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Histone modifying and chromatin remodelling enzymes in cancer and dysplastic syndromes

Richard J. Gibbons*

MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DS, UK

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Inactivation of tumour suppressor genes is central to the development of cancer. Although this inactivation was once considered to be secondary to intragenic mutations, it is now clear that silencing of these genes often occurs by epigenetic means. Hypermethylation of CpG islands associated with the tumour suppressor genes was the first manifestation of this phenomenon to be described. It is apparent, however, that this is one of a host of chromatin modifications which characterize gene silencing. Although we know little about what determines which loci are affected, our understanding of the nature of the epigenetic marks and how they are established has blossomed. There is no compelling evidence that cancer ever develops by purely epigenetic means, but it is apparent that perturbations in the apparatus which establish the epigenome may contribute to the development of cancer. This review will focus on the role of two classes of chromatin remodelling enzymes, those that alter histones by the addition or removal of acetyl and methyl groups and those of the SWI/SNF family of proteins that change the topology of the nucleosome and its DNA strand via the hydrolysis of ATP, and we shall examine the consequence of mutations in, or mis-expression of, these factors. In some cases, mutations in these factors appear to play a direct role in cancer development. However, their general role as important intermediaries involved in regulating gene expression makes them attractive therapeutic targets. In exciting developments, it has been shown that inhibition of these factors leads to the reversal of tumour suppressor gene silencing and the inhibition of cancer cell growth.

INTRODUCTION

Although a more thorough description of the epigenetic marks may be found elsewhere, it is helpful to summarize what is understood about the synergistic chromatin modifications associated with active or silenced genes. The process that has been most thoroughly characterized is the silencing of heterochromatin, the gene-poor, highly repetitive and largely inactive areas of the genome that remain condensed in interphase. The factors involved in this process were first identified in fission yeast, fruit flies and plants, but there appears to be a high degree of conservation, and it is highly likely that many of the factors and processes will also be acting in mammalian cells.

It has long been a mystery as to how the process of epigenetic silencing is initiated and how specific regions are targeted to become heterochromatin, but recently, the importance of small interfering RNAs (siRNAs) has come to light (reviewed by Mattick, this issue). Components of the dicer system which generate siRNA have been shown to be required in plants, and a role for RNA in the establishment of heterochromatin (1) as well as gene silencing (2) has been shown in mammalian cells. Waves of histone deacetylation, histone H3-K9 methylation and DNA methylation follow which in turn lead to the recruitment of non-histone proteins and protein complexes, which reinforce these changes and lead to the densely packed silent heterochromatin. This self-sustaining system probably helps to maintain the epigenetic marks following cell division. Genetic manipulations have been used to establish the hierarchy of epigenetic marks, and despite the limitations of this approach in mammalian cells, it appears that DNA methylation lies downstream of H3-K9 methylation. When, by experimental manipulation in the mouse, the major histone K9 methyltransferases are inactivated, there is a reduction in DNA methylation at major satellites (3) and several imprinted genes (4). Nevertheless, studies in other model systems have revealed a degree of cross-talk between these epigenetic marks; inactivation of

*To whom correspondence should be addressed. Tel: +44 1865 222632; Fax: +44 1865 222500; Email: richard.gibbons@imm.ox.ac.uk

