HHS Public Access

Author manuscript

Nat Rev Cancer. Author manuscript; available in PMC 2021 May 03.

Published in final edited form as:

Nat Rev Cancer. 2021 April ; 21(4): 257–275. doi:10.1038/s41568-020-00330-0.

Solid tumours hijack the histone variant network

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Abstract

Cancer is a complex disease characterized by loss of cellular homeostasis through genetic and epigenetic alterations. Emerging evidence highlights a role for histone variants and their dedicated chaperones in cancer initiation and progression. Histone variants are involved in processes as diverse as maintenance of genome integrity, nuclear architecture and cell identity. On a molecular level, histone variants add a layer of complexity to the dynamic regulation of transcription, DNA replication and repair, and mitotic chromosome segregation. Because these functions are critical to ensure normal proliferation and maintenance of cellular fate, cancer cells are defined by their capacity to subvert them. Hijacking histone variants and their chaperones is emerging as a common means to disrupt homeostasis across a wide range of cancers, particularly solid tumours. Here we discuss histone variants and histone chaperones as tumour-promoting or tumour-suppressive players in the pathogenesis of cancer.

Histone variants and their dedicated chaperones, which escort histones throughout the cell and deposit them into chromatin (FIG. 1), are altered in both paediatric and adult solid tumours (TABLE 1). The discovery of ‘oncohistones’, histone mutations found at high frequency in specific tumour types, has dramatically shifted the paradigm of histone variant function from cellular housekeeping and developmental processes to cancer initiation and progression. Ongoing efforts to understand the biological and clinical consequences of epigenome rewiring by abnormal histone incorporation may reveal novel tumour vulnerabilities that can be leveraged with anticancer therapeutics.

Histones play a fundamental role in the process of compacting DNA into the nucleosomal fibre, and actively proliferating cells ensure this function by loading large amounts of canonical histones onto DNA during replication (BOX 1). Histone variants, which share protein sequence homology with canonical histones, are encoded by single genes dispersed

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Author contributions

D.F. and F.G.G. researched data for the article: All authors substantially contributed to discussion of the content, and wrote, reviewed and edited the manuscript before submission.

Competing interests

The authors declare no competing interests.

Peer review information

Nature Reviews Cancer thanks M. Buschbeck and Z. Zhang for their contribution to the peer review of this work.

Supplementary information

Supplementary information is available for this paper at https://doi.org/10.1038/s41568-020-00330-0.

throughout the genome, synthesized and incorporated independently of DNA replication[1]. Canonical and variant histones can harbour a similar repertoire of post-translational modifications (PTMs) at key residues, which engage chromatin 'readers' to regulate chromatin accessibility, gene expression or other processes. What sets histone variants apart from their canonical counterparts is their unique biophysical properties, genomic localization patterns (FIG. 1) and binding partners, conferred by differences in primary amino acid sequence and structure[2–4]. Histone variants can replace existing canonical histones and their associated PTMs, thus remodelling the epigenome throughout the cell cycle and during development[5].

In recent years, a growing number of studies have connected histone variant mutation, deregulation or misincorporation to various aspects of tumour biology. Here we review the normal and cancer-associated functions of histone variants and their dedicated chaperones, delineate outstanding questions in the field and discuss potential therapeutic avenues. We focus our discussion on members of the diverse H2A and H3 histone variant families, including H2A.Z, macroH2A, H3.3 and centromeric protein A (CENP-A), where a strong body of evidence for their involvement in solid tumours is available. Other aspects of histone biology in cancer are reviewed in detail elsewhere[6–8].

The H2A histone family

H2A.Z

Functions and partners.—H2A.Z is a highly conserved histone variant with 60% similarity to H2 A. In mammals, two paralogues of H2A.Z exist: H2A.Z.1 and H2A.Z.2, encoded by the genes *H2AFZ* (also known as *H2AZ1*) and *H2AFV* (also known as *H2AZ2*), respectively[9,10]. These are expressed in most tissues, with H2A.Z.1 being predominant. While H2A.Z isoforms differ by only three amino acids distributed throughout the protein, they have different effects on chromatin properties and display tissue-specific functions[11–16]. Overall, H2A.Z has been associated with transcriptional activation (FIG. 1), possibly due to the presence of an extended acidic patch that decreases the electrostatic interaction between DNA and histone[17]. Additionally, H2A.Z can co-occur with H3.3 in the same nucleosome, generating a highly salt-labile particle enriched at regions of high histone turnover, which likely facilitates the access of transcription factors to the underlying DNA[18,19]. Similarly to other histones, H2A.Z is subjected to PTMs such as acetylation, methylation and ubiquitylation that differentially modulate gene expression; for example, hyperacetylated H2A.Z is found near transcription start sites (TSSs) and at enhancers of active genes, correlating with transcriptional activation[20,21].

Deposition of H2A.Z into specific genomic regions is achieved by either of the ATP-dependent chaperone complexes SNF2-related CBP activator protein (SRCAP) or p400–TIP60 (FIG. 1a). Both complexes contain several subunits essential for proper H2A.Z deposition and distribution throughout the genome, many of which are shared between the two complexes (reviewed elsewhere[22]). Besides histone deposition, these complexes can also modify histones. For example, TIP60 is a lysine acetyltransferase that is required for H2A.Z acetylation in mammals[23,24], as well as for canonical H2A acetylation as a prerequisite for H2A.Z loading[25]. The p400–TIP60 and SRCAP complexes also contain

'reader' activities that bind to specific histone PTMs. For instance, GAS41, common to both complexes, contains a YEATS domain that binds diacetylated H3 residues directing H2A.Z deposition[26,27]. On the other hand, eviction of H2A.Z is orchestrated by the INO80 and ANP32E chaperone complexes (FiG. 1a). ANP32E has been shown to negatively regulate H2A.Z enrichment around the TSS, at enhancers and at insulators[28], while both the ANP32E and INO80 complexes remove H2A.Z from sites of DNA damage to allow efficient homologous recombination[29,30].

H2A.Z has been studied mostly in the context of transcriptional regulation; however it is also implicated in chromosome segregation, pericentric heterochromatin formation during the cell cycle, and cellular fate[26,31–33]. Regarding the last of these, H2A.Z is incorporated in bivalent chromatin domains in embryonic stem cells (ES cells) via GAS41, and allows the recruitment of Polycomb repressive complex 2 (PRC2) to lineage-specific genes[26,34,35]. Taken together, these data show that H2A.Z is an important player in arbitrating cell fate transitions and lineage commitment, which are directly relevant to cancer.

H2A.Z: transcriptional regulator of cancer-associated genes.—While H2A.Z.1 and H2AZ.2 are not frequently mutated, their expression is commonly upregulated in several tumour types (FiG. 2; TABLE 1). Our group demonstrated a specific role for H2A.Z.2 in sustaining the proliferation of melanoma cells in vitro, whereby the transcription factor E2F and the bromodomain and extra-terminal (BET) protein BRD2 are recruited to cell cycle genes in an H2A.Z.2-dependent fashion to promote their expression[13]. Similar effects on cell proliferation were observed in hepatocellular carcinoma (HCC) cell lines. Here, H2A.Z.1 selectively modulates cell division through suppression of cell cycle inhibitors, while also being required for expression of cyclin-dependent kinases (CDKs)[36]. In colon cancer cell lines, H2A.Z.1 expression is positively regulated by the WNT–β-catenin pathway, and its depletion impairs proliferation and induces expression of enterocyte differentiation markers[37].

A large body of evidence highlights the importance of H2A.Z.1 in hormone-regulated cancers, such as breast and prostate cancer (FIG. 2b; TABLE 1). Here, H2A.Z.1 incorporation can be guided to specific genomic regions by a variety of transcription factors, such as MYC, oestrogen receptor-α (ERα) or androgen receptor (AR), allowing specific and context-dependent gene expression regulation by H2A.Z.1 (REFS[38–40]). In the context of ERα$^+$ breast cancer, modelled in MCF-7 cells, the presence of canonical oestrogen response elements in the *H2AFZ* promoter enhances expression synergistically with MYC binding[41,42]. This signalling is further amplified by p400–TIP60-mediated H2A.Z.1 incorporation at enhancers of ERα-regulated genes and recruitment of the transcription factor FOXA1, promoting increased expression of these genes in a positive-feedback loop[43]. Also in ERα$^+$ breast cancer, the lysine methyltransferase SMYD3 methylates H2A.Z at lysine 101, stabilizing the variant at the TSS of the gene encoding cyclin A1 (*CCNA1*) driving cell cycle progression[44]. Due to its direct regulation by ERα, it is assumed that H2A.Z.1 plays a recurrent role in hormone-positive breast cancers versus other breast cancer subtypes, while the role of H2A.Z.2 remains unknown.

A similar mechanism of action occurs in prostate cancer. Although the *H2AFZ* gene itself is not controlled by androgen response elements (AREs), H2A.Z.1 is present at several AREs driving cancer progression[45,46]. Androgen signalling increases MYC binding at the *H2AFZ* promoter, leading to H2A.Z.1 overexpression in a positive-feedback loop[47]. H2A.Z.1 accumulates at androgen-activated enhancers and promotes gene expression in prostate cancer cells, contributing to the stabilization of a poised enhancer state that persists after withdrawal of androgen stimulation[46]. Acetylated H2A.Z can drive abnormal gene expression, and TIP60 has been implicated in acetylating H2A.Z in ARE regions near enhancers[48]. In addition, H2A.Z acetylation is a key modification present at the TSS of active genes in prostate cancer cell lines, while unacetylated H2A.Z in promoters of tumour suppressor genes negatively regulates their expression[49].

Besides acetylation, H2A.Z can also undergo monoubiquitylation by the PRC1 ubiquitin ligase RING1B, an activity associated with gene repression[50]. Deubiquitylation by USP10 promotes the transition of the gene encoding prostate-specific antigen (*PSA*, also known as *KLK3*) and other genes from a poised state to being actively transcribed driving tumorigenesis[51]. Thus, in both breast cancer and prostate cancer, H2A.Z.1 drives transcription of key oncogenic factors and its function is modulated by its PTM status.

