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Regulation of chromatin structure via histone post-translational modification and the link to carcinogenesis

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Abstract The loss of genome integrity contributes to the development of tumors. Although genome instability is associated with virtually all tumor types including both solid and liquid tumors, the aberrant molecular origins that drive this instability are poorly understood. It is now becoming clear that epigenetics and specific histone post-translational modifications (PTMs) have essential roles in maintaining genome stability under normal conditions. A strong relationship exists between aberrant histone PTMs, genome instability, and tumorigenesis. Changes in the genomic location of specific histone PTMs or alterations in the steady-state levels of the PTM are the consequence of imbalances in the enzymes and their activities catalyzing the addition of PTMs ("writers") or removal of PTMs ("erasers"). This review focuses on the misregulation of three specific types of histone PTMs: histone H3 phosphorylation at serines 10 and 28, H4 mono-methylation at lysine 20, and H2B ubiquitination at lysine 120. We discuss the normal regulation of these PTMs by the respective "writers" and "erasers" and the impact of their misregulation on genome stability.

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1 Introduction

The continued advancement of high-resolution imaging, genome-wide approaches, and next generation sequencing has spawned a resurgence in research designed to identify and characterize the pathogenic origins of various human disease states, including cancer. Over a century ago, the pioneering observations of Theodor Boveri suggested that genome instability or more specifically abnormal chromosome constitutions are pathogenic events that drive the tumorigenic process. A century's worth of genetic, biochemical, cytological, and cell biological research has validated this initial observation, and it is now widely accepted that the loss of genome integrity contributes to the development of tumors. Surprisingly, although genome instability is associated with virtually all tumor types including both solid and liquid tumors, the aberrant molecular origins that drive this instability are only poorly understood. What is now becoming clear, however, is that epigenetics and specific post-translational histone modifications have essential roles in maintaining genome stability under normal conditions. For example, many post-translational histone modifications exhibit roles in regulating chromatin structure and consequently may influence any biological process requiring the DNA template including DNA replication and repair, gene expression, and chromosome segregation. Thus, a strong relationship exists between aberrant post-translational histone modifications, genome instability, and tumorigenesis. Indeed, research conducted over the past decade has shown that alterations in specific post-translational histone modifications, stemming from aberrant expression and/or regulation of the enzymes that regulate these modifications,

correlate with the development and progression of many tumor types. In fact, the abundance of specific modifications and/or modifiers within tumors is now being routinely explored as a biomarker for the disease. Consequently, it is first essential to characterize the normal roles specific post-translational histone modifications have in maintaining genome stability under diverse environmental and biological conditions, before the specific roles their aberrant expression have in the development of cancer can be determined. Only once this information is gleaned will it be possible to begin to devise and develop the next generation of novel therapeutic strategies that are specifically designed to exploit these aberrant epigenetic origins.

2 Histone post-translational modifications and their impact on chromatin

The fundamental repeating structure of chromatin is the nucleosome. The nucleosome is comprised of approximately 146 bp of DNA wrapped ~1.7 times around an octameric core particle containing two histone H2A/H2B dimers flanking a central histone $(H3/H4)_2$ tetramer [1]. The canonical histones are small, highly basic nuclear proteins that share a common structure. This includes a central highly structured histone fold domain (or globular domain) that is required for histone–histone and histone–DNA interactions, as well as "unstructured" carboxy- and amino-terminal tails (Fig. 1a). It is the histone tails that extend out from the octameric core that generally serve as the primary substrates for residue-specific, post-translational modifications (PTMs). Histones are among the most highly post-translationally modified proteins, and they are the substrates for at least 11 different types of PTMs including phosphorylation, methylation, and ubiquitination [2–4]. The highly conserved amino acid sequence of histones and their propensity to accept specific PTMs is evolutionarily conserved, implying that these histone PTMs are essential for the maintenance of eukaryotic cell function.

Although the classical function of histones is DNA packaging, the chromatin structure is highly dynamic and can undergo local or global conformational changes. These structural changes are highly regulated and impacted by specific PTMs which render the chromatin amenable or resistant to a myriad of nuclear processes that utilize DNA/chromatin as a template, including transcription, DNA replication and repair, and chromosome segregation [5, 6]. In general, chromatin can be envisioned to impart its regulatory effects by differentially regulating the level of DNA compaction and exposure to DNA-binding proteins. At certain stages of the cell cycle or in response to certain stimuli, chromatin must decondense to facilitate access to appropriate factors involved in processing the genetic information (e.g., transcription, replication, and repair). Conversely, chromatin folding must increase at specific times to render the DNA inaccessible to certain factors, or during mitosis maximal folding must be achieved so that the genetic material can be accurately segregated to daughter cells [6]. This precise level of regulation is primarily achieved through the abundance and impact of specific histone PTMs. The PTMs impact chromatin structure by altering histone–histone and histone–DNA interactions and through their regulation and recruitment of critical protein complexes.

3 The regulation and abundance of histone PTMs impacts genome stability

The spatial and temporal distribution of a given histone PTM is regulated by the balance of the enzymatic activities of the proteins that add ("writers") or remove ("erasers") a specific PTM [7] (Table 1). The overall abundance also depends on the availability of the appropriate substrate and the protein complexes, or "readers" that interpret the PTMs to evoke specific functional and context-dependent outcomes [8]. Alterations in the steady-state levels of a given PTM can be brought about through various pathogenic events (e.g., specific somatic mutations, amplification, or deletions) that typically impact the expression, function, and/or regulation of the genes encoding the "writers," "erasers," or "readers." As a direct consequence, the steady-state level of a given histone PTM will be altered which in turn will impact normal chromatin structure and function to have far-reaching implications for the various biological processes that utilize the DNA template. Failure to accurately regulate chromatin condensation or decondensation can lead to increased DNA damage, aberrant gene expression, and errors in mitotic fidelity, which all contribute to genome instability. Genome instability is a general term used to describe an increase in the rate of change or damage to the genome. It can arise through a diverse array of mechanisms including promoter hypermethylation [9], DNA repair defects [10], and chromosome instability (CIN), which is classically defined as an increase in the rate at which whole chromosomes or large parts thereof are gained or lost (reviewed in [11]). More recently, the definition of CIN has expanded to include structural alterations (e.g., translocations, inversions, duplications, deletions, etc.), and thus, both numerical and structural chromosomal changes are associated with CIN. Genome instability is a common hallmark and pathogenic event associated with many human syndromes and disease states. For example, genome instability is a characteristic of ataxia telangiectasia [12], xeroderma pigmentosa [13], Nijmegen breakage syndrome [14], and virtually all tumor types [15, 16].

