTF, a core subunit of the NURF complex, facilitates stabilization of NURF complexes, which, in turn, carry out nucleosome remodeling, leading to the formation of more-open chromatin strucutures and trasnscriptional activation. **(b)** Cross-talk between phosphorylation of H2A.X (γ-H2A.X) and INO80 remodeling complexes during the DNA double-strand break (DSB) repair response in yeast. Upon insults, such as ionizing radiation (IR), that lead to the formation of DSBs, spreading of γ-H2AX along the region flanking the DSB is induced via Mec1/Tel1 kinases, which are part of DNA-damage checkpoint mechanisms. Phosphorylation of Ser129 of γ-H2AX recruits INO80 remodeling complexes, which, in turn, initiate nucleosomal remodeling and facilitate DNA accessibility to DNA-repair machinery. Restoration of the chromatin state after repair of a DSB is presumably achieved via desphosphorylation of γ-H2A.X by a phosphatase or by H2A–H2B dimer exchange by an ATP-dependent remodeling complex, although detailed mechanisms remain unclear (see question marks).

specific set of genes [23–25]. Therefore, all ATP-dependent chromatin-remodeling complexes can lead to transcriptional activation and repression, and the precise outcome of their action is dependent on the particular chromatin context. However, detailed mechanisms of context-dependent transcriptional regulation by these ATPase chromatin remodelers remain to be elucidated. Nonetheless, recent studies have provided new insights into how some of the specificity of transcriptional regulation can be achieved. For example, as shown in Figure 2a, a member of the ISWI family of ATP-dependent chromatin-remodeling complexes, the NURF remodeling complex, has been identified as one component required for activation of *Hox* genes and maintenance of *Hox* gene expression patterns during development [26]. This specialized transcriptional regulation is brought about, at least in part, by methylation of lysine 4 on histone H3 (H3K4Me3), which recruits and stabilizes the NURF complex via direct association with the plant homeodomain (PHD) finger of the NURF subunit, BPTF (bromodomain and PHD-finger transcription factor) [26]. More interestingly, disruption of the *Drosophila* NURF complex (either of the catalytic subunit, ISWI, or the BPTF homologous subunit, NURF301) causes misregulation of homoetic gene expression and interferes with hematopoietic development, which leads to the transformation of larval blood cells and melanotic tumors [20]. As shown in Figure 2b, during DNA double-strand break (DSB) repair in yeast, H2A.X, a specialized member of the H2A family, is rapidly phosphorylated at Ser129 (γ-H2A.X), and this phosphorylation leads to a connection between the INO80 chromatin-remodeling complex and DSBs via direct interaction of γ-H2A.X and Arp4, a subunit of the INO80 complex, to facilitate DSB repair [25,27,28].

Besides a shared role in transcriptional regulation, these ATP-dependent chromatin-remodeling complexes also have unique functions in other cellular processes. The ISWI-family remodelers have been shown to play central roles in chromatin assembly after DNA replication and maintenance of higher-order chromatin structures [19,29]. The INO80- and SWI/SNF-family remodelers participate in DNA DSB repair and nucleotide-excision repair (NER) and thereby connect chromatin remodeling with DNA-damage response [25,30,31]. The methyl-CpG-binding domain protein 3 (MBD3), a component of the NuRD/Mi-2/CHD complex, is required for the maintenance of pluripotency of embryonic stem cells [32]. Similarly, the ISWI family of chromatin remodelers is required for the maintenance and self-renewal of germline and somatic stem cells in the *Drosophila* ovary [33]. Hence, ATP-dependent chromatin-remodeling complexes regulate a wide

Table 1. Human ATPase-dependent chromatin-remodeling complexes and cellular functions

