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Chromatin: a molecular interface between cancer and aging

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Abstract

To prevent cancer, mammals have evolved potent tumor suppression mechanisms, including senescence and apoptosis. These processes depend on regulation of chromatin. Chromatin-dependent tumor suppressor pathways are activated in pre-malignant cells and tissues harboring cancer-causing genetic alterations, and also in normal aged tissue, the latter likely due to accumulation of genetic and cellular damage. Paradoxically, however, disruption of chromatin structure may also promote cancer. Apparent defects in chromatin structure accumulate with age, the biggest single risk factor for cancer. Evidence suggests that these age-associated perturbations in chromatin structure contribute to the age-associated increase in incidence of cancer. Thus, alterations in chromatin structure can both suppress and promote the onset of cancer, and both activities are inextricably linked to aging.

Introduction

The basic repeating unit of chromatin structure is the nucleosome, comprised of about 146bp of DNA wrapped around an octamer of histones, assembled from an (H3/H4)₂ heterotetramer and two H2A/H2B heterodimers [1]. This nucleosomal structure is the platform for complex variations, modifications, binding of proteins and higher order folding patterns that impact all DNA and cell functions. Specifically, functionally distinct histone variants, such as H3.3, macroH2A, H2AZ and H2AX, can be substituted for the canonical histones, nucleosome positioning and turnover is regulated by chromatin-remodeling enzymes, histones are extensively post-translationally modified, and modified and unmodified histones bind to a multitude of regulatory chromatin binding proteins. Even DNA itself is modified by methylation on cytosine, to regulate its functions. Classically, chromatin has been structurally and functionally divided into two broad states: euchromatin is decondensed in interphase of the cell cycle and contains the majority of actively transcribed genes, while heterochromatin remains condensed throughout the cell cycle and contains mostly inactive genes.

Chromatin, like DNA itself, has a two-sided relationship to cancer. On the one hand, programmed chromatin changes contribute to tumor suppression to block the development of cancer, caused by oncogenic mutations in DNA and other forms of cellular damage that accumulate with age and/or in response to environmental insults. On the other hand, as a complex structure and key regulator of cell behavior, chromatin is itself a target for the molecular damage that initiates cancer (Figure 1).

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Programmed chromatin changes in tumor suppression

At least two cell autonomous tumor suppressor processes have been defined and extensively studied, namely apoptosis and senescence. Chromatin regulation is an important component of both processes.

Chromatin activates senescence—Primary cells that acquire diverse forms of potentially cancer-causing molecular damage, including activated oncogenes and inactivated tumor suppressors, can become senescent. Senescence is characterized by an irreversible proliferation arrest that blocks proliferation of the nascent tumor cells. In addition, senescent cells display a marked secretory program, involving immune regulators, that appears to promote killing of damaged senescent cells by the innate system [2].

Senescence is triggered through a complex cascade of signals and effector programs. Key upstream signals include: DNA damage signals caused by defective chromosomal telomeres, resulting from excess rounds of cell division; and oncogene-induced MAP-kinase signaling, leading to error-prone DNA replication and consequent DNA damage. These signals converge on the p53 and p16INK4a-pRB tumor suppressor pathways, and cell remodeling processes, including autophagy and marked reorganization of chromatin structure. Ultimately, these signals, effectors and cell remodeling processes culminate in proliferation arrest and activation of the secretory program [2].