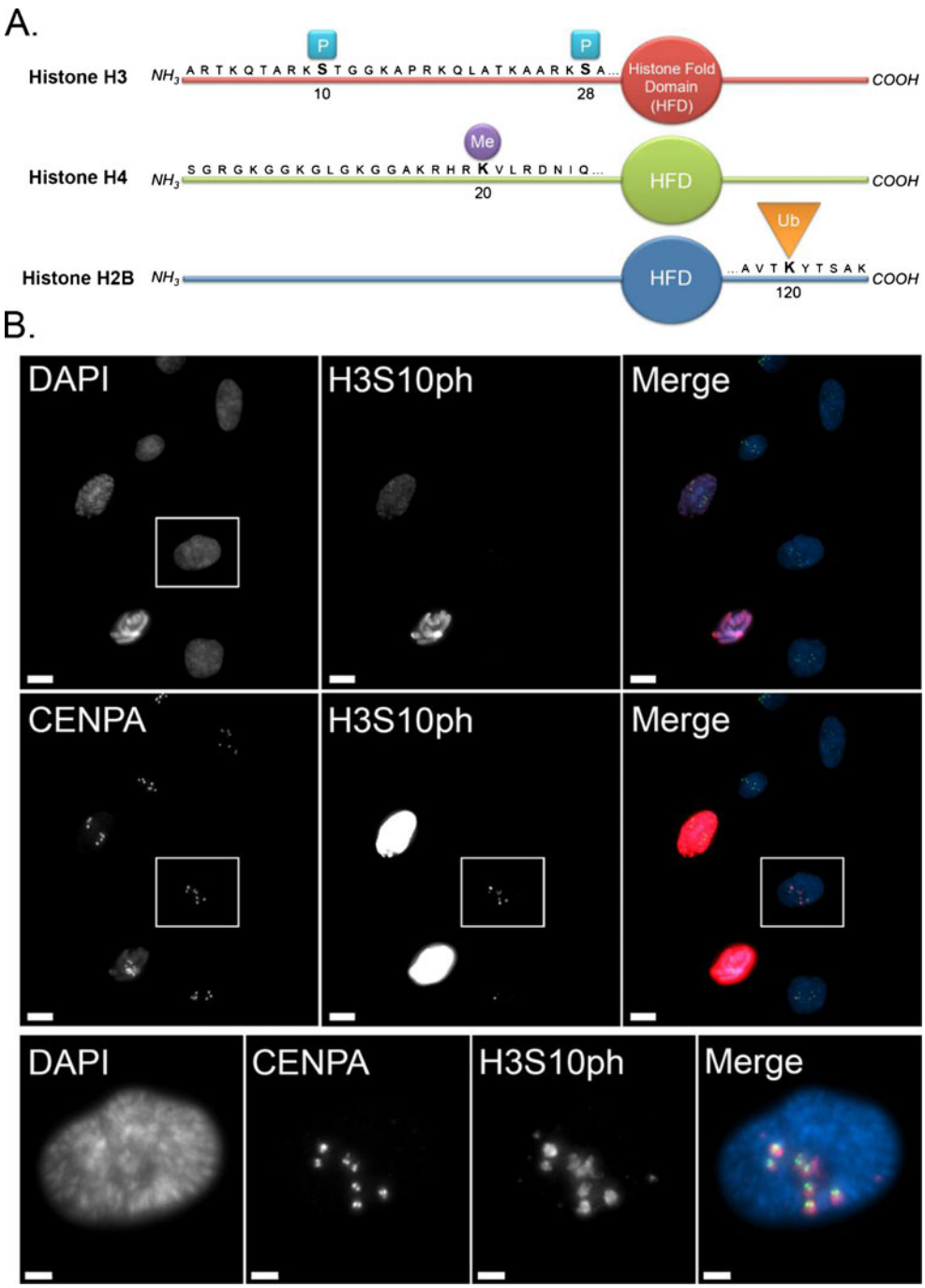

Fig. 1 Histone modifications and their implications in chromatin structure. **a** Schematic representation of the specific histone modifications discussed in the review, namely phosphorylation (*P*), methylation (*Me*), and ubiquitination (*Ub*). Amino acids are represented by their single amino acid code and the modified residues discussed are identified in *bold* with the position indicated below the respective residue. **b** Micrographs depicting the initial increase in H3S10ph that occurs within the pericentric heterochromatin in G2 cells. Asynchronous cells were paraformaldehyde-fixed, co-immunofluorescently labeled for anti-H3S10ph (*red*) and anti-centromeric protein A (*CENPA*; *green*), and counterstained with DAPI (*blue*). For illustrative purposes, the H3S10ph panel has been presented twice; the *top middle H3S10ph panel* is optimized to present the staining pattern within the two mitotic cells, while the *lower panel* is optimized (i.e., longer exposure time) to present the H3S10ph staining pattern initiating within the G2 cell (*bounding box*). The *bottom four panels* present high-resolution 3D projections of the G2 cell. Note the spatial proximity of the CENPA and H3S10ph signals within the G2 cell

The aberrant chromatin location or steady-state level of a specific histone PTM, brought about through imbalances in the enzymes and activities of the "writers" and "erasers," has the potential to impact and drive the development of numerous human conditions and, in particular, cancer. This review focuses on the misregulation of three specific types of histone PTMs, namely histone H3 phosphorylation at serines 10 and 28, H4 mono-methylation at lysine 20, and H2B ubiquitination at lysine 120 (see Fig. 1a). Here we discuss normal regulation of these PTMs by the respective "writers" and "erasers" (Table 1). We also discuss the impact the misregulation of these PTMs has on genome stability with particular reference to their known or putative roles in tumorigenesis and metastatic potential.

4 The enigmatic role of H3 phosphorylation in gene expression and DNA compaction

Histone H3 phosphorylation occurring within the amino-terminal tails (Fig. 1a) at serines 10 (H3S10ph) and 28 (H3S28ph) is of particular interest as these PTMs are

Table 1 Enzymes that regulate the abundance of select histone modifications

Histone	Residue	Modification	"Writer"	Substrate	"Eraser"
H3	Serine 10	Phosphorylation	AURKB	ATP, GTP	PPA1
			MSK1/MSK2	ATP, GTP	PPA1
H3	Serine 28	Phosphorylation	AURKB	ATP, GTP	PPA1
			MSK1/MSK2	ATP, GTP	PPA1
H4	Lysine 20	Mono-methylation	PR-Set7	s-adenosyl methionine	PHF8
H2B	Lysine 120	Mono-ubiquitination	UBE2A (E2)	ubiquitin	USP22
			RNF20/RNF40 (E3)	ubiquitin	

AURKB Aurora kinase B, *MSK1/MSK2* mitogen and stress activate kinase 1/2, *PPA1* protein phosphatase type A1, *PR-Set 7* PR-SET domain containing protein 7, *PHF8* plant homeodomain finger protein 8, *UBE2A* ubiquitin-conjugating enzyme 2A, *RNF20/RNF40* RING finger 20/40, *USP22* ubiquitin-specific protease 22

essential for biological processes that occur within apparently opposite chromatin states. In interphase cells, these two specific phosphorylation events are associated with and essential for chromatin remodeling of the upstream promoter regions such that immediate early gene (IEG) transcription can occur [17], while in mitotic cells H3 S10/S28 phosphorylation is fundamental to the formation of the higher-order chromatin structure and accurate segregation of chromosomes into daughter cells (reviewed in [18]). To account for the apparent contradictory roles, one has to consider the spatiotemporal distribution and abundance of H3S10ph and H3S28ph, which is a highly regulated process that depends on the balance of the enzymatic activities for the kinases that covalently link a phosphate group from nucleoside triphosphate (e.g., ATP or GTP) to the acceptor serine and the phosphatases that remove them (see Table 1 and below). In addition, the distinct cellular contexts in which they occur must be taken into account; the opposing chromatin states and PTMs occur at distinct stages of the cell cycle (i.e., interphase versus mitosis), the overall abundance of the PTMs is dramatically different (i.e., lower abundance in interphase versus maximal abundance in mitosis), and the genomic distribution is fundamentally distinct (gene-specific association versus the entire length of the chromosome arms). Finally, it is also important to note that these PTMs do not occur in isolation but rather occur within the context of the constellation of many additional PTMs (e.g., acetylation, methylation, ubiquitination, etc.) that not only occur on the same histone tail but also occur on other histones within the same, or spatially proximal nucleosomes.

5 H3 phosphorylation is critical for immediate early gene expression in interphase

During interphase, H3S10/H3S28 phosphorylation is restricted to specific gene loci and thus occurs less extensively and on fewer nucleosomes than the mitotic-associated phosphorylation events (Fig. 1b) [19, 20]. Exposure of mammalian cells to extracellular signals such as growth factors, phorbol esters, acetaldehyde, or UV irradiation stimulates either the RAS-MAPK-ERK1/2 or p38 MAPK signaling cascades resulting in the activation of mitogen and stress-activated kinases 1 and 2 (MSK1/MSK2) [21–23]. Studies in mammalian cell lines including mouse fibroblasts, human breast cancer cells, and Coffin–Lowry fibroblasts have shown that MSK1, and to a lesser extent MSK2, are responsible for the upstream promoter region-specific H3S10ph/H3S28ph of IEGs including *FOS* and *JUN* [17, 21, 24–26]. The presence of H3S10ph and/or H3S28ph in the upstream promoter regions regulates the recruitment of the "readers" including the 14-3-3ε/ζ adaptor protein, transcription factors, and chromatin-remodeling complexes, which in turn recruit and phosphorylate RNA polymerase II to ultimately induce IEG transcription [17, 27, 28]. Despite the fact that serines 10 and 28 are both phosphorylated by MSK1/MSK2, immunohistochemistry and chromatin cross-linking studies demonstrate that these two PTMs are on separate H3 tails and separate stretches of nucleosomes [17, 29, 30]. It is possible that the differential distribution of H3S10ph and H3S28ph may in part be regulated by other pre-existing histone PTMs, nucleosome-bound proteins, or different MSK1/2 complexes [30]. This would allow for the fine-tuned regulation of proliferation, differentiation, or apoptosis as dictated by the environmental conditions of each cell and may account for allele- or cell-specific maintenance of IEG expression [31–33].

The IEG products, FOS and JUN, form a heterodimeric transcription factor complex called AP-1, which mediates subsequent transcription of downstream genes and regulates numerous cellular processes such as proliferation, differentiation, and apoptosis [34–36]. Because of these critical roles, misexpression of *FOS* and *JUN* are pathogenic events that contribute to tumorigenesis. Indeed, their misregulation is implicated in the oncogenic transformation of certain cell

types, including osteoblasts and chondroblasts [37, 38]. In addition, *FOS* overexpression in murine models has been shown to induce skeletal osteocarcinoma formation, with *JUN* co-expression functioning to enhance the oncogenic potential of FOS [35, 39]. Finally, *FOS* overexpression has been shown to increase basal mutation rates in mouse embryonic fibroblasts [40] and induce CIN, which is a known driver in the tumorigenic process [15, 41].