H2A.Z chaperones in cancer.—Deregulation of histone chaperone complexes can contribute to tumour development, and in the case of H2A.Z, both its chaperone complexes, SRCAP and p400–TIP60, are affected. Components of the SRCAP complex, including the SRCAP helicase, YL1, GAS41, RUVBL1 and RUVBL2, are upregulated in several tumours, such as ovarian tumours, breast tumours, thyroid tumours, prostate tumours, melanoma, bladder tumours and glioma (cBioPortal for Cancer Genomics[52]). Although pan-cancer studies report mutations in these components (cBioPortal for Cancer Genomics[52]), functional effects of the mutations have not been described to date. In contrast, downstream consequences of altered expression of SRCAP complex components have been described. GAS41, for example, is essential to modulate deposition of H2A.Z at cell cycle and DNA replication genes in non-small-cell lung cancer cells, where it promotes expression of these genes, and in turn, proliferation[53].

The level of the TIP60 subunit of the p400–TIP60 complex is also altered in cancer. In contrast with subunits of the SRCAP complex however, TIP60 exhibits a tumour-suppressive role. In human colorectal cancer cell lines, downregulation of TIP60 brings about a low ratio of TIP60 to p400, which reduces the DNA damage response, resulting in decreased apoptosis and increased proliferation[54]. In a mouse model of colon cancer, TIP60 loss promoted β-catenin activity, whereas concomitant p400 loss rescued this phenotype, suggesting opposing roles for these two complex subunits and highlighting the importance of the TIP60/p400 ratio for tumour outcome[55].

While there are many reports of increased levels of H2A.Z in tumour cells, it remains to be established whether deregulation of the deposition machinery, including chaperones, is responsible for the gain or redistribution of H2A.Z isoforms across the genome. The interplay between H2A.Z, its PTMs and its chaperones will need to be further unravelled if we are to harness these therapeutically.

Therapeutic opportunities for H2A.Z-driven tumours.—Direct therapeutic targeting of H2A.Z and its chaperones remains elusive; however, strategies to counteract H2A.Z function are emerging. Since H2A.Z acetylation may promote tumorigenesis and is found in hyperacetylated nucleosomes[13,56], BET inhibitors (BETi) are a potential therapeutic strategy to counter this effect. BETi block the ability of BET family proteins (BRD2, BRD3, BRD4 and BRDT) to bind acetylated H4, among other acetylated histone residues, through their bromodomains[57]. Our laboratory showed that H2A.Z.2 depletion from cell cycle genes in combination with JQ1 (a BETi) selectively drove melanoma cell apoptosis due to the inability of BRD2 to bind acetylated histones at promoters of these genes, hindering expression and thus reducing proliferation[13]. In intrahepatic cholangiocarcinoma cell lines, knockdown of H2A.Z.1 promoted tumour cell sensitivity to cisplatin by amplifying drug-induced cell cycle arrest and apoptosis[58], possibly by means of H2A.Z's recently discovered role in regulating DNA replication origin firing[59]. Taken together, these data show that H2A.Z loss sensitizes tumour cells to both conventional and epigenetic therapies, suggesting that H2A.Z levels might be useful to stratify patients for therapy. As we continue to learn more about the structure and function of H2A.Z chaperones, therapeutic possibilities emerge, such as the development of small molecules to inhibit epigenetic readers and writers of chaperone complexes, or inhibitors of protein-protein interactions. To this end, the crystal structure of the H2A.Z/H2B dimer and YL1 (H2A.Z/H2B-YL1) was recently solved[60,61]; as YL1 is a subunit of both the SRCAP complex and the p400–TIP60 complexes, targeting its interaction with H2A.Z isoforms might be a potential therapeutic opportunity for H2A.Z-driven cancers.

MacroH2A

Structure and function.—MacroH2A is a divergent member of the H2A family characterized by a tripartite structure, containing a H2A-like domain, a lysine-rich linker region and a distinct macrodomain that protrudes from the nucleosome[62,63] (FIG. 1). MacroH2A1.1 and macroH2A1.2 are isoforms resulting from alternative splicing of the *H2AFY* gene, and macroH2A2 is a third isoform, encoded by *H2AFY2* (the genes were recently renamed *MACROH2A1* and *MACROH2A2*, respectively)[64–66]. MacroH2A variants are enriched on the inactive X chromosome, in senescence-associated heterochromatin foci, on repetitive DNA sequences and at inactive genes, and often colocalize with H3K27me3 or H3K9me3 genomic occupancy, collectively suggesting a role in transcriptional repression and heterochromatin structure[65,67–74] (FIG. 1; BOX 2).

Of the three isoforms, only the macroH2A1.1 macrodomain is capable of binding ADP-ribose metabolites and ADP-ribosylated proteins, including poly(ADP-ribose) polymerase 1 (PARP1)[75–77]. When bound to macroH2A1.1 in its ADP-ribosylated form, PARP1's enzymatic activity is inhibited, affecting its downstream processes such as transcriptional repression, DNA damage repair and stress response[78–80]. MacroH2A1.1 has also been shown to limit nuclear NAD^+ consumption by inhibiting PARP1, which in turn hinders mitochondrial function[81]. In addition, macroH2A1.1 interacts with *O*-acetyl-ADP-ribose, a by-product of the NAD^+-dependent deacetylases (sirtuins)[82], as well as autoribosylated sirtuins[77]. MacroH2A1.2 and macroH2A2 also inhibit PARP1 activity in vitro[83], but it

remains to be determined whether these can also bind metabolic cofactors, or what the consequences of this binding may be in vivo and in the context of cancer.

In the developing mouse embryo, macroH2A is highly enriched in the trophectoderm and in differentiated cell types[84]. MacroH2A prevents the expression of key pluripotency genes in somatic cells when reprogrammed towards pluripotency, and accordingly, depletion of macroH2A isoforms enhances reprogramming efficiency[72,85,86]. The finding that macroH2A 'locks' the cell in a differentiated state suggested that it might prevent cellular plasticity, thus acting as a tumour suppressor. While this holds true for many solid tumours, in specific contexts, contradictory findings have been reported, as discussed next.

MacroH2A: a putative tumour suppressor.—Like H2A.Z, macroH2A expression is altered in cancer, but the genes encoding macroH2A variants are not frequently mutated (FIG. 2; TABLE 1). MacroH2A levels are decreased in tumours relative to normal tissues across a spectrum of cancers[87,88]; however, whether the levels of the macroH2A isoforms impact tumorigenesis differentially is unresolved. In general, macroH2A1.1 is considered tumour suppressive, as downregulation of this variant is observed in several tumour types (TABLE 1). Splicing factors control the switch between macroH2A1 splice variants in tumour cells. QKI, for example, regulates splicing of *H2AFY* in favor of the macroH2A1.1 isoform over macroH2A1.2, and is lost in cancer, leading to decreased levels of macroH2A1.1 (REFS[88–90]). The RNA helicases DDX17 and DDX5 are transcriptional co-regulators of ERα that splice *H2AFY* producing the macroH2A1.2 variant (thus decreasing relative macroH2A1.1 levels), and promote breast cancer cell invasiveness in vitro[91]. In splicing factor-mutant myelodysplastic syndromes, macroH2A1 alternative splicing leads to a reduction in macroH2A1.1 levels, impacting differentiation along the haematopoietic lineage[92]. Beyond splicing regulation, macroH2A1.1 is ubiquitylated by the E3 ligase SKP2 in breast cancer cells, leading to its degradation and consequent tumour progression by increased CDK8 expression[93]. Additional support for its tumour-suppressive function comes from evidence that ectopic expression of macroH2A1.1 suppresses epithelial-mesenchymal transition in human mammary epithelial cells[94].

Although there is general consensus on macroH2A1.1, the role of macroH2A1.2 in cancer is largely context dependent. While alternative splicing in several tumour types revealed macroH2A1.2 to have an oncogenic role, suppression of metastasis by macroH2A1.2 has also been described. Both in breast cancer cells and in prostate cancer cells, it inhibits the expression of osteoclastogenic soluble factors which are required for remodelling of the metastatic niche[95,96]. In melanoma, ectopic expression of macroH2A1.2 in the highly metastatic B16 cell line dramatically reduced the lung metastatic burden in mice[97]. Hence, more studies are required to delineate the mechanism of action of each macroH2A1 splice variant, which may have cell type-specific or even cancer subtype-specific consequences.

MacroH2A1 has also been studied extensively in liver cancer and precursor lesions such as hepatic steatosis, with somewhat conflicting findings. MacroH2A1-deficient mice present a non-penetrant phenotype in which a subset of mice develop steatosis[98]; however, this observation was not reported in two other liver-focused studies[99,100], raising the possibility of mouse strain-specific effects. Furthermore, while macroH2A1 and macroH2A2 double

knockout mice have altered expression of hepatic genes related to lipid metabolism[101], any overt lipid-related pathology which could explain the steatosis phenotype was lacking. In contrast, macroH2A levels were increased in steatosis/HCC models. MacroH2A1.1 and macroH2A1.2 were upregulated in mice fed a high-fat diet with PTEN-driven HCC compared with control mice, and in human steatotic liver, cirrhotic liver and HCC samples[102,103]. In line with this, macroH2A1 was shown to prevent senescence induced by a DNA-demethylating agent in HCC cell lines[103] (BOX 2). However, the same group found macroH2A1 knockdown in HCC cell lines promotes insensitivity to confluency-dependent and serum starvation-dependent inhibition of proliferation[104], a common feature of cancer stem cells, raising questions as to how these conflicting observations hold true in the same tumour type.

Regarding a role for macroH2A2 in cancer, our team demonstrated that this variant is downregulated in metastatic melanoma, and plays an active role in suppressing proliferation and metastasis[97]. While less well studied than macroH2A1 variants, low levels of macroH2A2 are also associated with lung cancer recurrence, breast cancer proliferation and anal neoplasm progression in patient samples[87,105].

No dedicated histone chaperones have been identified for macroH2A variants, and current evidence largely suggests that the genome-wide distribution pattern of macroH2A is a consequence of its active exclusion[74] (FIG. 1). Co-transcriptional exclusion of macroH2A is enabled by the facilitates chromatin transcription (FACT) complex[74], a general H2A chaperone. Increased expression of FACT is associated with poorly differentiated and more aggressive tumours, such as triple-negative breast cancer and $HER2^{+}$ breast cancer[106], yet it remains to be determined whether FACT upregulation is linked to macroH2A exclusion in this context.