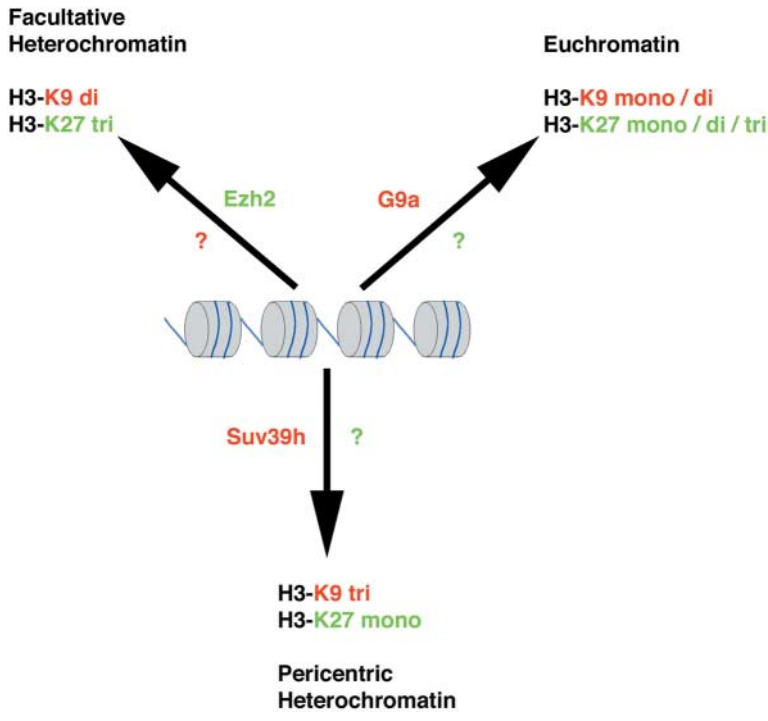

Figure 1. The major chromatin compartments, pericentric heterochromatin, facultative heterochromatin (the inactive X-chromosome) and euchromatin have particular histone H3-K9 and H3-K27 methylation patterns brought about by specific HMTs. Methylation status at lysine 9 (K9) and the HMTs responsible are shown in red; methylation status at lysine 27 (K27) and the HMT responsible are shown in green. H3-K9 trimethylation by Suv39h is thought to depend on prior H3-K9 monomethylation. Question marks indicate where the HMT is unknown. [This figure is adapted from a model presented in Kanno *et al.* (56).]

the plant CpG maintenance methyltransferase, MET1, leads to an alteration in the distribution of H3-K9 methylation (5).

The establishment of a heritable pattern of gene expression or 'cellular memory' is best characterized in pericentric heterochromatin, but attention is turning to how facultative heterochromatin, the hallmark of the inactive X-chromosome, is established and how gene silencing or activation occurs in euchromatin (reviewed in 6,7). It has long been known that the polycomb group (PcG) and trithorax group (trxG) of proteins are involved in maintaining gene expression patterns in euchromatin during development (8). For example, PcG proteins maintain the 'off state' and trxG proteins the 'on state' of the clustered homeotic box genes after the pattern of transcription is initially set by the transient expression of specific transcription factors. PcG complexes contain intrinsic histone H3-K27 methyltransferase activity and this is thought to play a part in maintaining cellular memory. H3-K9 methylation also appears to be involved in silencing some genes in euchromatin; the DNA binding factor retinoblastoma protein (pRb) has been shown to recruit the histone H3-K9 methyltransferase SUV39H1 to the promoters of cell-cycle control genes (9). In contrast, H3-K4 methylation is associated with actively transcribed genes, and reciprocal patterns of H3-K4 and H3-K9 methylation characterize euchromatic and heterochromatic chromosomal domains in yeast and plants (10,11). In facultative heterochromatin, the presence of the non-coding RNA, XIST, earmarks an X-chromosome for inactivation (12), and this leads to the recruitment of a PcG protein complex to the inactive X and methylation of H3-K27 (13). These different chromatin compartments are not only characterized by specific histone modifications (e.g. methylation of H3-K9 versus H3-K27) but also by the degree of methylation at specific lysine residues (mono-, di- or tri-methylation). The enzymes which are responsible for establishing these marks display remarkable specificity for the level of methylation they catalyze, and these enzymes vary for different types of chromatin (Fig. 1). Finally, it has been shown that different histone variants are associated with particular types of chromatin.

Histone methyltransferases

The N-terminal tail of histone H3 is subjected to methylation at multiple lysine residues by at least 17 methyltransferases (reviewed in 7). This family of proteins is characterized by the possession of the evolutionary conserved SET domain. It has recently been found that proteins other than histones may be substrates for these enzymes [e.g. p53 is subjected to regulation via lysine methylation (14)], so it is important not to assume that all their effects are mediated via histones. Nevertheless, a growing number of these proteins have been shown to promote or inhibit tumourigenesis through their histone methyltransferase (HMT) activity.

