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Visions & Reflections

Histones as tumour suppressor genes

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Introduction

Histones are the basic units utilized by cells to compact their genome into the nucleus. Two copies of each of four core histones – H2A, H2B, H3 and H4 – form an octamer around which approximately 150 bp of DNA is wrapped to form the nucleosome core particle. This basic level of packaging can be further assembled into higher orders of chromatin structure. This higher-order level of compaction is mediated, at least in part, by the presence of linker histones. However, far from being a homogeneous repetitive assembly, chromatin is a heterogeneous and dynamic structure. One aspect that adds to this variability is the existence of variants of core histone and multiple linker histones. These proteins make up the minority of the total histone complement in the cell, and can show both striking and subtle differences from their core counterparts (for example, see [1]).

It has been proposed that there are a number of unifying traits present in cancer cells [2]. These include an ability to evade apoptosis, self-sufficiency in growth signals, insensitivity to anti-growth signals, sustained angiogenesis, limitless replicative potential, and tissue invasion and metastasis. A gene that, in its wild-type form, encodes a protein that prevents one or more of these traits will function as a tumour suppressor gene. However, in addition to directly preventing these particular traits, a tumour suppressor gene can also function to maintain genes in their wild-type form, and thus indirectly prevent the acquisition of these traits. Thus, in order for cells to acquire the six traits listed above, the loss of genomic stability acts as an enabling characteristic [2] (fig. 1).

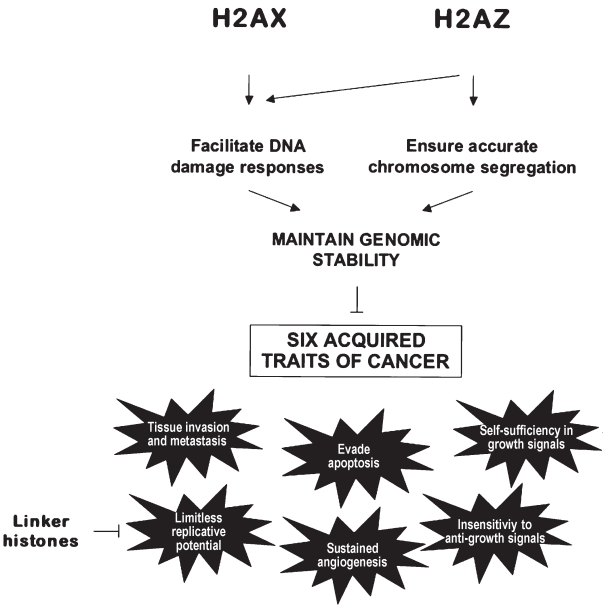

Figure 1. Histones and their multiple effects on the potential development of tumourigenesis.

The histone H2A variant H2AX has already been demonstrated to act as a tumour suppressor gene in mice by preventing the loss of genomic stability. In addition to this histone, there is emerging evidence to suggest that other histones may also play a role in preventing tumourigenesis. In particular, the H2AZ histone variant has been implicated in the maintenance of genomic stability. Finally, data suggest that linker histones have the capacity to directly prevent one of the six hallmarks of cancer: limitless replicative potential. These emerging studies highlight the central and active role that chromatin proteins play in maintaining genomic stability.

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Histone H2AX

The histone H2AX variants exist as single-copy genes in higher eukaryotes, are not transcriptionally coupled to S-phase like core histone H2A genes, and comprise between 2 and 25% of the total histone H2A complement (for review, see [3]). The H2AX proteins have an extended C-terminal tail with an SQ motif at S139 that is phosphorylated in response to DNA damage [3]. Hereafter, we will refer to the phosphorylated form of this protein as H2AXS139ph in accordance with the recently proposed histone modification nomenclature [4], although it is often referred to as γ-H2AX in the literature.

In lower eukaryotes, the core histone H2A genes that are transcriptionally coupled to S-phase and make up the bulk of the histone complement in the cell have a C-terminal tail that is of similar length to the core histone H2A proteins of higher eukaryotes. Notably, however, an SQ motif is often found in the same position relative to the stop codon as that seen in H2AX variants. Moreover, in the systems studied, the SQ motif of these core histone H2A proteins is also phosphorylated in response to DNA damage [5–7], suggesting that the role of this motif in DNA damage response is conserved throughout eukaryotic evolution.

The function of H2A or H2AX phosphorylation in DNA damage response has been the subject of numerous studies. In mammalian systems, H2AXS139ph has been shown to co-localize at the sites of DNA damage with a number of proteins involved in the repair and signalling of damaged DNA. In the absence of the H2AX gene, these proteins are no longer able to accumulate at sites of damage (for review, see [8]). Consistent with this, the mutation of the SQ motif in budding yeast H2A also results in the inability of proteins implicated in DNA repair response to accumulate at the sites of DNA breaks [8]. Interestingly, H2AX is not absolutely required for DNA double-strand break repair, since both the homologous recombination (HR) and non-homologous end-joining (NHEJ) activities are still present, if not necessarily at wild-type levels, in H2AX–/– mice [8]. Again, consistent with this, both HR and NHEJ activities are detectable in budding yeast lacking the SQ motif [5]. It is likely that H2AXS139ph and H2AS129ph function to facilitate DNA double-strand break repair by enhancing the rate or accuracy of the event.