Family and complexes	Remodeling-complex subunits	Complex functions	Refs
SWI/SNF family			
BAF	BRM or BRG1, SNF5/INI1, BAF155, BAF170, BAF250, BAF53, β-actin, BAF60a, BAF57	Tumor suppressor, cell-cycle progression, DNA replication, development, differentiation, elongation, signaling, splicing, DNA-damage repair	[11,17,66]
PBAF	BRG1, SNF5/INI1, BAF155, BAF170, BAF180, BAF53, β-actin, BAF60a		[11,17,66]
BRM	BRM, SNF5/INI1, BAF155, BAF170, BAF250, BAF53, BAF60a		[17]
BRG1-complex I	BRG1, SNF5/INI1, BAF155, BAF170, BAF250, BAF53, BAF60a		
BRG1-complex II	BRG1, SNF5/INI1, BAF155, BAF170, BAF250, BAF53		
EBAFa	BRG1, SNF5/INI1, BAF155, BAF170, BAF250a, BAF53, β-actin, BAF60a, ENL, EBAF70, EBAF100, EBAF140		
EBAFb	BRG1, SNF5/INI1, BAF155, BAF170, BAF250b, BAF53, β-actin, BAF60b, ENL, EBAF70, EBAF100, EBAF140		
ISWI family			
ACF/WCRF	SNF2H, WCRF180/ACF1	X-chromosome regulation, cohesion, embryonic development and differentiation, transcriptional activation and repression, DNA replication, DNA repair response	[11,19,66]
CHRAC	SNF2H, ACF1, CHRAC17, CHRAC15		[19,66]
RSF	SNF2H, p325		[19,66]
WICH	SNF2H, WSTF		[19,66]
SNF2H/Cohesin	SNF2H, Mi-2, Rad21, HDAC1, HDAC2, MTA1, MTA2, SA1/SA2, RbAp46, RbAp48, MBD2, MBD3, SMC1, SMC3		[19,66]
NURF	SNF2L, BPTF, RbAp46, RbAp48		[11,66]
NURD/Mi-2/CHD family			
NuRD/Mi-2/CHD	Mi2-α/CHD3 or Mi2-β/CHD4 or CHD1–2 or CHD5, HDAC1, HDAC2, RbAp46, RbAp48, MTA1 or MTA2 or MTA3, MBD2 or MBD3	Tumor suppressor, transcriptional repression and silencing, transcriptional activation, pluripotency of embryonic stem cell	[11,55]
INO80 family			
INO80	hINO80, Tip49a, Tip49b, BAF53a, Arp5, Arp8, hIes2, hIes6, Amida, NFRKB, MCRS1, FLJ90652, FLJ20309		[11,66,67]
TRRAP/Tip60	P400, Tip49a, Tip49b, BAF53a, actin, GAS41, DMAP1, YL-1, Brd8, TRRAP, Tip60, MRG15, MRGX, FLJ11730, MRGBP, EPC1, ING3		[11,67]
SRCAP	SRCAP, Tip49a, Tip49b, BAF53a, Arp6, GAS41, DAMP1, YL-1, ZnF-HIT1		[11,67]

Abbreviations: ACF, ATP-utilizing chromatin-assembly and remodeling factor; BAF, BRG1-associated factor; CHRAC, chromatin-accessibility factor; EBAF, ENL (a fusion partner of MLL in mixed-lineage leukemia)-associated BAF-containing complex; ENL, eleven-nineteen leukemia gene; MBD, methyl-CpG-binding domain protein; PBAF, polybromo and BRG1-associated factor; RSF, remodeling and spacing factor; SA1, stromal antigen 1; SMC1, structural maintenance of chromosomes 1A; WICH, WSTF-ISWI chromatin remodeling; WSTF, Williams syndrome transcription factor.

range of cellular processes, including transcription regulation, DNA-damage response, DNA replication and cellular identity determination. Dysregulation of any of these processes can result in neoplastic transformation and tumorigenesis (Table 1). As detailed below, we will focus on emerging evidence that connects dysregulation of the chromatin-remodeling complexes to the pathogenesis of cancer.

ATP-dependent chromatin remodeling and cancer

The SWI/SNF complex

Among the subunits of the SWI/SNF complexes, the core subunit, SNF5, and the catalytic subunits, BRG1 and BRM, present the most convincing examples of connections between SWI/SNF complexes and tumorigenesis. Examples from other subunits in the SWI/SNF complexes are also emerging [34].