Since one of the major downstream effects of MSK1/MSK2 activation is S10/S28 phosphorylation and the resulting transcription of IEGs (e.g., *FOS* and *JUN*), it is expected that alterations in MSK1/MSK2 activity and the alteration in IEG transcription may be a pathogenic event. Evidence in support of this comes from the highly metastatic Ha-*Ras* transformed murine fibroblast cell line Ciras-3, which exhibit increased MSK1/MSK2 activity and H3S10ph/H3S28ph levels [29, 42]. These cells possess less-condensed chromatin and exhibit CIN [31]. In addition, recent gene re-sequencing efforts have identified somatic aberrations (e.g., non-synonymous single nucleotide polymorphisms, amplifications, and homozygous deletions) in both *MSK1* and *MSK2* in a diverse array of tumor types including colorectal, ovarian, and prostate cancers (Table 2) [43–45]. MSK1 has been shown to promote cell proliferation in immortalized human keratinocytes and in a human epidermoid carcinoma cell line [46]. Furthermore, *in vivo* studies performed in mouse models, which utilize multistage skin tumor-inducing chemical treatments, support the observed *in vitro* role of MSK1/MSK2 in skin cell transformation. Chang et al. [47] showed that MSK1/MSK2-deficient mice developed fewer tumors following tumor-inducing chemical treatment in comparison to the wild-type control mice. These data provide support for a role of MSK1/MSK2 signaling in positively regulating cell proliferation and promoting skin carcinogenesis [47]. In addition, epidermal growth factor or phorbol ester-induced cell transformation has been shown to require MSK1-mediated H3S10ph. Induction of dominant-negative MSK1 or H3 mutant cell lines, in which S10 phosphorylation is hindered, or treatment of these tumor-induced cells with the MSK1-inhibitor H89, results in the dramatic reduction of cell transformation and tumor development [48]. Further, inhibition of MSK1/2 or knockdown of MSK1 in Ha-*Ras*-transformed mouse fibroblasts attenuated anchorage-independent growth, a measure of the metastatic potential of cancer cells [49]. Taken together, these results strongly suggest that MSK1/MSK2-dependent H3 phosphorylation events leading to IEG transcription are required for cell transformation. Certain MSK1/MSK2 mutations found in sequenced human tumors may therefore represent driver mutations for carcinogenesis and eventually MSK1/MSK2 may serve as effective, novel chemotherapeutic targets.

Table 2 Cancer-associated mutational data for genes encoding select histone modifying enzymes

Histone PTM	Gene[a]	Tumor type(s)	Mutation type(s)
H3S10p	*AURKB*	BR, CR, P, S, SO	nsSNP, AMP, DEL
	MSK1 (*RPS6KA5*)	BR, CR, L, P, S, SO	nsSNP, AMP, DEL
	MSK2 (*RPS6KA4*)	BR, L, P, S, SO	nsSNP, AMP
	PP1A	BR, L, P, S, SO	nsSNP, AMP
H4K20me1	*PR-SET7* (*SETD8*)	BR, CR, L, P, S, SO	nsSNP, AMP, DEL
	PHF8	BR, CR, L, SO	nsSNP
H2Bub1	*UBE2A* (E2)	BR, CR, L	nsSNP
	RNF20 (E3)	BR, CR, L, S, SO	nsSNP, AMP, DEL
	RNF40 (E3)	BR, CR, L, S, SO	nsSNP, AMP
	USP22	BR, CR, L, SO	nsSNP, AMP, DEL

Data compiled from the Catalog of Online Somatic Mutations in Cancer (www.sanger.ac.uk/perl/genetics/CGP/cosmic) and cBio Cancer Genomics Portal (www.cbioportal.org) [43–45]. The presented data are only included if it was from studies with mutation data

BR breast, *CR* colorectal, *L* lung, *P* prostate, *S* sarcoma, *SO* serous ovarian, *nsSNP* non-synonymous single nucleotide polymorphism, *AMP* amplification, *DEL* homozygous deletion

[a] Alternative gene name listed in parentheses

6 Mitotic H3 phosphorylation is essential for chromatin compaction and segregation

In contrast to the low levels of gene-specific H3 phosphorylation events detailed above, the evolutionarily conserved mitotic phosphorylation of H3S10 and S28 occurs along the entire lengths of all chromosomes and is essential to maintain chromosome stability [19, 50]. Indirect immunofluorescence revealed that the "mitotic" phosphorylation events actually initiate within the pericentric heterochromatin in late G2 cells (Fig. 1b). As cells enter the early stages of mitosis, H3S10 phosphorylation propagates down the length of each chromosome arm and attains maximal abundance at metaphase. The global dephosphorylation of H3S10ph begins as cells enter anaphase and is completed by the end of telophase [50, 51]. Although the spatial and temporal kinetics are similar for H3S28, there are a few subtle differences. For example, the initial increase in H3S28ph begins in prophase, the overall global abundance of H3S28ph is lower than that of H3S10ph [19], and the dephosphorylation event occurs more rapidly and is completed earlier in telophase [52].

The mitotic-associated increases in H3S10 and H3S28 phosphorylation are evolutionarily conserved and in mammals are mediated by the chromosomal passenger protein

Aurora B kinase (AURKB) [19, 53]. Chemical inhibition or altered AURKB expression and function significantly decreases the global abundance of mitotic H3S10ph and H3S28ph and correlates with an increase in aberrant chromosome condensation and segregation errors [54, 55]. Furthermore, microinjection of excessive AURKB substrate (i.e., H3S10 peptides) produces a G2 arrest and suggests that H3S10ph is required for chromosome compaction and entry into mitosis [56]. Collectively these data suggest that AURKB expression and activity is essential for cell cycle progression and to maintain genome integrity.

Misregulation and/or altered AURKB function will inherently affect the abundance of H3S10ph and H3S28ph, which underlies the development of aneuploidy and drives tumorigenesis. Indeed, recent gene re-sequencing efforts have determined that *AURKB* is somatically mutated in numerous tumor types (Table 2), while other studies have shown that AURKB overexpression and elevated levels of H3S10ph are both associated with CIN and highly invasive tumors [57, 58]. In fact, immunohistochemical analysis of advanced colorectal cancer tumors revealed increased levels of AURKB expression in tumors that were subsequently shown to be positively correlated with lymph node metastases [59]. Additional studies in oral, prostate, endometrial, and thyroid cancers studies have also shown that AURKB overexpression correlates with CIN, highly aggressive tumor-types, and poor prognosis [60–63], which suggests that AURKB and H3S10ph or H3S28ph may be a therapeutic target. In support of this concept, Harrington and colleagues [64] demonstrated that a small molecule inhibitor of AURKB was able to significantly and selectively reverse the growth of transplanted human malignant cells that overexpressed AURKB in xenograft models.

The dramatic decrease in H3S10ph and H3S28ph that is observed in the later stages of mitosis is predominantly regulated through the activity of protein phosphatase type 1 (PP1) [65, 66]. Interestingly, PP1 appears to exhibit stronger affinity for S28 than S10, which possibly explains the more rapid S28 dephosphorylation during the late mitotic stages [19]. Of particular note, the inhibition of PP1 by calyculin A treatment in late G2 cells, when H3S28ph is normally undetectable, results in an aberrant increase in H3S28ph. Not only does this observation support PP1 as a H3 phosphatase [19] but it also suggests that the balance between the AURKB and PP1 activities are normally highly regulated. Accordingly, genome stability will be compromised if PP1 activity is altered either through mutation or misregulation. As with AURKB, gene re-sequencing efforts have identified a number of somatic mutations including non-synonymous single nucleotide polymorphisms and gene amplifications in a variety of tumor types (Table 2). In addition, immunohistochemistry and qRT-PCR recently revealed statistically significant increase in PP1 expression in highly malignant, grade IV glioblastomas relative to controls [67]. In particular, these findings suggest that excessive PP1 activity may contribute to tumor development and progression and further suggest that PP1 expression levels and the abundance of H3 phosphorylation may harbor prognostic or therapeutic value.

7 Regulation of H3 phosphorylation and implications in cancer and therapeutics

The proper regulation and balance of the enzymatic activities associated with the "writers" (MSK1, MSK2, AURKB) and "erasers" (PP1) are essential for a variety of processes that occur throughout the cell cycle and are essential for genome stability—they are instrumental in interphase to maintain appropriate responses to mitogenic signals, while in mitosis they are essential for chromosome compaction and segregation. As a result, knowing how these activities are regulated and altered in certain cancer-contexts may hold the key to their value and use as prognostic and therapeutic markers, or even reveal an Achilles' heel that can be exploited for therapeutic targeting.

8 The fine balance of histone methylation and demethylation enzymes

As with all histone PTMs, the abundance of H4K20me1 is achieved through the balance of the enzymatic activities of the "writers" (i.e., histone methyltransferases, HMTs) and the "erasers" (histone demethylases, HDMs). In general, lysine HMTs utilize *S*-adenosyl methionine as a substrate to covalently attach methyl groups onto the ε-amino group of lysine residues that are predominantly contained within the N-terminal tails of histones H3 and H4 [68–70]. Histone lysine methylation, however, is distinct from other PTMs in that it can exist in mono-, di-, and tri-methylated forms. The sequential addition of methyl groups can occur, but is not an essential requirement due to the capacity of some HMTs to mono-, di-, or tri-methylate unmodified lysine residues [71, 72]. The fact that different methylation levels can exist at a single site adds an additional layer of complexity and regulation that is unrivaled by other histone PTMs. It is now well accepted that the subtle structural differences imparted by the three distinct levels of lysine methylation often confer unique functional properties. Although histone methylation was initially believed to be a stable epigenetic mark, a number of HDMs have been identified that are capable of removing the various methyl groups attached to histones. As with all PTMs, it is the collective abundance, regulation, and context of surrounding PTMs that ultimately direct the functional output of a specific histone lysine methylation event [73].