Chromatin regulation contributes to various aspects of this program. Upregulation of p16INK4a and activation of the pRB pathway is controlled by chromatin in various ways (Figure 2). Genetic inactivation of the chromatin-remodeling factor, SNF5, occurs in virtually all pediatric rhabdoid tumors [3]. SNF5 exerts its role as a tumor suppressor, in part, by activating expression of p16INK4a and inducing senescence [4]. In cells expressing p16INK4a, the histone variant H2AZ localizes to a chromatin boundary that prevents invasion of gene-silencing heterochromatin into the p16INK4a gene. This boundary is lost in some breast cancer cells in which p16INK4a expression is silenced, presumably facilitating their escape from senescence [5]. Most notably, a member of the PRC2 polycomb repressor complex, EZH2, is a histone methyltransferase that methylates histone H3 on lysine 27 to generate H3K27Me3 and silence expression of p16INK4a [6,7]. Expression of EZH2 is downregulated in senescent cells, allowing the histone demethylase, JMJD3, to remove H3K27Me3 [8,9]. In concert with MLL1-mediated methylation of histone H3 on lysine 4 to create H3K4Me3, this results in upregulation of p16INK4a expression [10,11]. Expression of EZH2 is downregulated during tissue aging *in vivo* [12,13], and expression of p16INK4a is upregulated during tissue aging and in premalignant lesions containing senescent cells [14-18]. However, in cancer, EZH2 is often upregulated and expression of p16INK4a is lost [6,19]. Thus, the EZH2-p16INK4a axis promotes senescence, thereby acting as a barrier to cancer, in response to activated oncogenes or other forms cellular damage that are associated with aging.

Another chromatin regulatory module implicated in cell senescence is the H3K9Me3-HP1 complex. Three isoforms of HP1, HP1 α , β and γ , bind to H3K9Me3 and, to varying degrees, all three HP1 isoforms and H3K9Me3 are found in transcriptionally silent heterochromatin [20]. In senescent cells, the H3K9Me3-HP1 complex is thought to facilitate silencing of proliferation-promoting genes [21]. Both H3K9Me3 and HP1 are upregulated in premalignant senescent cells *in vivo* and inactivation of this pathway accelerates tumorigenesis [14,16,22].

Two other histone H2A variants, macroH2A and H2AX, also contribute to senescence. The histone variant macroH2A is incorporated into heterochromatin of senescent cells *in vitro* and *in vivo* [23,24]. MacroH2A is thought to contribute to silencing of proliferation-

promoting genes in senescent cells, although the precise mechanism by which it achieves this is unclear. Consistent with senescence being a barrier to tumor progression, the presence of macroH2A in tumors correlates with better disease outcome [24].

Histone variant H2AX is a candidate tumor suppressor gene [25]. This variant is distributed through the chromatin of normal proliferating cells and becomes phosphorylated at its C-terminus by ATR and ATM checkpoint kinases in response to DNA damage. Phosphorylated H2AX, γ H2AX, then acts as a platform for recruitment of DNA damage repair and cell cycle checkpoint proteins. *In vivo*, γ H2AX is increased in early neoplastic lesions that contain senescent cells and also in aging tissues [22,26-28]. In both cases, γ H2AX is likely to contribute to senescence and proliferation arrest of potentially cancer-initiating damaged cells.

In sum, programmed changes in chromatin structure contribute to the senescent cell phenotype and, consequently, tumor suppression. This program is initiated in response to activated oncogenes and other diverse molecular stresses, including those that accumulate with normal cell and tissue aging (Figure 1).

Chromatin activates apoptosis—In response to diverse forms of cancer-causing DNA damage and other cellular stresses, cells can undergo a programmed cell death called apoptosis, which eliminates potentially harmful damaged cells.

Central regulators and effectors of the apoptosis program include the p53 tumor suppressor, cytochrome c released from mitochondria and caspase type proteases, whose concerted activity sets in train the hallmarks of apoptosis, namely chromatin condensation, membrane blebbing and DNA fragmentation [29].

Specific combinations of histone modifications contribute to apoptosis. For example, phosphorylation of H2B by Mst1 kinase to generate H2BS14P facilitates apoptotic chromatin condensation and/or DNA fragmentation [30]. In addition to facilitating cell cycle checkpoints and associated DNA repair, DNA damage-induced γ H2AX can also promote apoptosis. Remarkably, the choice between DNA repair and cell death is determined by another modification of γ H2AX; if γ H2AX is also tyrosine phosphorylated on Y142 (γ H2AXY142P), then DNA repair is inhibited and the cell is directed down a death pathway [31,32].