The SWI/SNF core subunit SNF5

SNF5 is one of the core subunits required for the ATP-dependent remodeling activity of the SWI/SNF complex. Increasing evidence from studies in human genetics and murine models supports SNF5 as a tumor suppressor [35]. The SNF5 gene is found to have undergone bi-allelic loss in the majority of human malignant rhabdoid tumors (MRTs) [36], and some 'proximal-type' epithelioid sarcomas [37]. MRTs are a highly aggressive group of tumors that usually occur in early childhood in various locations, including the kidney, lung, soft tissue and brain [35]. In addition, germline mutations in SNF5 have been identified in young children with MRTs and choroid plexus carcinoma from cancer-prone families [35,38]. These studies are consistent with Knudson's two-hit model of oncogenesis and suggest that SNF5 functions as a tumor suppressor.

The tumor-suppressive function of SNF5 has been further confirmed with a series of genetically targeted murine models [39–42]. $SNF5^{-/-}$ mice are embryonically lethal by day 7 [39–41]. By 15 months of age, $SNF5^{+/-}$ mice are prone to develop tumors that mostly resemble human MRTs [39–41]. Notably, all tumor cells have undergone loss of the remaining wild-type allele of SNF5 [40]. To overcome embryonic lethality and study the effect of complete loss of SNF5, chimeric mice with a subset of cells harboring inactivating mutations of both alleles of SNF5 were developed in an $SNF5^{+/-}$ background. [42]. These mice are viable and 100% of them develop either mature $CD8^+$ T cell lymphoma or rhabdoid tumors with a median onset of 11 weeks [42]. This reflects an unusually rapid course of cancer development when these chimeric $SNF5^{-/-}$ mice are compared with $p53^{-/-}$ mice (median 20 weeks) or $p16^{Ink4a-/-}$ mice (median 60 weeks) [35,42].

How SNF5 exerts its tumor-suppressive function remains an area of active investigation. Emerging evidence has linked SNF5 to the regulation of cell-cycle progression.

Reintroduction of human SNF5 into cultured SNF5$^{-/-}$ MRT cells induces cell arrest at G0/G1, cell senescence or apoptosis [43,44]. The G0/G1 arrest is mediated through functional retinoblastoma (pRb) and p16^{Ink4a} tumor suppressors [44,45]. In addition to its anti-proliferative effects, SNF5 has also been shown to control mitotic checkpoints, regulate cellular ploidy and maintain chromosomal stability via the p16^{Ink4a}-cyclin D/CDK4-pRb-E2F pathway [46]. SNF5$^{-/-}$ mouse embryonic fibroblasts (MEFs) exhibit hypersensitivity to genotoxic stress, such as UV irradiation and doxorubicin treatment, suggesting a role for SNF5 in the DNA-damage response [47]. Thus, SNF5 might prevent tumorigenesis by regulating cell proliferation, controlling cell-cycle progression, maintaining chromosomal stability and participating in DNA-damage repair. Nonetheless, it remains unclear whether the tumor-suppressor function of SNF5 requires SWI/SNF-mediated chromatin remodeling.

The SWI/SNF ATPase subunits – BRG1 and BRM

BRG1 and BRM, the ATPase subunits that are mutually exclusive in the SWI/SNF complexes, harbor tumor-suppressor properties [35]. Bi-allelic loss of the human BRG1 gene has been reported in prostate, lung, breast and pancreatic cancer cell lines [35]. Concomitant loss of both BRG1 and BRM has been observed in ~30% of non-small-cell lung cancer (NSCLC) cell lines and in ~10% of primary NSCLC [48]. BRG1$^{-/-}$ mice die during early embryogenesis, whereas BRG1$^{+/-}$ mice are prone to develop tumors of epithelial origin [49]. BRG1 appears to be haploinsufficient in this model because tumors do not exhibit loss or mutation of the other allele [35,49]. BRM$^{-/-}$ mice are viable and do not develop tumors [50]. However, MEFs isolated from these mice display increased cell proliferation and deficiency in G0/G1 arrest in response to DNA damage [50]. *In vitro*, BRG1 and BRM have been demonstrated to interact with several tumor suppressors, including pRb and BRCA1 [51].