Most studies on the tumour-suppressive function of macroH2A were performed in cell lines, patient samples or xenograft models. However, unlike the other histone variants, simultaneous ablation of all macroH2A isoforms in mice is not lethal and mice do not develop spontaneous tumours[101], suggesting macroH2A loss on its own does not initiate tumorigenesis. In a constitutive knockout model, loss of all macroH2A variants led to increased proliferative and clonogenic potential of intestinal organoids. Although their repopulating capacity after experimental injury was reduced, when crossed to an *Apc*-mutant intestinal adenoma mouse model, the increased number of reserve stem cells did not translate into increased adenoma initiation[107]. Nevertheless, since macroH2A deregulation occurs in other tumours such as breast cancer, HCC and melanoma, in vivo studies modelling these tumour types are essential to unravel the contribution of this histone variant to tumour initiation and progression.

Therapeutic targeting of macroH2A.—Chromatin incorporation and removal of macroH2A involves essential multitasking protein complexes such as FACT and ATRX, which are not specific for macroH2A[71,74]; therefore, targeting these factors can be challenging. However, macroH2A1.1 and macroH2A2 levels can be used as valuable biomarkers of metastatic or undifferentiated cancers that could guide the use of targeted therapies[87,88,97]. For example, FDA-approved PARP inhibitors are currently being used

against cancers with deficient homologous recombination repair[108], and might be a targeted therapeutic strategy against macroH2A1.1-deficient tumours to restore PARP inhibition, and in turn reverse the consequences of macroH2A loss for DNA repair and metabolism.

The H3 histone family

H3.3

Functions and partners.—Encoded by two paralogues, *H3F3A* and *H3F3B* (recently renamed *H3-3A* and *H3-3B*, respectively), H3.3 differs from canonical H3.1 and H3.2 by only four and five amino acids, respectively. H3.3 is expressed throughout the cell cycle and across all cell types, and forms most of the H3 pool in post-mitotic cells[109–111]. H3.3 has two distinct chaperone systems that place it into euchromatin and heterochromatin, respectively (FIG. 1 a). The HIRA (histone cell cycle regulation-defective homologue A) complex deposits H3.3 in regions of high nucleosome turnover, such as active genes and gene regulatory elements, through its ability to bind nucleosome-depleted DNA, RNA polymerase II or single-stranded DNA-binding proteins[112–114]. HIRA-mediated H3.3 deposition plays both structural and gene regulatory roles, preventing persistence of nucleosome gaps in chromatin[113], promoting transcriptional recovery after DNA damage[115] and maintaining a bivalent state at developmental gene promoters in ES cells[116]. Numerous gene expression programmes induced during development involve H3.3 deposition at promoters and enhancers (reviewed elsewhere[117]), which may causally contribute to transcriptional activation. Together with H2A.Z, H3.3 decreases nucleosomal stability and primes chromatin decompaction[19,118], while phosphorylation of S31 (an H3.3-specific residue) at stimulation-induced genes recruits chromatin remodelling activities to enhance accessibility of the transcription machinery[119].

Death domain-associated protein 6 (DAXX) in complex with ATRX (α-thalassaemia/mental retardation syndrome X-linked) deposits H3.3 at heterochromatin regions, including pericentric and telomeric repeats, endogenous retroviruses and imprinted genes[112,120–122]. In contrast to HIRA, H3.3 deposited by DAXX–ATRX at repetitive elements forms a substrate for K9 trimethylation to ensure silencing of telomeric repeat RNA and retrotransposition[112,121–124]. The HIRA and DAXX–ATRX chaperone complexes as well as H3.3 itself are involved in cellular senescence (BOX 2).

Within the last decade, next-generation sequencing studies of tumours have identified multiple mutations within the histone genes (FIG. 2a). H3.3 stands out due to its remarkably high frequency of recurrent point mutations in a set of rare cancers in children and adolescents. These somatic heterozygous but clonally homogenous mutations disrupt PTMs of key histone tail residues, with pervasive effects on the chromatin landscape and gene expression (TABLE 1). Such characteristics demonstrated for the first time a ‘driver’ role for histone variants in tumorigenesis, and these mutated variants have been coined ‘oncohistones’[125]. This includes H3.3 K27M, K36M and G34R/W substitutions in paediatric gliomas and skeletal tumours, which appear to be the most frequent and most well studied, and constitute our primary focus in the following sections.

These oncohistone mutations function in large part by blocking histone modifier enzymatic activity (see later); however, such enzymes do not necessarily display preference for H3 family members. Therefore, whether mutation in one of the H3.3 genes impacts the oncohistone pathology differently than mutation in a canonical H3 gene remains unresolved. Besides mutation, H3.3 may also contribute to tumour progression through changes in its levels. For example, the balance between H3.3 and replicative H3 deposition could modulate metastatic behaviour through transcriptional reprogramming[126]. Identifying the defining features of H3.3 that contribute to tumour progression, including genomic distribution by virtue of its specific chaperones as well as its relationship to the developmental time window and proliferative state of the cell, remains of great interest to the field.

H3K27M in paediatric high-grade gliomas.—The H3.3 K27M mutation was first identified in diffuse intrinsic pontine glioma (DIPG), glioblastoma, thalamic high-grade glioma (HGG) and spinal HGG in children and adolescent patients[127–136] (FIG. 2). These disorders are molecularly similar and share a devastating prognosis due to their undifferentiated character and diffuse spread, leading the WHO to classify them under a single disease, ‘diffuse midline glioma K27M-mutant’[137]. K27M mutations are infrequent in paediatric HGGs affecting the cerebral hemispheres and in adults[127,128,130,131,135,138].

The K27M mutation mainly affects *H3F3A*[127,128,130,131,135] (FIG. 2a), with less frequent mutations in H3.1 paralogues *HIST1H3B*[129] and *HIST1H3C*[131,133] or the H3.2 gene *HIST2H3C*[139]. The mutation occurs as an AAG>ATG transition in *H3F3A* and *HIST1H3B* (cBioPortal for Cancer Genomics[52]), and would require a much less likely dinucleotide substitution to generate the same outcome in *H3F3B*-encoded K27, which is is encoded by an AAA codon. The organization of canonical histone genes in multiple, highly similar copies complicates efforts to map mutations, which may contribute to an underestimation of their prevalence in cancer[140].

EZH2, the catalytic subunit of PRC2, trimethylates H3K27 to maintain gene silencing and cellular identity[141]. Despite representing a small fraction of the total H3 pool, K27M decreases global levels of H3K27me3, suggestive of a gain-of-function EZH2 inhibitor[142–145]. Exactly how this occurs remains a matter of debate. Together with increased interaction of PRC2 with the mutant due to stronger binding of the K27M peptide in the active site, the hypothesis that K27M sequesters PRC2 and prevents K27 methylation of non-mutant H3 in *trans* was proposed[142,146]. Genome-wide profiling of H3K27me3 in K27M-mutant tumours and neural progenitor cells also showed H3K27me3 gains at numerous loci[142,144,147], inconsistent with complete PRC2 inhibition. Indeed, sharp peaks of H3K27me3 and the PRC2 subunit SUZ12 are present in K27M-mutant gliomas, notably at unmethylated CpG islands, which are high-affinity recruitment sites for PRC2 (REF.[148]). Such retained H3K27me3 peaks also display lower levels of H3.3K27M-mutant incorporation, potentially explaining this localized residual PRC2 activity[149]. However, the modification is unable to spread to form characteristic PRC2-mediated large repressive domains[148,150], which may be due to stable conformational changes of PRC2 induced by K27M. Indeed, H3K27M-mediated inhibition preferentially affects the PRC2 complex once it binds to nucleosomes and becomes allosterically activated by existing H3K27me3 (REF.

[150]). This mode of action would predict PRC2's capacity to propagate existing K27me3 to neighbouring nucleosomes is impaired by K27M.

The K27M mutation appears to occur early, as it is present in all primary and metastatic tumour cells of K27M-mutant gliomas and can be traced to the cell of origin through evolutionary reconstruction[125]. However, K27M is often accompanied by other driver mutations affecting canonical oncogenic and tumour suppressor pathways[131,133,135,136]. Whereas the dominant effect of K27M on H3K27me3 levels can be recapitulated across multiple unrelated cell types[143,148,150], its contribution to tumorigenesis is highly context dependent. First, K27M is almost exclusively found in paediatric and adolescent tumours, which suggests it preferentially impacts a vulnerable developmental time window, consistent with the role of PRC2 in differentiation (FIG. 2; TABLE 1). Second, efforts to model the oncogenic effects of K27M in cell culture or in vivo revealed that it induced senescence in human fibroblasts and astrocytes[147] and demonstrated embryonic lethality in mice[151], while targeted expression of the H3.3K27M mutant in neural progenitor cells did not induce tumours on its own[151–153]. Third, K27M does induce proliferation in neural progenitor cells[147] or p53-deficient nestin-positive progenitors of the neonatal mouse brainstem[143], but generation of undifferentiated, invasive K27M-mutant midline gliomas in mice required cooperating oncogenic events during central nervous system development[151–153]. Importantly, animal models recapitulate global H3K27me3 loss, as well as instances of local enrichment, and gene expression profiles found in human K27M HGGs. Among PRC2 target genes, there is upregulation of developmental factors associated with neurogenesis, such as WNT and HOX family members, but also repression of *CDKN2A*, indicating enhanced self-renewal and proliferation[151–153]. Furthermore, many genes upregulated in K27M mouse tumours have bivalent promoters in K27 wild-type controls[153]. Although it remains unclear which of these transcriptional changes are necessary and sufficient for tumorigenesis, they serve to emphasize the developmental nature of K27M-associated pathology. K27M, and thus PRC2 inhibition, likely maintains HGG cells in an undifferentiated state. Deletion of the mutant *H3f3a* gene in mouse DIPG xenograft models rescues H3K27me3 spreading and abolishes tumour formation[148], and knockdown significantly decreases proliferation and restores a repressive chromatin state at bivalent genes, pushing cell identity towards the oligodendrocyte lineage[154].