9 Monomethylation of histone H4 at lysine 20 is essential for genome stability

H4K20me1 is another histone PTM that is essential to maintain genome stability and when altered is implicated in the pathogenesis of a number of tumor types (Fig. 1a). H4K20me1 (Table 1) is restricted to higher eukaryotes and is absent in lower eukaryotes indicating that this PTM evolved more recently [74]. In mammals, H4K20me1 is formed by an exclusive HMT, PR-Set7 (also known as SETDB8, SET8, and KMT5A) [75, 76]. Unlike other HMTs that can add or covalently attach multiple methyl groups at lysine residues, PR-Set7 is only capable of mono-methylating K20 [76–78]. In fact, based on 3D structural data, PR-Set7 is incapable of binding tri-methylated residues due to steric hindrance [74]. Immunofluorescence studies in human and mouse cells clearly show that the cell cycle-dependent distribution pattern of H4K20me1 mimics the temporal expression pattern of PR-Set7. PR-Set7 and H4K20me1 levels are virtually undetectable in G1, begin to increase throughout late S and G2 stages, and attain their peak during the middle stages of mitosis (i.e., prometaphase to anaphase), whereupon entry into the later stages of mitosis, the enzyme is rapidly degraded (resulting in concomitant decreases in H4K20me1), until basal levels are again reached by early G1 [79–81]. The global distribution of H4K20me1 varies depending on species, cell type, and cell cycle phase. In murine interphase cells, high-resolution microscopy revealed that the H4K20me1 is present at the periphery of the heterochromatic regions [81]. This spatial and temporal regulation pattern is similar to that observed for H3S10ph and H3S28ph, which suggests that it may be essential for cell cycle progression. Indeed, both S-phase and G2/M-phase arrests were observed in cells in which PR-Set7 silencing resulted in overall decreases in H4K20me1. Surprisingly, these and additional studies also implicated PR-Set7 and H4K20me1 in the regulation of mitotic chromosome condensation. It is thought that since H4K20 is positioned at the DNA-nucleosome junction, the presence or absence of H4K20me1 may directly impact chromatin structure by affecting internucleosomal contacts [82–84]. H4K20me1 indirectly regulates chromatin structure through the recruitment of protein complexes that mediate chromatin compaction such as condensin II [85]. Cells isolated from a murine model lacking PR-Set7 and H4K20me1 contained enlarged nuclei (characteristic of decondensed chromatin) and exhibited defects in DNA replication and increased levels of DNA damage [86]. Dominant negative experiments, which utilize catalytically dead PR-Set7, revealed that the mono-methylating activity of PR-Set7 is required to prevent these phenotypes and the presence of H4K20me1 is critical to maintain genome stability [79].

Aberrant PR-Set7 expression and/or function impacts the global abundance of H4K20me1, which, in turn, will produce a variety of aberrant phenotypes that promote tumor progression, development, and metastases. Recent gene resequencing efforts have determined that *PR-Set7* is somatically mutated in numerous tumor types (Table 2). But beyond altered function encoded by mutations, aberrant PR-Set7 expression and function also appears to be a pathogenic event, which stems from its role in gene regulation. Recently, Yang et al. [87] showed that PR-Set7 physically associates with TWIST, the master regulator of the epithelial–mesenchymal transition, to form a functional transcription factor complex. Under normal conditions, the epithelial–mesenchymal transition involves the induction of N-cadherin and silencing of E-cadherin genes in epithelial cells, so as to reduce cell–cell contacts and promote a mobile and invasive phenotype. The TWIST/PR-Set7 complex is recruited to TWIST target gene promoters, where PR-Set7 mono-methylates K20 (H4). This methylation event results in the silencing of E-cadherin, but surprisingly also induces N-cadherin expression. Overexpression of PR-Set 7 in breast epithelial cells up-regulates N-cadherin expression, while simultaneously down-regulates E-cadherin expression thereby promoting the epithelial–mesenchymal transition [87] and suggests that PR-Set7 may be a therapeutic target. Although the contrasting functional outcomes associated with H4K20me1 detailed above are paradoxical, they have been previously observed with other histone PTMs (see H3S10ph and H3S28ph above). It was recently shown for example that H4K20me1 physically recruits lethal 3 malignant brain tumor 1 (L3MBTL1) to specifically silence target genes [88], while in other instances H4K20me1 is enriched within promoter or coding regions of transcriptionally active genes [89–91]. Although the mechanism underlying these contradictory roles is unclear, it may be due to the surrounding histone PTM context [89, 90] or the presence or absence of cell-type specific PTM "readers" such as L3MBTL1.

10 Aberrant H4 demethylase activity is associated to tumors

The HDM responsible for the removal of H4K20me1 (Table 1) is plant homeodomain-finger protein 8 (PHF8) [85]. PHF8 is a member of the Fe^{2+}/2-oxoglutarate-dependent family of demethylases, which utilize 2-oxoglutarate, molecular oxygen, and Fe^{2+} as cofactors [92]. Mutation of the Fe^{2+} binding site of the plant homeodomain inhibited H4K20me1 demethylation, indicating that these domains are required for H4K20me1 demethylation [85]. Furthermore, RNAi-based silencing of PHF8 produced cell cycle arrests in G2/M and disrupted the G1/S-phase transition [85, 93], which further

supports a role for H4K20me1 in cell cycle progression. In addition, cell motility and invasion assays also support a role for PHF8 in cell migration, invasion, and inhibition of apoptosis [93]. Consequently, altered expression and/or function of PHF8 and the concomitant effect on H4K20me1 abundance would adversely impact a multitude of biological pathways that collectively are all implicated in the tumorigenic process.

The recent advances in characterizing PHF8 and its role(s) in a variety of cellular processes have strongly implicated aberrant expression and function in tumorigenesis. At the genomic level, gene re-sequencing efforts have identified a number of non-synonymous single nucleotide polymorphisms in a variety of tumor types (Table 2). Although these require functional validation, it can be assumed that certain mutations may adversely impact normal PHF8 function. Along those lines, Björkman and coworkers [93] recently determined that PHF8 is significantly overexpressed in clinical prostate cancer samples relative to controls and that PHF8 overexpression correlates with a more aggressive tumor type and consequently a poorer prognosis. They also demonstrated that RNAi-based silencing of PHF8 decreased cell proliferation within several highly proliferative prostate cancer cell lines but had little effect on benign or normal prostate cells. These findings suggest that PHF8 may exhibit a role in cellular proliferation and prostate cancer development. As such, PHF8 is an excellent candidate for therapeutic intervention in tumors where it is overexpressed. Along those lines, it has previously been shown that 2–4-pyridinecarboxylic acid, a Fe^{2+}/α-ketoglutarate-dependent oxygenase targeting compound, is capable of repressing PHF8 [94, 95], perhaps providing a starting point for the development of novel prostate cancer therapeutics.

11 Ubiquitination, a large and unique covalent histone modification

Histone ubiquitination, like phosphorylation and methylation, is implicated in a number of evolutionarily conserved cellular processes including transcription, DNA repair, and genome stability. It should be noted that histone ubiquitination, unlike canonical protein poly-ubiquitination, does not mark histones for proteolytic degradation via the 26S proteasome or subcellular targeting, but rather is intimately associated with the regulation of various other pathways and processes. Similar to the PTMs described above, the presence of ubiquitinated histones depends on the balance between the enzymatic activities of the enzymes that covalently add ubiquitin to lysine residues (see below), those that remove it, and the availability of ubiquitin itself. However, unlike the small covalent additions of phosphate or methyl groups, ubiquitin is a 76-amino acid polypeptide that adds approximately 8.5 kDa to the overall mass of a histone (~11–15 kDa). Of the four canonical histones, H2A, H2B, and H3 have been identified as ubiquitin substrates *in vivo* [96]. Currently, histone ubiquitination has only been observed within the C-terminal domains and only at a single lysine residue within histones H2A (K119) and H2B (K120) *in vivo* [97–99]. Although H2A can exist in either a mono- or poly-ubiquitinated form, H2B exists predominantly as a mono-ubiquitinated form that is referred to as H2Bub1 (Fig. 1a). In multicellular eukaryotes, both H2A and H2B are ubiquitinated, whereas in lower eukaryotes such as budding yeast, only H2B is capable of being ubiquitinated, which suggests that H2Bub1 likely exhibits critical roles in evolutionarily conserved cellular processes.