The observation that loss of BRG1, BRM or SNF5 all result in malignancies of different severity and phenotypes suggests that each of these subunits might possess specialized roles in addition to their participation in the SWI/SNF remodeling complexes. Data connecting SWI/SNF to cancer continues to accrue, but the mechanisms underlying the cancer pathways that are relevant to SWI/SNF-mediated chromatin remodeling remain to be defined.

The NuRD/Mi-2/CHD complex

The NuRD/Mi-2/CHD ATPase subunit CHD5

CHD family members, CHDs 1–5, make up the ATPase subunit of the NuRD chromatin-remodeling complexes [8,52]. 1p36.3 is a genomic region frequently deleted in neuroblastoma and other malignancies of epithelial and hematopoietic origins [53,54]. Bagchi and colleagues have recently demonstrated that CHD5 is a tumor-suppressor gene at human 1p36 by utilizing genetically engineered mice that harbor rearrangement of the corresponding human 1p36 locus [54]. CHD5$^{+/-}$ mice are prone to spontaneous tumors, including lymphoma and squamous cell carcinoma. At the cellular level, CHD5 is found to regulate proliferation, apoptosis and senescence in a dosage-dependent manner via the p19^{Arf}-p53 pathway [54]. It is hypothesized that CHD5 and its associated NuRD complex maintain a chromatin state that favors active transcription of p19^{Arf}. Because CHD5$^{+/-}$ mice have a stronger phenotype than p19$^{Arf\ -/-}$ mice, other mechanisms of tumor suppression are likely to be important.

The NuRD/Mi-2/CHD subunits – MTA proteins

Metastasis-associated gene 1 (MTA1) was originally identified in rat and human metastatic breast cancer-cell lines [55]. MTA1 and its homologues, MTA2 and MTA3, are part of the NuRD/Mi-2/CHD remodeling complexes [55]. MTA1 overexpression is closely associated with invasive behavior and has been observed in >30% of primary esophageal, colorectal and gastric carcinomas [55]. In breast cancer, MTA1 is a target of growth-factor-signal transduction in the HER2 pathway [55]. MTA1s, a naturally occurring variant of MTA1, is overexpressed in breast tumors with low or no nuclear estrogen receptor (ER); MTA1s inhibits nuclear signaling by sequestering ER in the cytoplasm, promoting a non-genomic response of ER and thereby stimulating tumorigenesis in breast cancer [56].

MTA3 is an ER-dependent component of the NuRD/Mi-2/CHD remodeling complex, and its expression significantly correlates with ER expression in breast cancer [57]. In response to ER signaling, MTA3 directly inhibits transcription of Snail, a master regulator of epithelial-to-mesenchymal transitions (EMTs) [57]. EMT is a critical step in tumor metastasis. Therefore, downregulation of MTA3 in ER-negative breast cancer might lead to aberrant overexpression of Snail and result in a metastatic phenotype. Moreover, MTA3 interacts with Bcl-6, a proto-oncogene and a transcriptional repressor that prevents maturation of B lymphocytes into plasma cells in the germinal center. Introduction of Bcl-6 and MTA3 into plasma cells results in de-differentiation toward a B cell state [58]. Bcl-6 is the most frequently mutated gene in non-Hodgkin's lymphomas, and inhibitors targeting the MTA3 and Bcl-6 interaction might have a therapeutic benefit. Nonetheless, it remains unclear whether the cancer phenotype associated with aberrant MTA3 expression requires NuRD/Mi-2/CHD chromatin-remodeling activities.