H3K36M in chondroblastoma.—Strikingly, a K36M mutation of H3.3 was identified in 73 of 77 cases of chondroblastoma[155], a benign tumour of the cartilage of bone growth plates in adolescents and young adults (FIG. 2b). The mutation appears diagnostic as it can distinguish chondroblastoma from histologic mimics[156]. The high frequency of mutation, coupled with the lack of other recurring mutations and genomic aberrations, implies a driver role for H3K36M. *H3F3B* was preferentially mutated; only 7% of H3.3 mutations occurred in *H3F3A*[155]. Subsequent studies identified K36M in the H3.1 genes in paediatric soft tissue sarcomas and adult head and neck squamous cell carcinomas[157,158], albeit at much lower frequencies.

Studies based on transgenic expression of K36M in various cell types[143,157], knock-in K36M immortalized human chondrocytes and chromatin immunoprecipitation followed by sequencing of primary tumours[159] revealed that the mutation decreased overall levels of

H3K36 dimethylation and trimethylation, suggesting a dominant-negative effect similar to K27M. Accordingly, K36M inhibits deposition of both the dimethyl mark by NSD2 (but not other K36 methyltransferases) and the trimethyl mark by SETD2 (REFS[157,159]). Given that SETD2 binds with stronger affinity to the mutant peptide and nucleosome, as proposed for PRC2 at K27M, K36M likely sequesters methyltransferase activity[157,159]. Importantly, the mutation not only affects modification of K36 but also enhances K27 methylation by reducing the antagonistic effect of K36 methylation on EZH2 (REF[160]). Loss of K36me3 instructs the acquisition of K27me3 at nearby intergenic regions and leads to a redistribution of PRC1 (REF.[157]). PRC1-catalysed repression is therefore lost at its target genes, several of which promote self-renewal and lineage specification of mesenchymal stem cells, thereby impairing their differentiation into chondrocytes. Accordingly, exogenous expression of K36M in mesenchymal progenitor cells drives sarcoma growth in mouse allografts[157].

H3.3 G34 mutations in gliomas and skeletal tumours.—G34R, and less frequently G34V, mutations were identified in more than 10% of HGGs (FIG. 2a), and in contrast to K27M, occur more frequently in tumours of the cerebral hemispheres in teenagers and young adults[127,129,130]. In more than 90% of giant cell tumours of the bone (GCTB), G34 is mutated to W and less so to L[155], and like K36M in chondroblastoma, it is highly specific for GCTB while absent in histologically similar tumours[156]. Nearly all of these mutations affect *H3F3A*, which cannot be explained by codon usage alone — the two H3.3 genes and three H3.2 genes share the same codon at this position, and the same R and V substitutions could arise in H3.1-encoding genes through either an identical or an adjacent point mutation. In gliomas, the G34 substitutions are consistently accompanied by mutations in *ATRX* or *DAXX*, which may promote alternative lengthening of telomeres (ALT; see later)[127,130,131,135]. Nevertheless, it is unclear whether the G34 mutation impacts ATRX–DAXX function in these tumours and would select for the concomitant inactivation of this complex. Similarly to K27M, G34R/V mutations often co-occur with established cancer drivers[127,131,135].

While G34 is not a substrate for modification, it lies close to K36, and is mechanistically distinct from K-to-M mutations. It was reported that G34R/V mutants blocked K36 methylation only in *cis* and therefore did not have a dominant-negative effect on K36me3 (REFS[143,161]). This can be explained by the structure of the SETD2 K36 methyltransferase, whose narrow substrate channel can accommodate only the minimal side chains of G34 within the H3 tail, thereby preventing G34-mutant histones carrying invariably bulkier side chains from accessing its active site[162,163]. Intriguingly, a targeted G34R mutation of a single copy of *H3f3a* in mouse ES cells led to local gains of K36me3 at lysine-specific demethylase 4 (KDM4)-enriched loci. G34R displayed increased affinity for KDM4 and inhibited its demethylase activity towards its K36me3 substrate, leading to accumulation of its other substrate, H3K9me3 (REF[164]). The importance of the K36me3 axis for gliomagenesis is additionally underscored by the fact that *SETD2* itself is frequently mutated in paediatric HGGs[165], and driver mutations in the isocitrate dehydrogenase genes *IDH1* and *IDH2*, which occur in a distinct set of gliomas lacking H3.3 mutations[166], promote tumorigenesis through abnormal production of an oncometabolite that inhibits DNA and histone demethylases, including KDM4 (REFS[167,168]). GCTB mutant G34W/L

similarly blocks K36 methylation in *cis* when overexpressed, through a mechanism involving SETD2 inhibition[169]. The same in vitro study showed impaired K27 methylation and binding of HIRA to G34-mutant histones. G34W-mutant tumour cells isolated from GCTB displayed increased proliferation and invasive capacity, which could be recapitulated by overexpression of the mutant in an osteosarcoma cell line[170]. Furthermore, selective depletion of the mutant in GCTB cell lines revealed it was necessary to sustain their proliferation and migration in vitro and in vivo[171].

Therapeutic opportunities for H3.3-mutant tumours.—The presence of the K27M mutation predicts shorter survival in DIPG[128] and other HGGs[172,173]. Detection of K27M either by sequencing, immunohistochemistry (using H3K27M antibodies) or consequent decrease in H3K27me3 is equivalent[139,173], carrying a uniformly fatal prognosis independent of the anatomic site or histologic grade[172]. Small-molecule inhibitors targeting histone-modifying enzymes allow direct modulation of K27 methylation, or indirect modulation by targeting complementary histone PTMs to reverse the mutant gene expression profile. Therefore, K27M mutation provides a therapeutic opportunity in HGGs. For example, inhibition of the K27 demethylase JMJD3 in K27M-mutant brainstem glioma cell lines[174] or menin, a subunit of the MLL–SET1 methyltransferase complex[175], in an ES cell-derived model[147] decreased viability and proliferation of tumour cells both in vitro and in murine xenografts. Importantly, antitumour activity was dependent on the presence of mutated K27M, and efficiently targeted the brainstem, highlighting the capacity of these drugs to cross the blood—brain barrier[147,174]. Even though global loss of K27me3 is found in DIPG, the use of EZH2 inhibitors impaired proliferation and induced senescence in primary patient-derived K27M DIPG cell cultures[176]. Given that several tumour suppressor genes (for example, *CDKN2A*) retain residual H3K27me3 in DIPG cells[149,152,176,177], it is likely that EZH2 inhibition is synthetically lethal in the presence of K27M owing to re-expression of these tumour suppressors.

Concurrent with global H3K27me3 loss, K27M tumours may harbour an increase in H3K27 acteylation (H3K27ac) at genomic regions enriched in the mutant histone[177]. Given that H3K27ac loci are co-occupied by BRD2 and BRD4, therapeutic use of BETi was investigated in this context. In one study, BETi use led to impaired proliferation of DIPG cells in vitro and orthotopically by downregulating the sonic hedgehog pathway[177]. This was challenged in another study where the presence of K27M in DIPG-derived isogenic cell systems led not to BETi sensitivity but to pervasive H3K27ac deposition across the genome, including at repetitive elements[178]. The resulting derepression of endogenous retroviruses is a therapeutic vulnerability that was further amplified using DNA demethylating agents and histone deacetylase inhibitors, increasing the immunogenicity of these tumours through viral mimicry[178]. Finally, a K27M-derived tumour neoantigen has been identified which elicits a $CD8^+$ T cell response in patients with DIPG. Engineered antigen-specific T cells directed at this epitope caused glioma cell killing in vitro and controlled tumour growth in vivo[179], providing a proof of principle that engineered T cell approaches may be feasible in the future. While efforts to target H3.3 mutations have focused on K27M glioma, epigenetic drugs that counterbalance the consequences of K36 or G34 mutations at the PTM level are promising candidates for future research.

DAXX and ATRX mutations

Mutations in the H3.3 chaperone complex DAXX—ATRX are common in tumours of neural or neural crest origin, such as pancreatic neuroendocrine tumours, glioma and neuroblastoma[127,180–182] (TABLE 1). In addition, DAXX is upregulated in The Cancer Genome Atlas (TCGA) datasets compared with normal tissue, and in metastatic versus primary tumour samples[183]. Upregulation of DAXX carries a negative prognostic value in prostate and ovarian cancer[184–186].

DAXX is enriched in promyelocytic leukaemia bodies and has been reported to interact with various nuclear proteins besides ATRX, suggesting additional functions beyond H3.3 deposition[183]. ATRX is a chromatin remodeller that contains an ADD domain, a DAXX-interacting region and an SNF2 helicase domain, and also has DAXX-independent functions[187]. ATRX interacts with macroH2A to exclude it from subtelomeric regions[71] and contributes to silencing of the inactive X chromosome and PRC2 target genes[188].

To attain replicative immortality, a prerequisite for tumour growth, aberrantly proliferating cells prevent telomere shortening either by activating mutations of the telomerase reverse transcriptase gene (*TERT*) or via the process of ALT. ALT is observed, for example, in pancreatic neuroendocrine tumours, glioblastoma, oligodendrogliomas, neuroblastoma, and osteosarcomas, and is highly correlated with loss or mutations in *ATRX* or *DAXX*[180,181,189] (TABLE 1). ATRX disruption occurs in a mutually exclusive manner with *TERT* activation[190] and is associated with larger tumour size, higher risk of metastasis and higher tumour grade[191]. Impaired ATRX or DAXX function was required to sustain ALT in a cancer cell line, as reintroduction of either protein potently suppressed telomere lengthening[189,192]. The involvement of ATRX in this process likely stems from its enrichment at G-quadruplex structures, whose replication and transcription it facilitates[192–194]. At telomeres, where these structures are enriched, absence of ATRX leads to collapse of stalled replication forks, which would trigger homology-directed DNA repair and ultimately ALT[192]. Depletion of another H3 chaperone, ASF1 (BOX 1), induces hallmarks of ALT in immortalized and transformed human cell lines, suggesting that improper H3 incorporation at telomeres plays a causal role in ALT[195].