EZH2, a H3-K27 methyltransferase, is part of the PcG complexes PRC2 and PRC3 (reviewed in 15). Such protein complexes are often directed to specific sites by DNA binding factors, and SUZ12, another component of the complexes, plays this role and is also required for the complexes'

full activity. The H3-K27 mark provides the docking signal for binding of the repressive polycomb complex PRC1 via the chromodomain of the polycomb. EZH2 is required for cell proliferation and appears to act downstream of the pRb-E2F cell-cycle control pathway (16). EZH2 is highly expressed in metastatic prostate cancer (17) and lymphomas (18); the level of expression correlates with the degree of aggressiveness of breast cancer (19), and amplification of the EZH2 gene is observed in a variety of cancers (16). Forced overexpression of EZH2 leads to an increase in the rate of cell proliferation (18), and knocking down expression by RNAi leads to reduced proliferation of prostatic cancer cells (17). SUZ12 is frequently re-arranged in endometrial stromal tumours (20) and is up-regulated in some colon, breast and liver cancers (21). Curiously, though up-regulation of EZH2 and SUZ12 is associated with cancer, it is down-regulation of another component of the PRC2 and PRC3 complexes, EED, which is associated with an increased incidence of carcinogen-induced lymphoma (22). This has led to the hypothesis that altered stoichiometry of the complex may be sufficient for an increased cancer risk, perhaps through the generation of non-functional complexes (15). It is important to note, however, that in the absence of transgenic mouse models with increased EZH2 expression, it is not clear whether overexpression of EZH2 is a cause or consequence of malignancy.

RIZ1 (PRDM2) is a H3-K9 methyltransferase (23) originally identified through its interaction with pRb (24). The gene maps to chromosome 1p36, a region commonly deleted in more than a dozen different types of human cancers (25). Biallelic inactivation of the *RIZ1* gene, through mutation or promoter methylation, has been identified in human gastric cancers (26), and RIZ1 expression is commonly silenced in many types of human tumours including breast cancer, liver cancer, colon cancer, neuroblastoma, melanoma, lung cancer and osteosarcoma (27–29). Furthermore, gene knockout mice that lack RIZ1 are prone to develop B cell lymphomas (30). Of particular interest is the finding that cancer specific mutations in RIZ1 reduce or abolish the histone methylase activity of the protein (23), suggesting an important role for this activity in tumour suppression.

SUV39H1 is a H3-K9 methyltransferase which is associated predominantly with pericentric heterochromatin. Approximately 30% mice null for SUV39H1 develop B cell lymphomas after 9–15 months of age (31). The karyotype of the tumour cells were largely hyperdiploid, and in some cases, non-segregation of chromosomes was observed leading to the presence of 'butterfly' chromosomes. It is possible that H3-K9 methylation at pericentric heterochromatin is required to protect genome stability. SUV39H1 is also targeted by pRb to the promoters of S-phase genes whose activity is required for cells to cycle (9). Local methylation at H3-K9 leads to repression of these genes, and this seems to be part of the control switch for exiting the cell cycle and entering differentiation (32). It is tempting to attribute the tumour suppressive effects of a number of these factors to their association with pRb. However, the spectrum of human and mouse tumours that result from inactivation of pRb and these factors are each very specific and have little overlap.

SMYD3 methylates histone H3 at K4, the epigenetic mark associated with gene activation. It appears to be targeted to particular genes via the specific DNA binding sequence 5′-CCCTCC-3′. SMYD3 forms a complex with RNA polymerase II through an interaction with the RNA helicase HELZ. It is expressed at its highest levels in normal skeletal muscle and testis, but microarray analysis showed that it is up-regulated in colorectal and hepatocellular carcinomas (33). Overexpression in cultured cells enhanced cell growth whereas knockdown of SMYD3 expression by siRNAs resulted in significant growth suppression in cancer cell lines. Artificial expression of SMYD3 in cells which normally lack it, led to activation of a number of genes, and this was associated with a local increase in H3-K4 methylation. It is presumed that overexpression of SMYD may lead to the misregulated expression of normally silent oncogenes and hence to cancer. However, as for EZH2, in the absence of transgenic mouse models with increased SMYD3 expression, it is not clear whether overexpression of SMYD3 is a cause or consequence of malignancy.