The genomic location and levels of ubiquitinated histones are highly regulated processes that are orchestrated through the balance of the enzymatic activities that add and remove ubiquitin. The addition of ubiquitin occurs via a multistep process that involves three distinct enzymatic reactions. First, the ubiquitin-activating enzyme (termed E1) activates ubiquitin in an ATP-dependent manner by forming a thiol ester linkage with the C-terminal glycine of ubiquitin. Ubiquitin is subsequently transferred from the E1 to the active site of an ubiquitin-conjugating enzyme (termed E2). The final step is mediated by the ubiquitin ligase (termed E3), which is required for target recognition (i.e., the specific histone lysine residue) and the formation of an isopeptide bond between the lysine residue of the target histone and the C-terminal glycine of ubiquitin [100]. The histone deubiquitination reaction is less complex and is generally mediated through the activities of ubiquitin-specific proteases (USPs). It is the balance between these two opposing enzymatic activities and the bioavailability of ubiquitin itself that regulates the global and gene-specific abundance of histone ubiquitination. What has now become clear is that disruptions in this balance have pathogenic consequences for a variety of human diseases including cancer. Next we specifically focus on H2Bub1, the enzymes that add or remove this PTM, its role in transcription, DNA repair and genome stability, and the implications for tumorigenesis.

12 Aberrant H2B ubiquitination and deubiquitination drive tumorigenesis

Alterations in the enzymes that regulate histone ubiquitination and deubiquitination pathways directly impact higher-order chromatin structure [101, 102] (i.e., beyond the single nucleosome level), which ultimately influences all cellular processes that employ chromatin as a biological template. Consequently, aberrant expression and/or function of ubiquitin/deubiquitin complex members have the potential to adversely impact these processes, many of which directly impact genome stability. Ubiquitination of mammalian H2B

occurs at K120 and is predominantly regulated through the enzymatic activities of ubiquitin-conjugating enzyme E2A (UBE2A or RAD6A) and the RNF20/RNF40 ubiquitin ligase complex (Table 1). RNAi-based silencing of these components results in concomitant decreases in the global abundance of H2Bub1 [103, 104]. Altered expression of UBE2A and RNF20/RNF40 is suggested to be a pathogenic event that contributes to the development and progression of various tumor types. For example, gene re-sequencing efforts from various tumor types have uncovered genetic alterations including non-synonymous single nucleotide polymorphisms, amplifications, and homozygous deletions (Table 2). Translational studies by Shema et al. [104] revealed that *RNF20* promoters are hypermethylated within breast cancer tumor samples relative to controls. Additional studies have also shown that reduced levels of RNF20 occur in testicular germ cell carcinomas (seminomas) [105] and parathyroid cancers [106]. At the very least, these correlative studies suggest that altered RNF20 expression and/or function, and presumably altered levels of H2Bub1, may be causal factors that contribute to cancer development and progression. Indeed, it was recently shown that the global abundance of H2Bub1 was decreased within a small cohort of malignant breast cancer samples relative to normal and benign controls, which is consistent with a role of H2Bub1 in tumor suppression [107].

In an analogous fashion to that detailed above, aberrant expression and/or function of the deubiquitination pathway members are also expected to affect the global abundance of H2Bub1. Multiple H2Bub1 deubiquitinating enzymes have been proposed (e.g., USP44, USP3, USP27X, USP51, and USP7), but many of these appear to be cell-type and cell-context specific. USP22, on the other hand, is ubiquitously expressed and is generally accepted as the predominant H2Bub1 deubiquitinating enzyme [102, 108, 109]. As with *RNF20*, a number of different somatic mutations within *USP22* (e.g., non-synonymous single nucleotide polymorphisms, amplifications, and homozygous deletions) have been identified in a variety of tumor types (Table 2). Recently, increases in USP22 expression, and the resulting decrease in H2Bub1, have garnered much attention. For example, Zhang et al. [110] noted that USP22 levels were elevated in human breast cancer samples relative to benign and normal controls. In fact, those patients with increased levels of USP22 levels had more aggressive tumors and poorer overall outcomes than patients with lower USP22 levels. Furthermore, they determined that USP22 levels were positively correlated with lymph node metastases, HER-2 positivity, Ki67 expression levels and breast cancer recurrence, leading the authors to propose that USP22 may serve as a prognostic indicator of breast cancer survival [110, 111]. Although not specifically evaluated, these results may suggest that in addition to USP22, perhaps H2Bub1 may also have prognostic value as it reflects not only the abundance of USP22 but also the abundance of RNF20/RNF40.

13 H2Bub1 promotes tumor suppressing transcriptional profiles

H2Bub1 is classically defined as an epigenetic mark that is associated with transcriptional activation and tumor suppression [112, 113]. The covalent addition of the large ubiquitin moiety onto the C-terminal tail of histone H2B is expected to have a significant impact on higher-order chromatin structure. The presence of nucleosomes within coding regions represents a challenge to transcriptional elongation [1, 114]. One can simply envision that H2B ubiquitination disrupts higher-order chromatin structure to render specific genomic regions accessible to transcription factors and factories thereby enabling gene transcription. Support for this concept came in 2003 [115] when it was established that H2B ubiquitination promotes transcriptional elongation by destabilizing nucleosomes through a process that is mediated by a histone chaperone complex called facilitates transcription through chromatin (FACT). These *in vitro* transcription studies performed on reconstituted chromatin revealed that RNF20-RNF40, in combination with FACT, is essential for efficient and optimal transcription [115]. It is believed that the FACT complex plays a major role in promoting H2A–H2B dimer displacement when the RNA polymerase II complex is transcribing through the nucleosome. The H2A–H2B displacement appears to partially overcome the transcriptional barrier imparted by the canonical nucleosome structure and allows for increased transcription efficiency. In agreement with the above mechanism, FACT in the presence of H2Bub1 resulted in the overall increase in RNA polymerase II elongation rates and significantly increased transcript lengths [116]. Moreover, a positive correlation between gene length and reduced sensitivity to retinoic acid gene induction was found in an RNF20-depleted neuronally committed human teratocarcinoma cell line [117]. Consistent with a role in transcriptional elongation, RNF20 is recruited to the elongating form of RNA polymerase II through an adaptor protein called WAC that is essential for transcription-associated H2B ubiquitination [118].

The information detailed above argues that altered expression and/or function of ubiquitinating or deubiquitinating complex members will impact the normal abundance of H2Bub1 and ultimately impact gene expression profiles. Indeed, alterations in the abundance of H2Bub1 impact gene expression in a manner that promotes oncogenic transformation. For example, Shema and colleagues [104] demonstrated that RNF20 silencing and concomitant decreases in H2Bub1 altered expression profiles of a subset of genes. This not only

included the down-regulation of specific tumor suppressor genes such as *TP53* but also the surprising up-regulation of several proto-oncogenes including *MYC* and *FOS*. Interestingly, this study demonstrated that the induction of IEGs was increased when RNF20 expression was knocked down. These results suggest that H2Bub1 regulates the level of IEG induction. The lack of RNF20 and H2Bub1 at IEGs may result in the increased levels of IEG products such as FOS and JUN, which support oncogenic and metastatic programs, similar to that observed in cancer cells with a deregulated RAS-MAPK-MSK pathway [49]. While there have been numerous studies that can account for the transcriptional down-regulation following RNF20 silencing, the mechanism(s) that account for the unexpected increase in gene expression are just beginning to be understood [119]. In addition to modifying tumor suppressor genes and proto-oncogene expression profiles, altered RNF20 expression also affects cell mobility and therefore may contribute to metastatic potential. RNAi-based silencing of RNF20, and presumably diminished H2Bub1, resulted in increased cell migration in a breast cancer cell line, while overexpression of RNF20 in an ovarian cancer cell line significantly decreased cell mobility [104]. Finally, H2Bub1 levels are also correlated with differentiation in multipotent stem cells, suggesting that altered H2Bub1 levels may impact the differentiated state. Karpiuk and colleagues [120] recently demonstrated that H2Bub1 levels increase during human mesenchymal stem cell differentiation, and when H2Bub1 levels are altered, this leads to a less differentiated phenotype in advanced tumors thus enhancing malignant and metastatic potential. RNF20 knockdown altered the expression profiles of differentiation-associated genes, which impaired human mesenchymal stem cell differentiation [120]. This may have important implications in a cancer-context as tumor cells deficient in H2Bub1 may fail to terminally differentiate, which leads to a high-grade tumor. Collectively, the above data strongly suggest that alterations in the enzymatic activities that regulate the global abundance of H2Bub1 are pathogenic events that contribute to malignant transformation and metastatic potential.