Mutually exclusive mutations of *ATRX* and *DAXX* were identified in pancreatic neuroendocrine tumours, the second most lethal type of pancreatic cancer[181], where they correlate with shorter overall survival[191,196–199]. In midline gliomas, *ATRX* mutations occur alone or together with H3.3 mutation, particularly G34R/V[127,131,135]. In neuroblastoma, point mutations, indels and large in-frame fusions were identified in *ATRX*, but not *DAXX* or the genes encoding H3.3 (REFS[182,200,201]), raising the possibility that *ATRX* mutation in neuroblastoma may be independent of H3.3 deposition. In both midline glioma and neuroblastoma, *ATRX* mutations are more common in older children (8 years old, up to adolescent age) and are associated with significantly shorter overall survival compared with patients with wild-type *ATRX*[128,182,202,203].

Therapeutic opportunities for ATRX *and* DAXX*-mutated cancers.*

Given the strong correlation between *DAXX* and *ATRX* mutations and ALT, loss of function of the complex could serve as a useful prognostic marker[191]. Telomere instability associated with replication stress in the absence of ATRX—DAXX could also constitute a vulnerability, which could be exploited by disabling homology-directed DNA repair. This principle has been demonstrated in vitro using inhibitors of the DNA damage response kinase ATR, which display selective cytotoxicity in ALT-positive osteosarcoma cell lines[204]. Similarly, inhibition of PARP1, whose activation was induced by fork stalling in the absence of ATRX in the developing brain, exacerbated DNA damage and attenuated proliferation in ATRX-deleted HeLa cells[205]. Further support for this exploitable vulnerability came from a recent, high-throughput compound screen which identified an increased susceptibility to ATM inhibitors and PARP inhibitors in ATRX-altered neuroblastoma models[206]. Furthermore, a novel therapeutically tractable connection has been established between *ATRX* in-frame fusions and EZH2 inhibitor sensitivity in neuroblastoma; we demonstrated that these gain-of-function *ATRX* mutations promote silencing of neurogenesis genes through REST activation and thus suppression of differentiation programmes, which can be reversed by EZH2 inhibition[201].

CENP-A

Essential for cellular proliferation.

CENP-A (encoded by the gene *CENPA*) is a divergent H3 variant found at a subset of nucleosomes associated with centromeric satellite repeat sequences[207] (FIG. 1). CENP-A has a dual role, directing the assembly of the kinetochore[208] and propagating centromere identity across mitosis and meiosis in an epigenetic manner[209]. In mammalian cells, CENP-A dynamics are tightly linked to cell cycle progression. Unlike canonical H3 or variant H3.3, CENP-A expression and translation peak in G2 phase[210,211], and deposition at centromeres is restricted to early G1 phase by the inhibitory activity of CDK1 and CDK2 (REFS[212,213]). Holliday junction recognition protein (HJURP) was identified as a CENP-A chaperone[214,215] that relies on structural differences in H3 variants to specifically recognize CENP-A[216]. HJURP activity is limited during the cell cycle by cyclin A, which inhibits its association with centromeres outside G1 phase[217]. CENP-A is indispensable for embryogenesis[218], and together with HJURP, depletion in vertebrate cell lines results in failure of kinetochore assembly and impaired chromosome segregation[214,215,219,220], illustrating their essential role in maintaining centromere function.

CENP-A and HJURP accumulate during transformation.

HJURP and CENP-A are upregulated in a plethora of solid tumours (FIG. 2b; TABLE 1). Increased *CENPA* and *HJURP* mRNA levels may distinguish tumour versus normal cells or late versus early lesions regardless of the cell of origin, as differential expression reaches significance in most solid tumours[221]. This, along with their mechanistic connection to cell cycle progression and correlation with Ki67 (REFS[222–225]), suggests high CENP-A and HJURP levels are indicators of aberrant cellular proliferation.

Survey of TCGA data across cancer types reveals very low rates of somatic *CENPA* (FIG. 2a) and *HJURP* mutation (cBioPortal for Cancer Genomics[52]). Thus, the accumulation of the two centromeric factors in tumours is likely a result of transcriptional deregulation or increased protein stability. The increased efficiency of centromere propagation when both the variant and the chaperone are abundant could contribute to the fitness of frequently dividing cancer cells[226]. Tumours harbouring deletion or gain-of-function point mutations in *TP53* have increased *CENPA* and *HJURP* expression[227]. This may be explained at least in part by the presence of binding motifs in *CENPA* and *HJURP* promoters, which restrict the peak of their expression to G2/M phase[228,229] by recruiting the p53–DREAM complex[227]. This can be recapitulated ex vivo, as upregulation of HJURP and CENP-A occurs in p53-deficient mouse embryonic fibroblasts and, conversely, p53 activation leads to downregulation of these proteins in human cancer cell lines. In addition to transcriptional regulation, when one partner is exogenously overexpressed or depleted, the other follows[227], suggesting reciprocal stabilization.

High levels of CENP-A can induce its promiscuous mislocalization into chromosome arms when exogenously overexpressed in HeLa cells[230,231], but the chaperone responsible for this is not HJURP. Excess CENP-A hijacks DAXX, which deposits an abnormal CENP-A–H3.3 hybrid nucleosome at loci where H3.3 is normally present[231]. However, in such exogenous experimental models, CENP-A expression is uncoupled from cell cycle control of its chaperone machinery, which could in turn contribute to its aberrant deposition. Moreover, in colorectal cancer cell lines displaying ectopic incorporation, increased CENP-A levels correlate with the upregulation of DAXX and ATRX, but not HJURP[232]. This imbalance appears to determine CENP-A mis-targeting, as overexpression of HJURP decreases the number of CENP-A extracentromeric peaks and, conversely, HJURP depletion can allow ectopic CENP-A accumulation even in cancer cell lines where the variant is not overexpressed[233]. However, in TCGA datasets for breast invasive carcinoma, lung adenocarcinoma and lower-grade glioma, *CENPA* and *HJURP* display a remarkable level of co-expression (Spearman correlation coefficients of more than 0.90; cBioPortal for Cancer Genomics[52]), likely reflecting their common mode of transcriptional regulation. Thus, studying their contribution to cancer progression requires identification of tumours where CENP-A and HJURP levels are uncoupled. In these instances, promiscuously incorporated CENP-A could contribute to tumorigenesis on the one hand by seeding the formation of neocentromeres, which promote genomic instability and rescue broken chromosome fragments[234]. On the other hand, accumulation of CENP-A at gene regulatory regions and transcription factor-binding sites[231,232] could interfere with gene expression, but both hypotheses are yet to be confirmed in cancer models.

Therapeutic opportunities for targeting CENP-A deposition.

High levels of HJURP and CENP-A correlate with tumour progression and carry a negative prognosis (TABLE 1); however, they also predict increased tumour sensitivity to adjuvant chemotherapy or radiotherapy[221,222], likely as a consequence of CENP-A involvement in cellular proliferation. Moreover, genomic instability, a potential consequence of perturbed CENP-A deposition, can either promote or hamper tumour progression[235]. CENP-A deposition may represent a vulnerability in the context of p53 loss. In a mouse embryonic

fibroblast model, the effect of *Hjurp* knockout was limited to proliferative arrest in wild-type *Trp53* cells, whereas in *Trp53*-null transformed cells and tumours it led to altered ploidy and cell death[227]. Highly proliferating cancer cells rapidly lose CENP-A from centromeres without active deposition[220,236], and loss of p53 will allow centromere-challenged cells to continue proliferating and accumulate catastrophic levels of aneuploidy[237]. Therefore, inhibiting CENP-A deposition might preferentially target transformed cells while sparing p53-proficient normal cells. Furthermore, aneuploidy resulting from perturbed centromere or kinetochore function generates replication-coupled DNA damage, proteotoxic stress and a senescence-associated secretory phenotype in human cells[238,239] (BOX 2), which can enhance the immune response elicited by the death of transformed cells[240].

No existing pharmacological compounds specifically target CENP-A deposition; however, multiple enzymatic activities are important for the propagation of centromere identity[241]. Unlike transcriptional regulatory PTMs found on other histones, CENP-A is modified to modulate its deposition and mitotic role. CDK1 and CDK2 complexes phosphorylate CENP-A and components of its deposition machinery to inhibit loading of the variant outside G1 phase[213,217,242–246], while phosphorylation of HJURP by the M-phase kinase PLK1 promotes CENP-A deposition[247]. Furthermore, ubiquitylation of CENP-A K124 facilitates its interaction with HJURP and proper centromeric targeting[248]. Perturbing the balance of these modifications could provide a chemical means to block CENP-A deposition. Importantly, PLK1 inhibitors have progressed into clinical trials, but their efficacy in cancer cells is impaired by a functional p53 response[249]. This suggests that p53-deficient tumours with a high degree of dependency on CENP-A incorporation might be vulnerable to such a therapeutic approach.

Perspectives

As described throughout this Review, histone variants contribute to various processes of the ‘hallmarks of cancer’[250], which has led us to adapt these to histone variant biology (FIG. 3a). For example, metabolic rewiring is an essential feature of cancer cells, and the ADP ribose-binding properties of macroH2A1.1 are emerging as a connection between metabolites and the epigenome that warrants further investigation in vivo. Given that the stages of tumorigenesis from initiation to growth, metastasis, dormancy, and treatment resistance are driven by distinct cellular pathways, we suspect that deregulation of histone variant functions is co-opted by transformed cells with distinct consequences along the evolutionary trajectory of a tumour (FIG. 3b). However, we lack knowledge of histone variant biology in important processes such as tumour cell dormancy and drug resistance.

We anticipate increased efforts for the foreseeable future to characterize the diversity of the histone mutational spectrum across cancer types, as well the impact of emerging histone point mutations on the transcriptome and epigenome, in particular by leveraging large cancer datasets. For example, while most H3 mutations have been found in solid tumours, such mutations are also emerging in haematological cancers, albeit at lower frequencies[251–255]. Moreover, a study of more than 3,000 unique patient samples from common cancer types revealed that all core histones are recurrently mutated in ~4% of tumours, but the functional contribution of these mostly novel substitutions remains to be characterized[140].