Analysis of a recurrent cryptic translocation, t(5:11)(q35;p15.5), in childhood acute myeloid leukaemia identified a component of one of the predicted fusion proteins to be a putative histone methylase, nuclear receptor-binding SET domain containing protein (NSD1) (34). Constitutional deletions or point mutations in NSD1 give rise to Sotos syndrome, a condition which gives rise to childhood overgrowth and learning difficulties (35). This is associated with an increased risk (170-fold) of developing cancer (36). A wide variety of neoplasms have been described in Sotos syndrome including Wilms tumour, neuroblastoma, acute lymphocytic or lymphoblastic leukaemia, hepatocellular carcinoma and small cell carcinoma of the lung. There are no reports regarding loss of the second NSD1 allele in these tumours. Given that Sotos syndrome is thought to arise from haploinsufficiency for NSD1, it is possible that the 5;11 translocation is associated with loss of NSD1 function.

The mixed lineage leukaemia (*MLL*) gene encodes a homolog of Trithorax in *Drosophila.* This SET domain containing gene is a common target for chromosomal translocations associated with human acute leukaemias (reviewed by Croce, this issue). As the SET domain is absent from the oncogenic fusion proteins, the HMT activity does not appear to play a part in what appear to be gain of function mutations. Nevertheless, rarer loss of function mutations of MLL have been described in leukaemia and suggest that perturbation of MLL function in the absence of a fusion partner may be sufficient to promote leukaemogenesis (reviewed in 37).

Histone acetylases

The acetylation of lysine residues located in the N-terminal tails of histone is one of the key features associated with active gene transcription (reviewed in 38). The addition of acetyl groups neutralizes the positive charge of lysine, which affects the interaction of the histone tails with DNA, RNA and protein. The acetyl group also provides a specific binding site for certain proteins via their bromodomain. Furthermore, modification by acetylation or methylation may compete directly, for example, at H3-K9 with completely

opposite functional consequences. Two classes of protein determine the acetylation status of histones—histone acetyltransferases (HATs) and histone deacetylases (HDACs). It has recently been shown that acetylation of lysine is much more widespread than realized and HATs can also acetylate non-histone proteins, including transcription factors and p53, so it is important not to assume that HATs exert their effects solely through the modification of histones (39). HAT activity has been shown to be intrinsic to a growing number of transcriptional cofactors. There are three principal groups GNAT, MYST and p300/CBP (reviewed in 40). They tend to be components of multiprotein complexes and are recruited to specific promoters via DNA bound transcription factors.

Perturbation of HAT activity may be seen in cancer. Rubenstein–Taybi syndrome (RTS) is a developmental disorder associated with mutations in CBP which inactivate the protein's HAT activity (41). Individuals with RTS have a 350-fold increased risk of developing cancer, usually childhood cancers of neural crest origin (42). Mouse knockouts heterozygous for CBP, which have a phenotype resembling RTS, eventually develop haematological malignancy (43). The other allele is somatically mutated in these malignancies. Although no germline mutations of the related protein p300 have been identified, missense or truncating mutations in p300 are seen in rare cases of colorectal and gastric tumours and in these, the second allele is often deleted (44). When embryonic stem cells null for p300 are injected into blastocysts, the chimeric mice obtained develop histiocytic sarcomas which are derived from the null cells (45). So it appears that both CBP and p300 function as classical tumour suppressors.