The INO80 complex

The INO80-family chromatin-remodeling complexes, the INO80 and SWR1 complexes, are evolutionally conserved in organisms from yeast to mammals. At present, functional studies have primarily been performed in yeast, and parallels in mammals must be drawn with some caution. As discussed in part I of 'Chromatin remodeling and cancer' [6], upon insults of DSBs, H2A.X, an H2A variant, is phosphorylated at Ser139 (γ-H2A.X) in mammalian cells (and at Ser129 in yeast) in the highly conserved C-terminal tail. This phosphorylation event plays essential roles in DSB repair, maintenance of genome stability and tumor suppression [59]. In yeast, it has been found that the INO80 family members are recruited to DNA DSB sites to mediate both homologous-recombination and non-homologous-end-joining repair pathways [25,28,60,61]. The

recruitment of INO80 remodeling complexes to DSB sites is mediated by direct interaction of γ-H2A.X and Arp4, a component of the INO80 complex [27,28] (Figure 2b). Disruption of INO80 or SWR1 remodeling complexes in yeast results in hypersensitivity to DNA-damaging agents such as UV and alkylators; a similar phenotype is seen in yeast strains carrying non-phosphorylatable H2A.X mutants [25]. Mechanistic studies in yeast indicate that INO80-mediated chromatin remodeling promotes DNA accessibility to repair machineries and also facilitates single-stranded DNA (ssDNA) formation, a critical step of the homologous-recombination repair pathway [25]. INO80 is also implicated in homologous recombination in plants [62]. Whether there is a parallel pathway linking chromatin remodeling and DNA-damage repair in mammalian cells remains to be elucidated.

Concluding remarks

Despite emerging evidence that closely connects ATP-dependent chromatin-remodeling complexes with tumorigenesis, direct evidence supporting a causal role of ATP-dependent chromatin-remodeling activity *per se* in oncogenesis remains to be established. For example, as discussed above, mutations in SNF5, BRG1 or BRM are intimately associated with tumorigenesis. Presumably, these mutations all result in the disruption of SWI/SNF-mediated ATP-dependent chromatin-remodeling activities at the cellular level; however, loss of SNF5, BRG1 or BRM results in different types of cancer of different severity (rhabdoid tumors and lymphoma for SNF5 mutation and epithelial tumors for BRG1 and BRM mutations). In light of this, is ATP-dependent chromatin-remodeling activity truly relevant to tumorigenesis? Do SNF5, BRG1 and BRM have different interacting partners beyond the SWI/SNF remodeling complexes and might these partners account for the different cancer phenotypes? Given the different cancer types, is there differential tissue-specific expression of these proteins, and is there cellular-lineage-dependent (ectoderm, mesoderm, endoderm) chromatin regulation by these proteins (Box 1)?

At the cellular level, it appears that SNF5 regulates cell-cycle progression and maintains chromosomal stability and ploidy through the tumor-suppressive $p16^{Ink4a}$-cyclin D/CDK4-pRb-E2F pathway. However, it remains unclear whether the chromatin-remodeling ATPase activities *per se* are involved in the transcriptional regulation of $p16^{Ink4a}$ and whether they are responsible for the cancer phenotype. 'Rescue experiments' by reconstitution of either a wild-type or an ATPase-defective mutant SWI/SNF remodeling complex in the $SNF5^{-/-}$ background might provide some insights. Because of the genome-wide regulatory role of the SWI/SNF ATP-dependent remodeling complexes, it is equally unclear whether cell-cycle-checkpoint pathways truly represent the crucially relevant oncogenic pathways or just 'conveniently' identified pathways. This question can be addressed by a 'rescue experiment' that examines whether reconstitution of $p16^{Ink4a}$ function can reverse the cell-cycle-defect phenotype in $SNF5^{-/-}$ murine models. Downregulation of tumor suppressor $p16^{Ink4a}$ probably represents only part of the tumor-suppressive mechanisms of SNF5 because $SNF5^{-/-}$ mice exhibit a much more dramatic tumorigenic phenotype than $p16^{Ink4a-/-}$ mice. Identification of gene promoters directly targeted by SNF/SWI remodeling complexes with genome-wide analysis, combined with gene-expression profiling analysis of wild-type versus null cells, will help to dissect oncogenesis-related direct targets.