Immunotherapies have brought about remarkable increases in survival in otherwise highly lethal cancers; however, ongoing efforts strive to understand tumour response or resistance and predict patient outcome. A handful of reports have linked macroH2A to regulation of cytokine and immune effector expression[95,256,257], leading us to hypothesize that variant-driven gene expression changes intrinsic to tumour cells or tumour-associated cells might shape the immune microenvironment. Therefore, it is important to assess the potential impact of histone variant dysfunction on genes involved in immune evasion, and ultimately on response to immunotherapy.

Our capacity to exploit histone variant dysfunction in cancers will rely on our developing novel, pharmaceutically viable ways to target histone variant deposition. As yet, this has proven exceptionally challenging, given that most histone chaperones lack enzymatic activities, and binding to their respective substrates occurs through large surfaces devoid of deep pockets where small-molecule inhibitors might intervene. Alternatively, given the roles of histone variants and chaperones in regulating cell identity, it is reasonable to consider whether loss of cell fate associated with their deregulation could constitute a vulnerability to epigenetic reprogramming agents in poorly differentiated tumours[147,174,201]. Awaiting these discoveries, histone variants could still find use as prognostic markers or predictors of therapy response, contributing to more personalized treatments and ultimately adding to overall patient survival and quality of life.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors thank members of the Bernstein laboratory for discussions relevant to this Review. This work was supported by NIH/NCI grant R01CA154683, NIH/NCI grant R01CA218024, NIH/NINDS grant R01NS110837 (E.B.) and the American Skin Association Ping Y. Tai Foundation Research Grant in Skin Cancer/Melanoma (D.F). The authors apologize to those whose work was not cited due to space constraints.

Glossary

Oncohistones
Missense mutations in a histone variant, most commonly H3.3, often altering a key post-translational modification site that leads to a tumorigenic gene expression programme driving specific malignancies in children or young adults.

YEATS domain
Histone post-translational modification reader module found in transcriptional regulator proteins that selectively binds to acetylated and crotonylated lysine residues.

Pericentric heterochromatin
Constitutively condensed chromatin localized adjacent to the centromere, formed on repetitive DNA satellite sequences repressed by DNA methylation and histone H3 lysine 9 trimethylation (H3K9me3).

Bivalent chromatin domains
Genomic regions that harbor both the repressive mark histone H3 lysine 27 trimethylation (H3K27me3) and the activating mark histone H3 lysine 4 trimethylation (H3K4me3) and poise a subset of genes silenced in embryonic stem cells for activation during subsequent cell fate decisions to drive normal development.

PRC2
Polycomb repressive complex 2 (PRC2) catalyses trimethylation of histone H3 lysine 27 to silence developmental genes during differentiation, and contributes to facultative heterochromatin formation at the inactive X chromosome and imprinted genes.

PRC1
Polycomb repressive complex 1 (PRC1) monoubiquitylates histone H2A lysine 119 to induce gene repression, in coordination with histone H3 lysine 27 trimethylation (H3K27me3) deposited by PRC2.

Poly(ADP-ribose)
polymerase An enzyme that attaches chains of ADP-ribose from an NAD^+ donor molecule to acceptor proteins (including poly(ADP-ribose) polymerases themselves) to regulate DNA repair and carbohydrate and lipid metabolism.

Triple-negative breast cancer
Breast cancer subtype that does not express oestrogen and progesterone receptors or HER2, and therefore is not suited to hormone therapy or HER2 inhibition.

Euchromatin
The loosely compacted, transcriptionally active and gene-enriched fraction of chromatin.

Alternative lengthening of telomeres
(ALT). Telomerase-independent mechanism that exploits homologous recombination of repetitive telomere sequences to promote telomere length maintenance in certain immortalized cell lines and tumours.

Promyelocytic leukaemia bodies
Membraneless nuclear compartments formed on a scaffold of promyelocytic leukaemia protein involved in telomere lengthening and DNA damage response.

ADD domain
Protein domain consisting of an amino-terminal GATA-like zinc-finger, a plant homeodomain finger and a long carboxy-terminal α-helix which binds histone H3 unmethylated at lysine 4 and trimethylated at lysine 9.

SNF2 helicase domain
Protein domain that uses the energy of ATP hydrolysis to apply torsional strain to DNA in order to remodel nucleosomes and other DNA—protein complexes.

G-quadruplex structures

Stable secondary DNA structures present in G-rich DNA held together by G-G base pairs formed within the same DNA strand.

ATM
Ataxia telangiectasia mutated (ATM) is a DNA damage kinase that phosphorylates itself and downstream effectors in response to DNA double-strand breaks to coordinate the cellular DNA damage response.

Kinetochore
Multicomplex protein structure localized at the centromere during mitosis to attach, orient and move sister chromatids along the mitotic spindle, ensuring accurate chromosome segregation.

Neocentromeres
Abnormal centromeres formed de novo at a location distinct from the primary constriction of a chromosome.

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Box 1 |

Replicative histones and their deposition pathway

Histones are distinguished by their expression pattern throughout the cell cycle and by a dedicated network of histone chaperones ensuring their transit throughout the cell and assembly into chromatin. Canonical histones H2A, H2B, H3 and H4, also known as replicative histones, form the nucleosomal core[287]. They peak in expression during S phase and are incorporated into chromatin in a DNA synthesis-coupled manner by specific chaperones. To meet the requirement for extensive incorporation throughout the genome during S phase[2,4], replicative histones have unique genomic organization and transcriptional control. They are present in multiple copies organized in clusters; they lack intronic regions and are not polyadenylated, but instead have a conserved RNA processing mechanism involving the stem-loop structure and purine-rich sequence at their 3’ end, providing a unique means to control their expression[1]. Chromatin assembly factor 1 (CAF1) is an H3–H4 chaperone complex that associates specifically with H3.1 and H3.2 replicative histones[288,289] and incorporates them into chromatin in tandem with replication fork passage, by directly associating with the DNA polymerase processivity factor known as proliferating cell nuclear antigen (PCNA)[290,291]. Equally important is ASF1, an H3-H4 chaperone capable of binding heterodimers containing all H3 variants, functioning as an intermediate to hand them over to CAF1 or HIRA (histone cell cycle regulation-defective homologue A) complexes[292–294]. By directly interacting with the MCM helicase, ASF1 regulates the reincorporation of pre-existing histones displaced by the replication fork and the integration of newly synthesized histones[294,295].

Box 2 |

Histone variants in senescence

Senescence is a potent tumour-suppressive mechanism limiting proliferation of aged or damaged cells, elicited by a variety of cellular stressors, including oncogenic stimuli. It involves an irreversible exit from the cell cycle as well as metabolic and microenvironment reprogramming, including a senescence-associated secretory phenotype (SASP). SASP reinforces cell cycle arrest and contributes to senescent cell clearance by the immune system, but it can also support protumorigenic inflammation[296]. In addition, senescent cells undergo dramatic chromatin reorganization through formation of senescence-associated heterochromatin foci (SAHF), enriched in repressive histone methylation (H3K9me3 and H3K27me3), heterochromatin binding protein 1 (HP1), and macroH2A[67,297]. While macroH2A is not critical for initiation of SAHF, it plays a role in their maintenance[67], and macroH2A1.1 specifically contributes to senescence by activating a subset of SASP genes[257]. Furthermore, H2A.Z regulates senescence induction, as its eviction from the *CDKN1A* promoter allows expression of its product p21, leading to growth arrest[43,298].

Our group demonstrated that the H3.3 amino-terminal tail undergoes proteolytic clipping by the protease cathepsin L1 (REF.[299]), thereby inducing an irreversible loss of post-translational modifications involved in transcriptional activation, including K4me3. Concurrent with loss of H3K4me3 by JARID1A- or JARID1B-mediated demethylation[300], this leads to downregulation of cell cycle genes and exit from the cell cycle[299]. H3 chaperones have a twofold impact on senescence. On the one hand, HIRA (histone cell cycle regulation-defective homologue A) and ASF1 contribute to recruitment of heterochromatin factors such as HP1 to SAHF[67]. However, it is unlikely they actively deposit H3.3 at these domains, as the histone variant is excluded from SAHF[299] and new H3.3 appears to be incorporated into promyelocytic leukaemia bodies mainly by death domain-associated protein 6 (DAXX) in oncogene-induced senescent cells[301]. On the other hand, HIRA is required for H3.3 deposition at active genes in senescent cells, which in turn prevents loss of H4 K16 acetylation, a mark required to inhibit oncogene-induced transformation[302].