The HATs, CBP, p300 and MOZ and MORF are occasionally involved in fusion proteins that arise from chromosomal translocations associated with leukaemia or secondary to treatment for myelodysplastic syndrome (reviewed by Croce, this issue). It appears that these fusion proteins represent gain of function mutants and that the leukaemogenic effect may be due to mistargetting of HATs, leading to aberrant acetylation and gene activation (reviewed in 40). For example, in the MLL–CBP fusion (which, it should be noted, lacks the SET domain of MLL required for its H3-K4 HMT activity), potential targets for acetylation are the *Hoxa7* and *Hoxa9* genes; the MLL fusion oncoprotein aberrantly maintains the expression of these *Hoxa* genes which are required for myeloid transformation through inappropriate self-renewal of myeloid progenitors that cannot terminally differentiate (46).

Histone deacetylases

HDACs catalyze the removal of acetyl groups from lysine residues and promote gene repression. The substrates include non-histone proteins as well as histones. There are some 18 HDACs encoded by the human genome, and these have been divided into three different classes (I, II, III) on the basis of their sequence homology to the yeast HDACs, reduced potassium dependency 3 (Rpd3), histone deacetylase 1 (Hda1) and silent information regulator 2 (Sir2), respectively (reviewed in 47). Class I and II HDACs are sensitive to the inhibitor trichostatin A (TSA), whereas class III is insensitive to TSA and requires the coenzyme NAD+ as a cofactor. Many HDACs exist as components in multiprotein complexes (e.g. NURD), which act as transcriptional co-repressors. These are then targeted to specific genomic regions by interactions with DNA binding factors. The best characterized of these interactions is the recruitment of HDACs to the hypermethylated CpG islands of tumour suppressor genes via methyl binding proteins. As a consequence, there is loss of acetylation from the histone tails, which by reinforcing gene silencing, appears to play a secondary role to methylation, as re-establishing acetylation by the use of HDAC inhibitors alone does not restore the expression of hypermethylated tumour suppressor genes.

A potentially more important role for HDACs in cancer, and one which occurs independently of DNA methylation, involves the recruitment of HDACs to tumour-suppressor genes via oncogenic DNA binding factors. In this way, HDACs appear to play a mediating role for these factors in specific forms of cancer. This has been demonstrated for the translocation-generated fusion protein AML–ETO, which is directed to its target genes via the DNA binding domain of AML1 (48). In contrast to the wild-type AML1 protein, the fusion protein actively suppresses transcription via the aberrant recruitment of HDAC containing co-repressors. As AML1 is required for differentiation of haematopoietic cells, AML1–ETO expression leads to a block in myeloid development and leukaemic transformation. A similar mechanism underlies the action of the fusion protein PML–RARα (49).

The silencing of tumour-suppressor genes through the aberrant targeting of HDACs to their gene promoters may be a more common phenomenon than was previously realized. $p21^{WAF1}$ encodes a cyclin-dependent kinase inhibitor which inhibits cell-cycle progression leading to arrest in G1. It has the characteristics of a tumour-suppressor gene: $p21^{WAF1}$ knockout mice develop tumours (50), $p21^{WAF1}$ expression is lost in numerous different tumours and overexpressing it in deficient cancer cell lines leads to growth arrest (51). It appears that in some tumours, $p21^{WAF1}$ is inactivated epigenetically by hypoacetylation of the promoter. Reactivation of the gene by HDAC inhibitors leads to hyperacetylation of histones at the promoter, and this is associated with inhibition of tumour cell growth (52). It is important to note, however, that these drugs have pleiotropic effects inducing differentiation, cell-cycle arrest and apoptosis, and it is not known for certain whether these effects are mediated by changes in histone acetylation at specific loci or because of some other undefined action. Nevertheless, there is great interest in using HDAC inhibitors alone or in combination with DNA demethylating agents to reactivate silenced tumour-suppressor genes, and a large number of clinical trials are underway to establish their safety and efficacy in cancer therapy.