The H2AX gene is not essential and genomic instability and sensitivity to DNA damaging agents are seen in its absence [9, 10], although there is only a modest increase in tumour incidence in knockout mice [9, 11]. Strikingly, these two groups found that the incidence of tumourigenesis is significantly increased in the absence of H2AX when the p53 tumour suppressor gene has been inactivated [11, 12]. Moreover, this increase is dose-dependent, and the $H2AX^{+/-}p53^{-/-}$ mice display an intermediate level of tumourigenesis between $H2AX^{+/+}p53^{-/-}$ and $H2AX^{-/-}p53^{-/-}$ mice. In both studies, the authors identified a number of chromosomal translocations associated with the tumours, suggesting that the H2AX gene functions to prevent inaccurate repair of DNA breaks [11, 12]. One mechanism by which H2AX could do this is by tethering the two broken ends together, perhaps by recruiting proteins that are capable of forming protein bridges across the broken ends. Alternatively, by facilitating the rapid or efficient repair of DNA breaks, H2AX may prevent recombination simply by reducing the persistence of recombinogenic DNA ends. These two possibilities are not necessarily mutually exclusive, and the anchoring mechanism may become particularly important for the repair of persistent DNA breaks. Notably, a number of recent studies have identified putative phosphorylation-specific H2A and H2AX interacting factors, including proteins involved in DNA damage repair and signalling pathways (for review, see [13]). Somewhat surprisingly, chromatin remodelling complexes were also identified (discussed briefly in more detail below, for review, see [13, 14]), raising the possibility that this phosphorylation event is important for providing a permissive chromatin environment for either the repair or anchoring of DNA breaks.

Histone H2AZ

Like H2AX, the H2AZ histone variant differs from core H2A primarily in the C-terminal tail [1]. However, unlike H2AX, it is essential for viability in mice, *Drosophila* and *Tetrahymena*. In contrast, the gene encoding the H2AZ protein in budding yeast, *HTZ1*, is not essential for viability. It was found that in budding yeast, Htz1 is important for the ability of cells to survive in the presence of DNA damage, suggesting a role in the maintenance of genome integrity [15–17]. The Swr1 complex (Swr-C) mediates the exchange of H2A-H2B dimers with Htz1-H2B dimers in nucleosomes [15]. Interestingly, Swr-C was demonstrated to interact with the phosphorylated SQ motif of the budding yeast H2A tail, pointing to a role for Swr1 in DNA damage responses [17]. Additionally, the exchange reaction by the *Drosophila* homologue of this complex (Tip60) is facilitated by phosphorylation of the SQ motif [18]. Together, these data suggest that the H2A(X) SQ motif, the Swr1/Tip60 complex and the H2AZ variants, at least in budding yeast and *Drosophila*, may work together to mediate DNA damage responses. If the mammalian homologues function similarly with H2AX to mediate DNA damage response, then it is possible that the H2AZ gene, by implication, may also function as a tumour suppressor gene.

In addition to its function in DNA damage response, a role in maintaining genomic stability has been identified

in mammalian cell lines [19]. Using RNA interference (RNAi) H2AZ was found to be important for normal mitotic segregation of chromosomes, and major nuclear and chromosomal abnormalities were detected in its absence [19]. This appears to be a conserved function of H2AZ, as it has subsequently been shown that the budding yeast variant is also important for proper chromosome segregation [20]. Both budding yeast and mammalian H2AZ variants have been found associated with centromeric or pericentric regions of the genome, respectively [19, 20], suggestive of a direct role in mediating chromosome segregation, perhaps through the formation of specialized chromatin structures. Therefore, regardless of whether the activity in mediating the repair of DNA damage is conserved in higher eukaryotes, the ability to prevent chromosome missegregation makes H2AZ a compelling candidate for a tumour suppressor gene.

Linker histones

In higher eukaryotes, there are numerous linker histone variants present in cells, and while each individual gene is not essential for viability, a critical threshold of linker histone concentration appears to be essential [21]. In budding yeast, the single linker histone gene is not essential for viability. In this system, it was demonstrated that the linker histone is inhibitory to homologous recombination [22]. One consequence of this inhibition of recombination was that in the absence of the linker histone, cells were far more successful at maintaining their telomeres by recombination [22]. If this activity is conserved in higher eukaryotes, it may have important ramifications for tumourigenesis. In order to acquire limitless replicative potential, one of the six hallmarks of cancer, cells must maintain their telomeres. In most tumours, this is done by reactivation of telomerase. However, in a significant proportion (around 20%), the cells use a homologous recombination-dependent mechanism, termed the ALT pathway [23]. If linker histones can function as a barrier to the ALT pathway, then it is possible that they can act as tumour suppressor genes by inhibiting the ability of cells to acquire limitless replicative potential. This is a highly speculative possibility, and it remains to be seen whether the activity uncovered in budding yeast is a conserved feature of linker histones in other organisms.

Concluding remarks

The discovery that H2AX functions as a tumour suppressor gene in mice has potential clinical ramifications. Interestingly, the human gene encoding the H2AX variant, H2AFX, is located in a region of the genome that exhibits loss of heterozygosity or deletion in a large number of human cancers [11, 12]. While it is still not known whether linker histones or the histone H2AZ variant also function in this capacity, it is tempting to speculate that they do, and it will therefore be of great interest to determine whether mutations in the genes encoding either H2AZ or linker histones are associated with human cancers. Additionally, it is likely that, as the central players in packaging and organizing our genetic information, other histones and chromatin components will also be involved in maintaining the integrity of the information contained within. It seems likely, therefore, that the number of chromatin components working to prevent the acquisition of the six hallmarks of cancer will continue to rise.

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