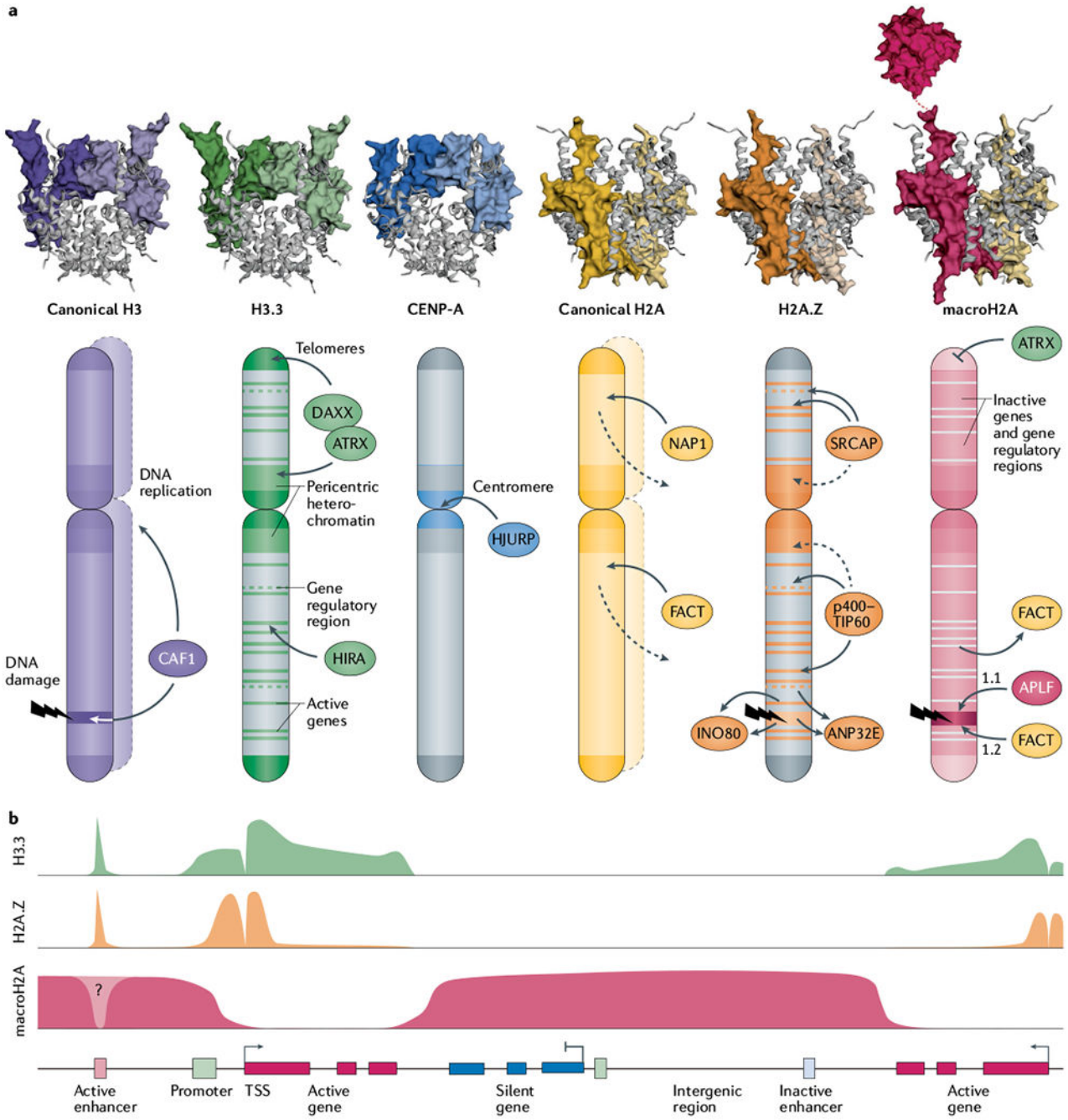

Fig. 1 |. Localization and deposition pathways of histone variants across the genome.

a | Histone variants are structurally similar to their canonical counterparts, with the exception of macroH2A's non-histone macrodomain, which protrudes from the nucleosome particle via its linker region. Histone variants are differentially enriched across genomic landmarks as a function of histone chaperones. The CAF1 (chromatin assembly factor 1) complex deposits canonical H3 variants H3.1 and H3.2 during DNA replication or repair. H3.3 is deposited at active genes and at gene regulatory and nucleosome-depleted regions by the HIRA (histone cell cycle regulation-defective homologue A) complex, and at pericentric

heterochromatin and subtelomeric regions by death domain-associated protein 6 (DAXX)–ATRX (α-thalassaemia/mental retardation syndrome X-linked). Centromeric protein A (CENP-A) is deposited at the active centromere by Holliday junction recognition protein (HJURP). NAP1 and the facilitates chromatin transcription (FACT) complex catalyse incorporation of canonical H2A during replication, and throughout the cell cycle, counterbalancing its continuous turnover (dashed lines). H2A.Z is deposited at active genes and regulatory regions by the SNF2-related CBP activator protein (SRCAP) and p400–TIP60 complexes, but is also enriched at pericentric heterochromatin through an unclear mechanism. The INO80 remodeling complex and ANP32E chaperone exchange H2A.Z for H2A at active genes, gene regulatory elements and DNA damage sites. APLF and FACT promote the accumulation of macroH2A1.1 and macroH2A1.2, respectively, at DNA damage sites. MacroH2A enrichment is negatively regulated by ATRX at telomeres and by FACT-mediated active eviction at transcribed genes. Crystal structures of canonical histones and H3.3 (REF.[283]), CENP-A[284], H2A.Z[12], macroH2A–H2A heterotypic nucleosome[285] and macroH2A macrodomain[76] were rendered with use of EzMol[286]. **b** | At gene-level resolution, H3.3 and H2A.Z are enriched at active regulatory elements (enhancers) and around the promoter of active genes, with the exception of the nucleosome-free region at the transcription start site (TSS). H3.3 is additionally present across transcribed gene bodies and transcription stop sites. MacroH2A forms large domains limited by actively transcribed regions.

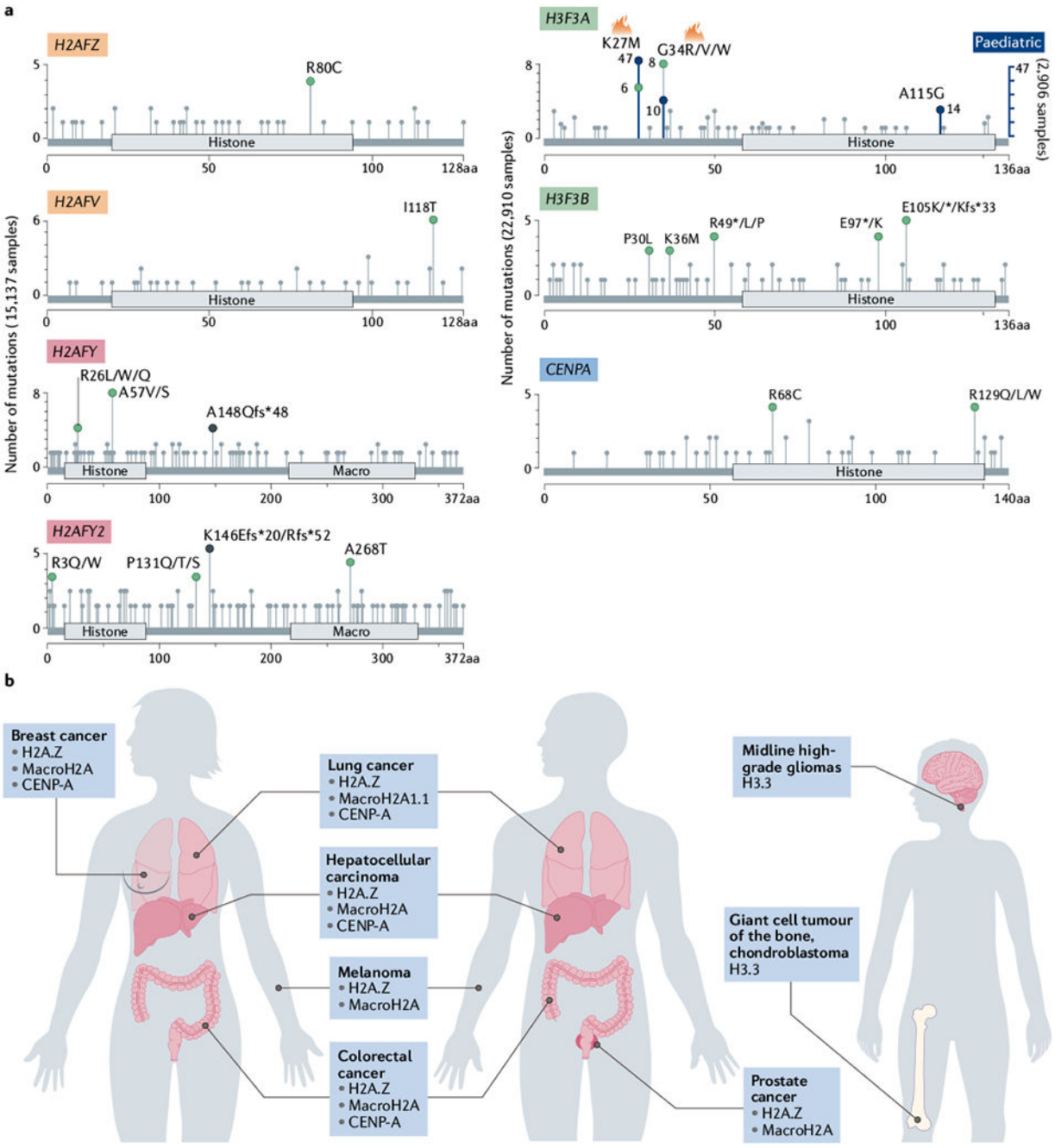

Fig. 2 |. Histone variant mutational spectrum and altered expression across solid tumours.

a | From pan-cancer studies (The Cancer Genome Atlas cohorts used are listed in Supplementary Box 1), the mutational spectra of histone variant genes *H2AFZ* (encoding H2A.Z.1), *H2AFV* (encoding H2A.Z.2), *H2AFY* (encoding macroH2A1; both isoforms, macroH2A1.1 and macroH2A1.2, are included), *H2AFY2* (encoding macroH2A2), *H3F3A* (encoding H3.3), *H3F3B* (encoding H3.3) and *CENPA* are shown. Most histone variants show a low frequency of mutation. *H3F3A* has hotspot mutations (denoted by flame symbol) that represent K27M/R and G34R/V mutations. While in adult cancers these mutations are

diluted, cohorts of paediatric tumours (blue axis; right) have higher mutation frequency in these hotspots. Histones are not drawn to scale. Green dots represent missense somatic mutations, while black dots indicate truncations. **b** | Histone variant alterations across the human body by location; H2A.Z, centromeric protein A (CENP-A) and macroH2A display altered regulation in diverse adult cancers, while H3.3 alterations are restricted to paediatric tumours. aa, amino acids.