Chromatin remodelling factors

Chromatin remodelling is required for all the key processes revolving around DNA metabolism such as gene expression, DNA replication, repair, chromosomal recombination and mitosis (53). Changes in nucleosomal structure and organization are brought about by ATP-hydrolyzing enzymes which commonly are part of multiprotein complexes. SWI-SNF

Table 1. The SNF2-like subfamilies modified from Eisen *et al.* (60)

Subfamily	Characteristic motif	*S. cerevisiae*	*Drosophila*	*A. thaliana*	Mammal	Proposed function
SNF2	Bromodomain	Snf2	Brahma	At5g19310	BRG1/SMARCA4	Activation and repression of transcription
		Sth1	Brm	At3g06010	BRM/SMARCA2	Activation and repression of transcription
ISWI	SANT-like	ISWI1	Iswi	At3g06400	SNF2H/SMARCA5	Maintaining chromosome structure
		ISWI2		At5g18620	SNF2L/SMARCA1	Maintaining chromosome structure
CHD1	Chromodomain	CHD1	Chd1	At2g1337	CHD1	Activation of transcription
					CHD2	?
				At2g25170	CHD3	? Methylation induced gene silencing
			Mi-2	At5g44800	CHD4	Repression of transcription
INO80	DBINO	Ino80	CG31212	At3g57300	INO80	DNA repair and gene expression
ERCC6		Rad26		At2g18760	ERCC6	Transcription-coupled DNA repair
RAD54		Rad54	okr		RAD54L	DNA repair by homologous recombination
			dXNP	At1g08600	ATRX	DNA methylation, ? regulation of transcription
DDM1		YFR038w		DDM1	HELLS/PASG/Lsh	DNA methylation
MOT1		MOT1	Hel89B	At3g54280	BTAF1	Repression of transcription

Examples of members of these subfamilies and their homologues in yeast, fly, plant and mammals are shown (as indicated by Homologene at http://www.ncbi.nlm.nih.gov). The proposed function is based on reviews by Havas *et al.* (79) and Meehan *et al.* (80).

type chromatin remodelling factors have been shown to be required at many of the steps required to establish heterochromatin (54–56). Mutations in these factors have been shown to affect both histone and DNA methylation (5,57,58), suggesting they play a role in both these processes. Their precise role has not been defined, but a common theme is that these chromatin remodelling factors probably facilitate access to DNA, for example, in RNA-mediated silencing, they may help the siRNAs find their homologous DNA sequence (56) and in DNA and histone methylation, they may help to direct which sites are modified. More recently, they have shown to be involved in replacement of canonical histones with variant histones (59).

These nucleosome remodelling motors all belong to the SWI2/SNF2 family of ATPases. This family is divided into at least eight subfamilies according to the degree of homology within the conserved ATPase domain and the presence of protein motifs in adjacent regions: SNF2, ISWI, CHD1, INO80, ERCC6, RAD54, DDM1 and MOT1 (Table1) (53,60). Only a few such proteins have so far been implicated in neoplasia, and where this occurs, it is important to draw a distinction between situations where the changes involving these proteins are a cause or consequence of cancer. Important evidence of a causal role is an increased susceptibility to cancer in animals with constitutional mutations in the genes of such proteins. As we shall see, the case for the proteins playing an important role in tumourigenesis is weak and often evidence for their involvement is circumstantial.

Mutations in PASG (also known as Lsh and HELLS), a member of the DDM1 subfamily that facilitates DNA methylation, have been identified in 40–60% cases of acute myelogenous leukaemia and acute lymphoblastic leukaemia (61). Mice knocked out for PASG die within a few hours after birth with reduced body weight and renal defects (62). A very intriguing observation was that, similar to the effect of mutations in its plant homologue, DDM1, the mouse knockout is associated with a marked reduction in global cytosine methylation (50–60% of wild-type levels) (63). This mainly affected satellite DNA and dispersed repetitive sequences; the effect on low copy sequences was more variable and stage-specific. Mice homozygous for an hypomorphic mutation of PASG have increased karyotypic instability, age prematurely and do not survive beyond 3–4 weeks, an age before tumours might be expected to develop (64). There are no reports of increased cancer susceptibility in heterozygotes. It would be interesting to see whether disrupting this gene postnatally, through an induced conditional knockout model, led to cancer predisposition.