14 The impact of H2B ubiquitination on chromatin structure and DNA damage repair

H2Bub1 has most recently been implicated in DNA double-strand break (DSB) repair [105, 121–123], which is an indispensible process required to maintain genome stability. The ability of H2Bub1 to disrupt higher-order chromatin structure is an essential process required for the timely repair of DNA DSBs [123]. In fact, RNAi-based silencing of RNF20 resulted in aberrant phenotypes that are commonly associated with a defective DNA damage response, such as increased sensitivity to radiomimetic drugs and delayed repair kinetics as indicated by the persistence of γH2AX and 53BP1 foci [123]. Furthermore, cells in which either RNF20 or RNF40 were independently or simultaneously silenced exhibited significant increases in DNA DSBs relative to controls, which strongly implicates H2Bub1 in DNA DSB repair [123]. Under normal conditions, DNA DSBs repair requires chromatin regions surrounding the break to undergo localized decondensation that appears to involve H2Bub1, at least in part. Using a novel *in vitro* fluorescence-based assay, Fierz and colleagues [101] demonstrated that H2Bub1 disrupts higher-order chromatin structure by impairing inter-fiber interactions, thus promoting a more open and biochemically accessible chromatin fiber. Presumably by rendering this chromatin more accessible to DSB repair proteins, a break can be accurately repaired in a timely manner. Indeed, diminished recruitment of several critical members of the two DNA DSB repair pathways (i.e., non-homologous end joining and homology recombination repair) to the sites of laser-induced DNA DSBs is observed within cells that have been depleted of RNF20/RNF40. Failure to accurately repair DNA DSBs within critical loci has the potential to alter gene expression profiles, produce chromosome translocations, and drive chromosome instability, which would ultimately underlie and contribute to the tumorigenic process.

15 Is there prognostic and therapeutic value for H2Bub1?

H2Bub1 is rapidly emerging as a histone PTM that has a particularly strong role in regulating chromatin structure to impact gene transcription, DNA repair, and chromosome stability to ultimately influence genome stability. Regardless of the exact contribution to tumor initiation and development, a clearer understanding of the frequency of H2Bub1 depletion with respect to cancer progression is required. Although a link to cancer progression has been proposed, further immunohistological studies regarding H2Bub1 in larger cohorts and different tissue types are needed. Information gleaned from these studies may provide novel information regarding H2Bub1 and its potential use as a prognostic marker. Finally, understanding the underlying biology behind aberrant H2Bub1 levels and its contribution to tumorigenesis may reveal novel drug targets and provide novel therapeutic strategies designed to better combat cancer.

16 Overall concluding remarks

This review highlights the role of histone PTMs, H3S10ph/HS28ph, H4K20me1, and H2Bub1 in genomic instability. There is still much to be learned about how these histone PTMs are "read" throughout the cell cycle and what structure

and/or function the histone PTM/reader impart on the chromatin region. Current evidence clearly demonstrates that the proper balance of the enzymatic activities associated with the "writers" and "erasers" (and the "readers") is essential to maintain genome stability. Beyond their fundamental roles in regulating chromatin structure, these enzymes and the PTMs that they regulate impact a multitude and diverse array of nuclear processes that collectively utilize chromatin as a template. These processes include DNA replication, DNA repair, gene transcription, and chromosome segregation, and the stringent control of these processes is fundamental to the maintenance of genome and chromosome stability. When these processes become altered, they contribute to and drive the tumorigenic process. As a result, it is critical to understand when, how, and why these activities are regulated or misregulated in certain cancer contexts, so that their real value as prognostic indicators or therapeutic targets can be realized.

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References

1. Luger, K., et al. (1997). Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature, 389*(6648), 251–260.
2. Cohen, I., et al. (2011). Histone modifiers in cancer: friends or foes? *Genes & Cancer, 2*(6), 631–647.
3. Martin, C., & Zhang, Y. (2007). Mechanisms of epigenetic inheritance. *Current Opinion in Cell Biology, 19*(3), 266–272.
4. Margueron, R., Trojer, P., & Reinberg, D. (2005). The key to development: interpreting the histone code? *Current Opinion in Genetics & Development, 15*(2), 163–176.
5. Horn, P. J., et al. (2002). Phosphorylation of linker histones regulates ATP-dependent chromatin remodeling enzymes. *Nature Structural Biology, 9*(4), 263–267.
6. Williamson, W. D., & Pinto, I. (2012). Histones and genome integrity. *Frontiers in Bioscience: A Journal and Virtual Library, 17*, 984–995.
7. Ruthenburg, A. J., Allis, C. D., & Wysocka, J. (2007). Methylation of lysine 4 on histone H3: intricacy of writing and reading a single epigenetic mark. *Molecular Cell, 25*(1), 15–30.
8. Kouzarides, T. (2007) SnapShot: histone-modifying enzymes. *Cell*, *131*(4), 822.
9. Issa, J. P. (2004). CpG island methylator phenotype in cancer. *Nature Reviews. Cancer, 4*(12), 988–993.
10. Kerzendorfer, C., & O'Driscoll, M. (2009). Human DNA damage response and repair deficiency syndromes: linking genomic instability and cell cycle checkpoint proficiency. *DNA Repair, 8*(9), 1139–1152.
11. Geigl, J. B., et al. (2008). Defining 'chromosomal instability'. *Trends in Genetics: TIG, 24*(2), 64–69.
12. Schroeder, T. M. (1982). Genetically-determined chromosome instability syndromes. *Cytogenetics and Cell Genetics, 33*(1–2), 119–132.
13. Huang, C. C., Banerjee, A., & Hou, Y. (1975). Chromosomal instability in cell lines derived from patients with xeroderma pigmentosum. *Proceedings of the Society for Experimental Biology and Medicine, 148*(4), 1244–1248.
14. Chrzanowska, K. H., et al. (2012). Nijmegen breakage syndrome (NBS). *Orphanet Journal of Rare Diseases, 7*, 13.
15. Lengauer, C., Kinzler, K. W., & Vogelstein, B. (1998). Genetic instabilities in human cancers. *Nature, 396*(6712), 643–649.
16. Negrini, S., Gorgoulis, V. G., & Halazonetis, T. D. (2010). Genomic instability—an evolving hallmark of cancer. *Nature Reviews. Molecular Cell Biology, 11*(3), 220–228.
17. Drobic, B., et al. (2010). Promoter chromatin remodeling of immediate-early genes is mediated through H3 phosphorylation at either serine 28 or 10 by the MSK1 multi-protein complex. *Nucleic Acids Research, 38*(10), 3196–3208.
18. Prigent, C., & Dimitrov, S. (2003). Phosphorylation of serine 10 in histone H3, what for? *Journal of Cell Science, 116*(Pt 18), 3677–3685.
19. Goto, H., et al. (2002). Aurora-B phosphorylates histone H3 at serine28 with regard to the mitotic chromosome condensation. *Genes to Cells: Devoted to Molecular & Cellular Mechanisms, 7*(1), 11–17.
20. Mahadevan, L. C., Willis, A. C., & Barratt, M. J. (1991). Rapid histone H3 phosphorylation in response to growth factors, phorbol esters, okadaic acid, and protein synthesis inhibitors. *Cell, 65*(5), 775–783.
21. Soloaga, A., et al. (2003). MSK2 and MSK1 mediate the mitogen- and stress-induced phosphorylation of histone H3 and HMG-14. *The EMBO Journal, 22*(11), 2788–2797.
22. Lee, Y. J., & Shukla, S. D. (2007). Histone H3 phosphorylation at serine 10 and serine 28 is mediated by p38 MAPK in rat hepatocytes exposed to ethanol and acetaldehyde. *European Journal of Pharmacology, 573*(1–3), 29–38.
23. Delcuve, G. P., Rastegar, M., & Davie, J. R. (2009). Epigenetic control. *Journal of Cellular Physiology, 219*(2), 243–250.
24. Caivano, M., & Cohen, P. (2000). Role of mitogen-activated protein kinase cascades in mediating lipopolysaccharide-stimulated induction of cyclooxygenase-2 and IL-1 beta in RAW264 macrophages. *Journal of Immunology, 164*(6), 3018–3025.
25. Davie, J. R. (2003). MSK1 and MSK2 mediate mitogen- and stress-induced phosphorylation of histone H3: a controversy resolved. *Science's STKE: Signal Transduction Knowledge Environment, 2003*(195), PE33.
26. Strelkov, I. S., & Davie, J. R. (2002). Ser-10 phosphorylation of histone H3 and immediate early gene expression in oncogene-transformed mouse fibroblasts. *Cancer Research, 62*(1), 75–78.
27. Macdonald, N., et al. (2005). Molecular basis for the recognition of phosphorylated and phosphoacetylated histone h3 by 14-3-3. *Molecular Cell, 20*(2), 199–211.
28. Winter, S., Fischle, W., & Seiser, C. (2008). Modulation of 14-3-3 interaction with phosphorylated histone H3 by combinatorial modification patterns. *Cell Cycle, 7*(10), 1336–1342.
29. Dunn, K. L., & Davie, J. R. (2005). Stimulation of the Ras-MAPK pathway leads to independent phosphorylation of histone H3 on serine 10 and 28. *Oncogene, 24*(21), 3492–3502.
30. Dyson, M. H., et al. (2005). MAP kinase-mediated phosphorylation of distinct pools of histone H3 at S10 or S28 via mitogen- and stress-activated kinase 1/2. *Journal of Cell Science, 118*(Pt 10), 2247–2259.
31. Dunn, K. L., et al. (2009). Increased genomic instability and altered chromosomal protein phosphorylation timing in HRAS-transformed mouse fibroblasts. *Genes, Chromosomes & Cancer, 48*(5), 397–409.