It is clear that alterations in many, if not all, chromatin-remodeling processes, including ATP-dependent chromatin remodeling (reviewed here), histone modifications (reviewed in part I of this series [6]) and DNA methylation (discussed elsewhere [2]), can contribute to oncogenesis. Chromatin remodeling not only regulates gene transcription but also participates in fundamental cellular processes that are intimately associated with oncogenesis. Such processes include DNA-damage repair, apoptosis and chromosome condensation and segregation. However, perturbations of each of the remodeling processes lead to different tumor phenotypes, as evidenced in mouse models and human cancer studies, implying that these processes act in a context-dependent manner. Despite emerging evidence that these chromatin-remodeling pathways interact with each other, it remains a future challenge for researchers to define clear links among histone modifications, DNA methylation and ATP-dependent chromatin remodeling, especially in the context of tumorigenesis. For example, new findings suggest that a catalytically inactive DNA methyltransferase, DNMT3L, reads a specific methylation 'signature' on histone H3, providing an intriguing link between histone modifications and DNA methylation [63].

On another level, epigenetic regulation (e.g. gene silencing of tumor suppressors) and genetic regulation (e.g. loss of tumor suppressors) probably cooperatively contribute to tumorigenesis [3]. What are the key cancer-causing 'determinant' steps? Which of these, if any, are upstream or downstream of one another? Given the complexity of epigenetic regulation, it is conceivable that each epigenetic regulatory process, that is, DNA methylation, histone modifications and ATP-dependent chromatin remodeling, as well as utilization of histone variants, contributes to tumorigenesis differently in a context-dependent manner. In a given type of cancer, there is increasing evidence that multiple regulatory processes are involved. For example, global methylation studies in colorectal cancer revealed long-range epigenetic silencing (a genomic region of more than 4 Mb on chromosome 2q14.2) mediated by three clusters of CpG island hypermethylation (~1 Mb each) and/or H3K9 methylation over the entire region [64]. Therefore, when one examines a sole regulatory process in oncogenic studies, it is important to keep in mind that multiple regulatory processes can lead to similar consequences, such as gene silencing in tumorigenesis. This is especially important

Box 1. Outstanding questions

- Is there a causal role for ATP-dependent chromatin-remodeling activity in oncogenesis?
- Is there tissue-specific expression of different ATP-dependent chromatin-remodeling complexes?
- What are the mechanisms of lineage-specific oncogenesis observed in mutations with different ATP-dependent chromatin-remodeling complexes?

when interpreting data, trying to develop epigenetic biomarkers or generating hypotheses for therapeutic interventions. Ideally, in light of the advances in global genomic and epigenomic technologies, it would be useful to correlate global cancer epigenomics to global gene expression by using chromatin immunoprecipitation on chip (ChIP on chip) of known histone modifications (e.g. H3K9 and H3K27 methylation in gene silencing and H3K4 methylation in gene activation), global CpG-island-methylation screening and microarray expression profiling. These types of approaches are proving valuable in the dissection of genetic and epigenetic signature genes and pathways for specific cancer types and in helping us understand how these epigenetic pathways work together to bring about malignant transformation [64,65]. Clearly, more collaborative research effort is needed if we are to study multiple cancer-related epigenetic pathways simultaneously to try to delineate the 'dominant' pathways for therapeutic-target development in cancer treatment. Additionally, it will be important to explore strategies where multiple epigenetic pathways are being targeted. Such strategies include the ongoing attempt to override epigenetic silencing with synergistic effects between HDAC inhibitors and DNA-demethylating agents [3]. Additionally, what are the clinically relevant substrates of HDACs, or of any other chromatin-modifying activities, with respect to distinct types of cancers (see below and Box 1 in part I [6]).

In closing, it is becoming clear that covalent (part I) and non-covalent (part II) mechanisms work together to introduce variation into the chromatin polymer and create far-reaching implications for human biology and human health, notably with regard to cancer. However, the extent to which histone proteins are true carriers of epigenetic information remains less clear, as do the exact mechanisms by which histone-based information might be inherited from one generation to the next. The extent to which histone proteins are the physiologically relevant substrate for any of the cancer phenotypes is also unclear. Future research efforts should be directed not only at investigating the basic mechanisms of chromatin remodeling in oncogenesis but also at integrating available basic research findings in clinical applications.

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