Recently, somatic loss of function mutations in ATRX, an RAD54-like protein, have been identified in a rare form of myelodysplasia which is a preleukaemic blood disorder (65). Alpha thalassaemia myelodysplastic syndrome (ATMDS) is a clonal myeloid disorder associated with dramatic down-regulation of alpha globin gene expression. The resulting blood picture is one of small, poorly haemoglobinized red cells. If leukaemia develops or, as occasionally happens, the affected cells spontaneously disappear from the circulation, it is presumably because of overgrowth by another neoplastic clone (66). It seems likely that the acquired alpha thalassaemia *per se* does not confer a selective advantage to the affected clone, rather somatic mutations in ATRX arise as part of the widespread background of genetic mutations that occur in malignant cells, and these are flagged up by their dramatic phenotype. Constitutional mutations in ATRX give rise to a syndrome associated with severe mental retardation and alpha thalassaemia (67). Significantly, there is no predisposition to cancer in this condition. Mutation of ATRX in ATMDS may be an example of the involvement of an innocent bystander, and it is possible that other paraneoplastic phenomena that lead to a striking phenotype arise in a similar fashion.

The SNF2 subfamily is thought to be involved in gene expression, and studies in yeast showed that ~5% genes were either activitated or repressed by the action of SNF2 (68). The mammalian homologues, BRM and BRG1, each participate in a number of chromatin remodelling complexes. They are involved in preventing cell-cycle progression through their interaction with pRb (69,70). Mutations in

BRG1 have been identified in pancreatic, breast, lung and prostate cell lines (71,72). Approximately 30% of non-small-cell lung cancer cell lines and 10% primary tumours lack expression of both BRG1 and BRM (73). *Brm*−/− mice, however, do not develop tumours (74). *Brg*−/− mice are embryonic lethal but heterozygous *Brg*+/− mice are viable and develop epithelial tumours at low incidence (75).

Although it is not itself a chromatin remodelling protein, there is strong evidence that one of the core subunits of the mammalian SNF2 complexes, SNF5, acts as a classical tumour suppressor. Bialleleic inactivating mutations of SNF5 are found in the majority of malignant rhabdoid tumours, a cancer of early childhood which arises primarily in the kidney and brain (reviewed in 76). In familial cases of this tumour, germline mutations have been identified in affected children and loss of the normal allele occurs in the tumours. Mutations in SNF5 have been reported in paediatric choroid plexus carcinomas and more rarely in meningiomas, medulloblastomas and primary neuroectodermal tumours of the brain. Similarly, in mice where *snf5* is homozygously knocked out in a proportion of the cells, 100% of the mice develop T-cell lymphomas or rhabdoid tumours within 11 weeks (77). One might expect that mutations in different subunits of a particular complex might give rise to the same phenotype [as seen for the components of the yeast SNF2 complex (78)]. BRM and BRG1 participate with SNF5 in such chromatin remodelling complexes, but the cancer phenotype associated with SNF5 mutations is more severe than that for BRM or BRG1 mutations. BRM and BRG1 have a high degree of homology and at least *in vitro* are interchangeable. It is possible that the milder phenotype for BRG1 and BRM is because there is a degree of functional redundancy between BRM and BRG1 *in vivo*. Alternatively, the tumour suppressor activity of SNF5 may be independent of its function in the SNF2 complex.

CONCLUDING REMARKS

Progress in determining the epigenetic marks which characterize the different forms of chromatin has been remarkably rapid. Covalent modifications of histones, which have not been mentioned in this review, such as phosphorylation, ubiquitinylation, ADP-ribosylation and sumyolation, are being shown to play important roles in gene regulation and DNA repair, cell-cycle control and apoptosis. It seems inevitable that perturbations in these epigenetic features will also be implicated in the development of neoplasia. The fact that changes in the epigenome are potentially reversible makes them important targets for therapeutic intervention, and an exciting goal will be to identify those key steps at which it is possible to reprogram a cancer cell to terminally differentiate or apoptose rather than proliferate.

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