32. Lim, J. H., et al. (2004). Chromosomal protein HMGN1 modulates histone H3 phosphorylation. *Molecular Cell, 15*(4), 573–584.
33. Perez-Cadahia, B., Drobic, B., & Davie, J. R. (2009). H3 phosphorylation: dual role in mitosis and interphase. *Biochemistry and Cell Biology = Biochimie et Biologie Cellulaire, 87*(5), 695–709.
34. Shaulian, E., & Karin, M. (2002). AP-1 as a regulator of cell life and death. *Nature Cell Biology, 4*(5), E131–E136.
35. Jochum, W., Passegue, E., & Wagner, E. F. (2001). AP-1 in mouse development and tumorigenesis. *Oncogene, 20*(19), 2401–2412.
36. Wagner, E. F. (2002). Functions of AP1 (Fos/Jun) in bone development. *Annals of the Rheumatic Diseases, 61*(Suppl 2), ii40–ii42.
37. Grigoriadis, A. E., et al. (1993). Osteoblasts are target cells for transformation in c-fos transgenic mice. *The Journal of Cell Biology, 122*(3), 685–701.
38. Ruther, U., Wagner, E. F., & Muller, R. (1985). Analysis of the differentiation-promoting potential of inducible c-fos genes introduced into embryonal carcinoma cells. *The EMBO Journal, 4*(7), 1775–1781.
39. Wang, Z. Q., et al. (1995). c-fos-induced osteosarcoma formation in transgenic mice: cooperativity with c-jun and the role of endogenous c-fos. *Cancer Research, 55*(24), 6244–6251.
40. van den Berg, S., et al. (1993). Overexpression of c-fos increases recombination frequency in human osteosarcoma cells. *Carcinogenesis, 14*(5), 925–928.
41. Lengauer, C., Kinzler, K. W., & Vogelstein, B. (1997). Genetic instability in colorectal cancers. *Nature, 386*(6625), 623–627.
42. Drobic, B., Espino, P. S., & Davie, J. R. (2004). Mitogen- and stress-activated protein kinase 1 activity and histone h3 phosphorylation in oncogene-transformed mouse fibroblasts. *Cancer Research, 64*(24), 9076–9079.
43. Forbes, S. A., et al. (2011). COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer. *Nucleic Acids Research, 39*(Database issue), D945–D950.
44. Forbes, S. A., et al. (2010). COSMIC (the Catalogue of Somatic Mutations in Cancer): a resource to investigate acquired mutations in human cancer. *Nucleic Acids Research, 38*(Database issue), D652–D657.
45. Cerami, E., et al. (2012). The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discovery, 2*(5), 401–404.
46. Schiller, M., et al. (2006). Mitogen- and stress-activated protein kinase 1 is critical for interleukin-1-induced, CREB-mediated, c-fos gene expression in keratinocytes. *Oncogene, 25*(32), 4449–4457.
47. Chang, S., et al. (2011). Mice lacking MSK1 and MSK2 show reduced skin tumor development in a two-stage chemical carcinogenesis model. *Cancer Investigation, 29*(3), 240–245.
48. Kim, H. G., et al. (2008). Mitogen- and stress-activated kinase 1-mediated histone H3 phosphorylation is crucial for cell transformation. *Cancer Research, 68*(7), 2538–2547.
49. Perez-Cadahia, B., et al. (2011). Role of MSK1 in the malignant phenotype of Ras-transformed mouse fibroblasts. *The Journal of Biological Chemistry, 286*(1), 42–49.
50. Hendzel, M. J., et al. (1997). Mitosis-specific phosphorylation of histone H3 initiates primarily within pericentromeric heterochromatin during G2 and spreads in an ordered fashion coincident with mitotic chromosome condensation. *Chromosoma, 106*(6), 348–360.
51. McManus, K. J., & Hendzel, M. J. (2006). The relationship between histone H3 phosphorylation and acetylation throughout the mammalian cell cycle. *Biochemistry and Cell Biology = Biochimie et Biologie Cellulaire, 84*(4), 640–657.
52. Goto, H., et al. (1999). Identification of a novel phosphorylation site on histone H3 coupled with mitotic chromosome condensation. *The Journal of Biological Chemistry, 274*(36), 25543–25549.
53. Crosio, C., et al. (2002). Mitotic phosphorylation of histone H3: spatio-temporal regulation by mammalian Aurora kinases. *Molecular and Cellular Biology, 22*(3), 874–885.
54. Kallio, M. J., et al. (2002). Inhibition of aurora B kinase blocks chromosome segregation, overrides the spindle checkpoint, and perturbs microtubule dynamics in mitosis. *Current Biology: CB, 12*(11), 900–905.
55. Murata-Hori, M., Tatsuka, M., & Wang, Y. L. (2002). Probing the dynamics and functions of aurora B kinase in living cells during mitosis and cytokinesis. *Molecular Biology of the Cell, 13*(4), 1099–1108.
56. Van Hooser, A., et al. (1998). Histone H3 phosphorylation is required for the initiation, but not maintenance, of mammalian chromosome condensation. *Journal of Cell Science, 111*(Pt 23), 3497–3506.
57. Katayama, H., et al. (2004). Phosphorylation by aurora kinase A induces Mdm2-mediated destabilization and inhibition of p53. *Nature Genetics, 36*(1), 55–62.
58. Ota, T., et al. (2002). Increased mitotic phosphorylation of histone H3 attributable to AIM-1/Aurora-B overexpression contributes to chromosome number instability. *Cancer Research, 62*(18), 5168–5177.
59. Tuncel, H., et al. (2012). Nuclear Aurora B and cytoplasmic Survivin expression is involved in lymph node metastasis of colorectal cancer. *Oncology Letters, 3*(5), 1109–1114.
60. Qi, G., et al. (2007). Aurora-B expression and its correlation with cell proliferation and metastasis in oral cancer. *Virchows Archiv: An International Journal of Pathology, 450*(3), 297–302.
61. Lee, E. C., et al. (2006). Targeting Aurora kinases for the treatment of prostate cancer. *Cancer Research, 66*(10), 4996–5002.
62. Kurai, M., et al. (2005). Expression of Aurora kinases A and B in normal, hyperplastic, and malignant human endometrium: Aurora B as a predictor for poor prognosis in endometrial carcinoma. *Human Pathology, 36*(12), 1281–1288.
63. Sorrentino, R., et al. (2005). Aurora B overexpression associates with the thyroid carcinoma undifferentiated phenotype and is required for thyroid carcinoma cell proliferation. *The Journal of Clinical Endocrinology and Metabolism, 90*(2), 928–935.
64. Harrington, E. A., et al. (2004). VX-680, a potent and selective small-molecule inhibitor of the Aurora kinases, suppresses tumor growth in vivo. *Nature Medicine, 10*(3), 262–267.
65. Hsu, J. Y., et al. (2000). Mitotic phosphorylation of histone H3 is governed by Ipl1/aurora kinase and Glc7/PP1 phosphatase in budding yeast and nematodes. *Cell, 102*(3), 279–291.
66. de Carvalho, C. E., et al. (2008). LAB-1 antagonizes the Aurora B kinase in *C. elegans*. *Genes & Development, 22*(20), 2869–2885.
67. Ladha, J., et al. (2010). Glioblastoma-specific protein interaction network identifies PP1A and CSK21 as connecting molecules between cell cycle-associated genes. *Cancer Research, 70*(16), 6437–6447.
68. Rea, S., et al. (2000). Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature, 406*(6796), 593–599.
69. Lachner, M., O'Sullivan, R. J., & Jenuwein, T. (2003). An epigenetic road map for histone lysine methylation. *Journal of Cell Science, 116*(Pt 11), 2117–2124.
70. He, Y., et al. (2012). Targeting protein lysine methylation and demethylation in cancers. *Acta biochimica et biophysica Sinica, 44*(1), 70–79.
71. Steward, M. M., et al. (2006). Molecular regulation of H3K4 trimethylation by ASH2L, a shared subunit of MLL complexes. *Nature Structural & Molecular Biology, 13*(9), 852–854.
72. Santos-Rosa, H., et al. (2002). Active genes are tri-methylated at K4 of histone H3. *Nature, 419*(6905), 407–411.
73. Wang, H., et al. (2001). Purification and functional characterization of a histone H3-lysine 4-specific methyltransferase. *Molecular Cell, 8*(6), 1207–1217.
74. Beck, D. B., et al. (2012). PR-Set7 and H4K20me1: at the crossroads of genome integrity, cell cycle, chromosome condensation, and transcription. *Genes & Development, 26*(4), 325–337.