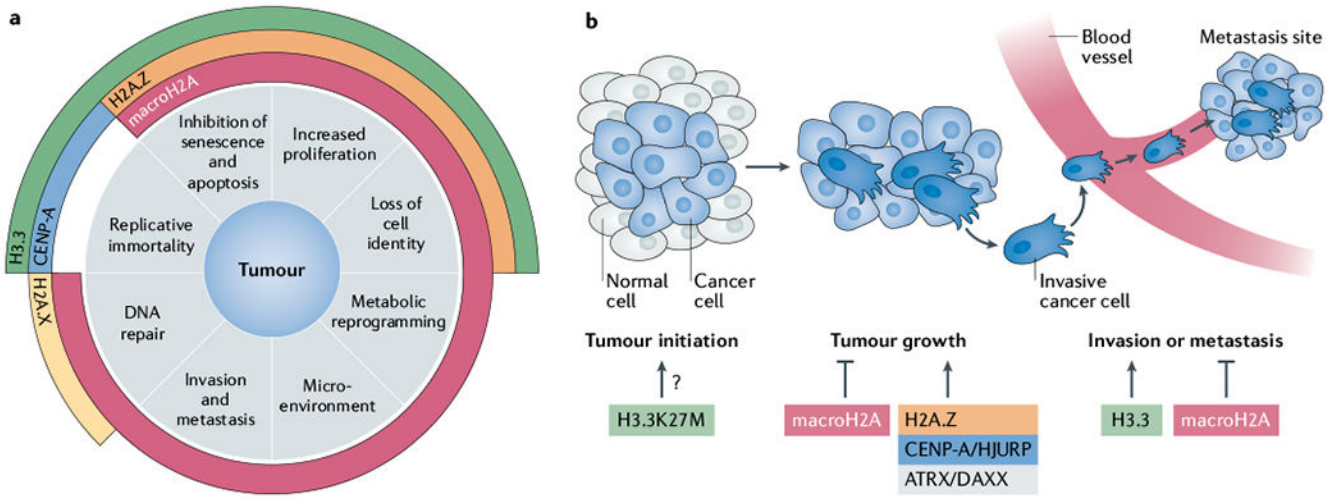

Fig. 3 |. Histone variant hallmarks in tumorigenesis.

a | The role of altered histone variants in tumorigenesis can be categorized into eight major hallmarks as shown. MacroH2A is associated with the highest number of hallmarks, underlying its highly versatile role in tumour development. Centromeric protein A (CENP-A) contributes to replicative immortality via its function in chromosome segregation. H3.3 alteration affects pathways involving replicative immortality due to its role in telomere maintenance, senescence, increased proliferation and loss of cell identity. Lastly, H2A.Z alterations affect mainly proliferation-associated pathways. **b** | Contribution of altered histone variants and their chaperones to the stages of tumour development. A large number of studies have addressed the impact of altered histone variants on proliferation, as shown in the ‘tumour growth’ phase. Although H3.3K27M cannot initiate transformation on its own, this mutation occurs very early during tumorigenesis. Few reports have linked histone variants to invasion or metastasis mechanistically; however, there is evidence that macroH2A may reduce tumour invasiveness and metastatic potential, while deposition of H3.3 in favour of H3.1 sustains induction of genes that promote epithelial–mesenchymal transition. ATRX, α-thalassaemia/mental retardation syndrome X-linked; DAXX, death domain-associated protein 6, HJURP, Holliday junction recognition protein.

Table 1 |

Tumour-specific alterations in histone variants and chaperones and their mechanisms of action

Histone variant or chaperone	Alteration in cancer	Clinical variables associated with alteration	Mechanism of action
Pan-tumour studies			
MacroH2A1.1 (REF.[88])	Downregulation	Increased Ki67 positivity, higher grade	MacroH2A1.1 loss promotes proliferation
H2A.Z[258]	Upregulation	High-grade tumours	Not known
CENP-A and HJURP[221,227]	Upregulation	Tumour vs normal cells, late vs early lesions, increased genomic instability, shorter patient survival	Not known
		TP53 gene deficiency	p53 represses the *CENPA* and *HJURP* genes, which are required to sustain centromere propagation
Breast cancer			
H2A.Z[41,42,259,260]	Upregulation	High grade	Oestrogen induces H2A.Z expression synergistically with MYC; H2A.Z is deposited at ERα target genes and poised enhancers
MacroH2A1/macroH2A1.1 (REFS[93,94])	Ubiquitylation/downregulation	Increased cancer cell proliferation, EMT	Degradation by SKP2 activates CDK8-dependent proliferation and migration pathways; reduced expression during EMT induction
MacroH2A1.2 (REF.[91])	Upregulation	Increased cancer cell invasion and migration	ERα cofactors DDX17 and DDX5 favour splicing of macroH2A1.2, increasing breast cancer cell invasiveness
CENP-A[224,261,262]	Upregulation	Higher grade, invasion, relapse, ERα-negative status	Not known
HJURP[222,262]	Upregulation	Higher grade, proliferation, shorter patient survival, radiosensitivity, ERα-and PR-negative status, increased Ki67 positivity	Not known
Melanoma			
H2A.Z.2 (REF.[13])	Upregulation	Higher proliferative potential	Promotes expression of E2F target genes
MacroH2A[97,263]	Downregulation	Increased proliferation and metastasis	Increased CDK8 Levels promote cell proliferation and metastatic burden
Hepatocellular carcinoma			
H2A.Z[36]	Upregulation	Increased cell proliferation	Promotes cell cycle and EMT gene expression
MacroH2A1 (REFS[103,104])	Downregulation	Maintenance of stem cell features	Resistance to proliferation inhibition by altering lipid and glucose metabolism
	Upregulation	Ageing, senescence-associated β-galactosidase marker	Escape from senescence through p38-IL-8 pathway
CENP-A[223]	Upregulation	Higher histological grade, Ki67 status, p53 positive	Necessary for cancer cell proliferation and survival

Histone variant or chaperone	Alteration in cancer	Clinical variables associated with alteration	Mechanism of action
HJURP[264]	Upregulation	Shorter patient survival, less differentiated, increased size and stage	Promotes cancer cell proliferation
Prostate cancer			
H2A.Z[46,49,56]	Acetylation/upregulation	Increased proliferation	Present at enhancers of genes associated with tumorigenesis, promotes AR-dependent transcription
SRCAP[40]	Upregulation	Increased proliferation	Associated with AR to regulate *PSA* promoter
MacroH2A1.1 (REF.[90])	Downregulation	Higher tumour Gleason score	Not known
Lung cancer (NSCLC unless otherwise specified)			
GAS41 (SRCAP or p400 complexes)[53]	Amplification and upregulation	Level elevated in NSCLC vs normal tissue	Promotes histone H2A.Z deposition to allow expression of cell cycle genes
MacroH2A1.1/macroH2A2 (REF.[87])	Downregulation	Higher tumour proliferation rate	MacroH2A leveis correlate with senescence
CENP-A[265]	Upregulation	Higher grade, proliferation, invasion of lung adenocarcinoma samples	Not known
HJURP[266]	Upregulation	Shorter patient survival	Not known
Colorectal cancer			
TIP60-p400 (REFS[54,55])	Decreased TIP60/p400 ratio	Decreased apoptosis, high proliferation	TIP60 prevents β-catenin signalling, while p400 promotes expression of WNT target genes
MacroH2A1 (REF.[267])	Downregulation	Increased proliferation	Increased expression of ZEB1 and DKK1, escape senescence
CENP-A[268]	Upregulation	Higher expression in tumour vs. normal tissue	Not known
Glioma			
MacroH2A[269]	Ubiquitylation/degradation	Increased Ki67 levels, presence of CD44 (cancer stem cell marker)	MacroH2A1 ubiquitylation by TRIM59 enhances STAT3 signalling activation and tumorigenesis
H3.3 (REFS[127–131,133,135,136,139,142–145,161–163])	Missense mutation	K27M: prevalent in paediatric midline gliomas; fatal prognosis, less differentiated, more invasive tumours	Inhibition of PRC2 and loss or redistribution of H3K27me3, altering expression of neurogenesis, differentiation or cell proliferation genes
		G34R/V: biased towards occurrence in the cerebral hemispheres in teenagers and young adults	Inhibition of SETD2 methyltransferase and decrease of H3K36me3
CENP-A[270]	Upregulation	Shorter patient survival in the mesenchymal subtype	Not known
ATRX[127,131]	Mutations	High-grade tumours in adolescent patients, associated with *TP53* and *IDH1* mutations, positive correlation with ALT	Not known
HJURP[271,272]	Upregulation	Grade, shorter patient survival	Necessary for cancer cell proliferation
Pancreatic neuroendocrine tumours			

Histone variant or chaperone	Alteration in cancer	Clinical variables associated with alteration	Mechanism of action
DAXX-ATRX[196,198,199]	Mutation	High-grade tumours, poor patient prognosis	*ATRX* and *DAXX* mutations associated with ALT
Neuroblastoma			
ATRX[182,200,201]	Mutation/in-frame fusions	Indolent disease, shorter patient survival	Suppression of neuronal gene expression programmes, sensitivity to EZH2 inhibitors
Ovarian cancer			
CENP-A and HJURP[273,274]	Upregulation	Higher tumour grade, higher tumour stage, shorter patient survival, metastasis	Not known
Endometrial cancer			
HJURP[275]	Upregulation	Shorter patient survival	Not known
Sarcomas			
CENP-A[225]	Osteosarcoma: upregulation	Increased tumour size and proliferation, chemoresistance, metastasis, shorter patient survival	Not known
ATRX[276–282]	Osteosarcoma: point mutations, deletions	ALT positivity, co-occurs with *TP53* mutations	*DAXX-KIFC3* gene fusion impairs DAXX function, associated with ALT
	Leiomyosarcoma: nonsense and frameshift mutations	ALT positivity, poor differentiation, shorter patient survival	Not known
	Pleomorphic and myxofibrosarcomas: loss of expression	Highly correlated with ALT	Not known
Giant cell-rich skeletal tumours			
H3.3 (REFS[143,155,157,159,169])	Missense mutation	K36M: chondroblastoma	Inhibition of K36 dimethylation and trimethylation by NSD2 and SETD2, redistribution of K27me3, impaired gene expression and DNA repair
		G34W/L: GCTB, increased proliferation and invasive capacity	Inhibition of K36 methylation by SETD2, impaired K27 methylation and HIRA binding

ALT, alternative lengthening of telomeres; AR, androgen receptor; ATRX, α-thalassaemia/mental retardation syndrome X-linked; CDK, cyclin-dependent kinase; CENP-A, centromeric protein A; EMT, epithelial–mesenchymal transition; ERα, oestrogen receptor-α; GCTB, giant cell tumour of the bone; HJURP, Holliday junction recognition protein; NSCLC, non-small-cell lung cancer; PR, progesterone receptor; PRC2, Polycomb repressive complex 2; SRCAP, SNF2-related CBP activator protein.