75. Rice, J. C., et al. (2002). Mitotic-specific methylation of histone H4 Lys 20 follows increased PR-Set7 expression and its localization to mitotic chromosomes. *Genes & Development, 16*(17), 2225–2230.
76. Nishioka, K., et al. (2002). PR-Set7 is a nucleosome-specific methyltransferase that modifies lysine 20 of histone H4 and is associated with silent chromatin. *Molecular Cell, 9*(6), 1201–1213.
77. Xiao, B., et al. (2005). Specificity and mechanism of the histone methyltransferase Pr-Set7. *Genes & Development, 19*(12), 1444–1454.
78. Couture, J. F., et al. (2005). Structural and functional analysis of SET8, a histone H4 Lys-20 methyltransferase. *Genes & Development, 19*(12), 1455–1465.
79. Houston, S. I., et al. (2008). Catalytic function of the PR-Set7 histone H4 lysine 20 monomethyltransferase is essential for mitotic entry and genomic stability. *The Journal of Biological Chemistry, 283*(28), 19478–19488.
80. Sims, J. K., et al. (2006). A trans-tail histone code defined by monomethylated H4 Lys-20 and H3 Lys-9 demarcates distinct regions of silent chromatin. *The Journal of Biological Chemistry, 281*(18), 12760–12766.
81. Biron, V. L., et al. (2004). Distinct dynamics and distribution of histone methyl-lysine derivatives in mouse development. *Developmental Biology, 276*(2), 337–351.
82. Lu, X., et al. (2008). The effect of H3K79 dimethylation and H4K20 trimethylation on nucleosome and chromatin structure. *Nature Structural & Molecular Biology, 15*(10), 1122–1124.
83. Davey, C. A., et al. (2002). Solvent mediated interactions in the structure of the nucleosome core particle at 1.9 a resolution. *Journal of Molecular Biology, 319*(5), 1097–1113.
84. Dorigo, B., et al. (2003). Chromatin fiber folding: requirement for the histone H4 N-terminal tail. *Journal of Molecular Biology, 327*(1), 85–96.
85. Liu, W., et al. (2010). PHF8 mediates histone H4 lysine 20 demethylation events involved in cell cycle progression. *Nature, 466*(7305), 508–512.
86. Oda, H., et al. (2009). Monomethylation of histone H4-lysine 20 is involved in chromosome structure and stability and is essential for mouse development. *Molecular and Cellular Biology, 29*(8), 2278–2295.
87. Yang, F., et al. (2012). SET8 promotes epithelial–mesenchymal transition and confers TWIST dual transcriptional activities. *The EMBO Journal, 31*(1), 110–123.
88. Kalakonda, N., et al. (2008). Histone H4 lysine 20 monomethylation promotes transcriptional repression by L3MBTL1. *Oncogene, 27*(31), 4293–4304.
89. Barski, A., et al. (2007). High-resolution profiling of histone methylations in the human genome. *Cell, 129*(4), 823–837.
90. Talasz, H., et al. (2005). Histone H4-lysine 20 monomethylation is increased in promoter and coding regions of active genes and correlates with hyperacetylation. *The Journal of Biological Chemistry, 280*(46), 38814–38822.
91. Vakoc, C. R., et al. (2006). Profile of histone lysine methylation across transcribed mammalian chromatin. *Molecular and Cellular Biology, 26*(24), 9185–9195.
92. Loenarz, C., & Schofield, C. J. (2008). Expanding chemical biology of 2-oxoglutarate oxygenases. *Nature Chemical Biology, 4*(3), 152–156.
93. Bjorkman, M., et al. (2012). Systematic knockdown of epigenetic enzymes identifies a novel histone demethylase PHF8 overexpressed in prostate cancer with an impact on cell proliferation, migration and invasion. *Oncogene, 31*(29), 3444–3456.
94. Rose, N. R., et al. (2010). Selective inhibitors of the JMJD2 histone demethylases: combined nondenaturing mass spectrometric screening and crystallographic approaches. *Journal of Medicinal Chemistry, 53*(4), 1810–1818.
95. King, O. N., et al. (2010). Quantitative high-throughput screening identifies 8-hydroxyquinolines as cell-active histone demethylase inhibitors. *PloS One, 5*(11), e15535.
96. Chen, H. Y., et al. (1998). Ubiquitination of histone H3 in elongating spermatids of rat testes. *The Journal of Biological Chemistry, 273*(21), 13165–13169.
97. Thorne, A. W., et al. (1987). The structure of ubiquitinated histone H2B. *The EMBO Journal, 6*(4), 1005–1010.
98. Osley, M. A. (2006). Regulation of histone H2A and H2B ubiquitylation. *Briefings in Functional Genomics & Proteomics, 5*(3), 179–189.
99. Wang, H., et al. (2004). Role of histone H2A ubiquitination in Polycomb silencing. *Nature, 431*(7010), 873–878.
100. Pickart, C. M. (2001). Mechanisms underlying ubiquitination. *Annual Review of Biochemistry, 70*, 503–533.
101. Fierz, B., et al. (2011). Histone H2B ubiquitylation disrupts local and higher-order chromatin compaction. *Nature Chemical Biology, 7*(2), 113–119.
102. Johnsen, S. A. (2012). The enigmatic role of H2Bub1 in cancer. *FEBS Letters, 586*(11), 1592–1601.
103. Kim, J., et al. (2009). RAD6-mediated transcription-coupled H2B ubiquitylation directly stimulates H3K4 methylation in human cells. *Cell, 137*(3), 459–471.
104. Shema, E., et al. (2008). The histone H2B-specific ubiquitin ligase RNF20/hBRE1 acts as a putative tumor suppressor through selective regulation of gene expression. *Genes & Development, 22*(19), 2664–2676.
105. Chernikova, S. B., et al. (2010). Deficiency in Bre1 impairs homologous recombination repair and cell cycle checkpoint response to radiation damage in mammalian cells. *Radiation Research, 174*(5), 558–565.
106. Hahn, M. A., et al. (2012). The tumor suppressor CDC73 interacts with the ring finger proteins RNF20 and RNF40 and is required for the maintenance of histone 2B monoubiquitination. *Human Molecular Genetics, 21*(3), 559–568.
107. Prenzel, T., et al. (2011). Estrogen-dependent gene transcription in human breast cancer cells relies upon proteasome-dependent monoubiquitination of histone H2B. *Cancer Research, 71*(17), 5739–5753.
108. Zhao, Y., et al. (2008). A TFTC/STAGA module mediates histone H2A and H2B deubiquitination, coactivates nuclear receptors, and counteracts heterochromatin silencing. *Molecular Cell, 29*(1), 92–101.
109. Zhang, X. Y., et al. (2008). The putative cancer stem cell marker USP22 is a subunit of the human SAGA complex required for activated transcription and cell-cycle progression. *Molecular Cell, 29*(1), 102–111.
110. Zhang, Y., et al. (2011). Elevated expression of USP22 in correlation with poor prognosis in patients with invasive breast cancer. *Journal of Cancer Research and Clinical Oncology, 137*(8), 1245–1253.
111. Liu, Y. L., et al. (2010). Increased expression of ubiquitin-specific protease 22 can promote cancer progression and predict therapy failure in human colorectal cancer. *Journal of Gastroenterology and Hepatology, 25*(11), 1800–1805.
112. Davie, J. R., & Murphy, L. C. (1994). Inhibition of transcription selectively reduces the level of ubiquitinated histone H2B in chromatin. *Biochemical and Biophysical Research Communications, 203*(1), 344–350.
113. Davie, J. R., & Murphy, L. C. (1990). Level of ubiquitinated histone H2B in chromatin is coupled to ongoing transcription. *Biochemistry, 29*(20), 4752–4757.
114. Li, B., Carey, M., & Workman, J. L. (2007). The role of chromatin during transcription. *Cell, 128*(4), 707–719.
115. Belotserkovskaya, R., et al. (2003). FACT facilitates transcription-dependent nucleosome alteration. *Science, 301*(5636), 1090–1093.

116. Pavri, R., et al. (2006). Histone H2B monoubiquitination functions cooperatively with FACT to regulate elongation by RNA polymerase II. *Cell, 125*(4), 703–717.
117. Fuchs, G., et al. (2012). RNF20 and USP44 regulate stem cell differentiation by modulating H2B monoubiquitylation. *Molecular Cell, 46*(5), 662–673.
118. Zhang, F., & Yu, X. (2011). WAC, a functional partner of RNF20/40, regulates histone H2B ubiquitination and gene transcription. *Molecular Cell, 41*(4), 384–397.
119. Shema, E., et al. (2011). RNF20 inhibits TFIIS-facilitated transcriptional elongation to suppress pro-oncogenic gene expression. *Molecular Cell, 42*(4), 477–488.
120. Karpiuk, O., et al. (2012). The histone H2B monoubiquitination regulatory pathway is required for differentiation of multipotent stem cells. *Molecular Cell, 46*(5), 705–713.
121. Shiloh, Y., et al. (2011). RNF20-RNF40: a ubiquitin-driven link between gene expression and the DNA damage response. *FEBS Letters, 585*(18), 2795–2802.
122. Nakamura, K., et al. (2011). Regulation of homologous recombination by RNF20-dependent H2B ubiquitination. *Molecular Cell, 41*(5), 515–528.
123. Moyal, L., et al. (2011). Requirement of ATM-dependent monoubiquitylation of histone H2B for timely repair of DNA double-strand breaks. *Molecular Cell, 41*(5), 529–542.