Overall, alterations in chromatin structure and function contribute to the apoptotic program and, presumably, its role in tumor suppression.

Other chromatin-mediated barriers to cancer—Senescence and apoptosis are not the only means by which organisms prevent the onset of cancer. For example, an organelle recycling process, autophagy, is also implicated in tumor suppression, perhaps through its ability to eliminate damaged macromolecules [33]; and there are non-cell autonomous routes to tumor suppression, such as immune surveillance [34]. Consequently, there are likely to be chromatin-mediated barriers to cancer that are not linked to either senescence or apoptosis. Interestingly, a number of chromatin regulators are misregulated in cancer and, seemingly, counter-regulated in aging. Examples include the NURD chromatin remodeling complex which is downregulated in aging and mistargeted in Acute Promyelocytic Leukemia (APL) [35,36], and H4K20Me3 which is upregulated in aging and downregulated in cancer [37,38]. Conceivably, this counter-regulation in aging and cancer reflects an age-associated tumor suppression barrier that is breached in cancer. None of these chromatin regulators has an established role in senescence or apoptosis, perhaps implicating them in other tumor suppression processes.

Age-associated defects in chromatin promote cancer

While some age-associated alterations to chromatin structure reflect a programmed chromatin level response to arrest the development of cancer, other age-associated chromatin changes actually appear to promote cancer (Figure 1). For example, in mammals, there is an age-associated decline in total genomic DNA methylation [39-41], that is thought to occur largely at repetitive DNA sequences [42]. This decline in DNA methylation levels is mirrored in tumor cells, since it is a hallmark of most types of cancer [43] and is believed to contribute to carcinogenesis, although the mechanism remains unknown [44].

Whilst genome-wide levels of methylation decrease with age in mammals, there is also a tendency for DNA methylation to increase at specific sites [45-53]. This occurs at CpG islands, which are mainly in the promoter regions of genes. CpG islands are CG rich sequences that are normally maintained in an unmethylated state. However aberrant methylation of these sequences is typically associated with stable and heritable gene silencing [1]. Indeed, hypermethylation and silencing of tumor suppressor genes is an early event in many cancers [54]. And, studies on specific tumor suppressor genes have indicated that some DNA methylation events that occur during aging might promote cancer. For example, one study showed that the p16INK4a gene and other tumor suppressors are methylated on aging [52].

More recent genome-wide approaches have strengthened the relationship between DNA methylation in aging and cancer. Two studies performed large-scale screens for genes that are hypermethylated in aging and demonstrated significant overlap between these regions and those hypermethylated and silenced in cancer [55,56]. Most likely, such chromatin changes are unprogrammed, erroneous events and a reflection of chromatin damage. Together, the studies reinforce the idea that age-related DNA hypermethylation might be a contributor to the striking increase in cancer incidence with age (Figure 1).

What is the molecular basis of this age and cancer-associated DNA hypermethylation? Surprisingly, previous studies have shown that not all genes that are hypermethylated in cancer are expressed in the normal counterpart tissue [57]. This suggests that stable gene silencing in cancer does not always result from stochastic hypermethylation and silencing, followed by adaptive clonal selection. In light of this, it is significant to note that genes that are hypermethylated in cancer and in aging both tend to be marked in stem cells (adult tissue stem cells and hES stem cells) by a characteristic chromatin signature. Specifically, they are marked with H3K27Me3 and proteins of the polycomb PRC2 complex, EED and SUZ12, which collaborate with EZH2 in deposition of H3K27Me3 [55,56,58-60]. Furthermore, some studies showed these hypermethylated genes to be marked by so-called “bivalent chromatin domains” in hES cells [55,58], a finding conserved in aging mice [61]. These bivalent chromatin domains contain histone modifications characteristic of both gene activation and gene repression, H3K4Me3 and H3K27Me3 respectively [62,63]. In pluripotent ES cells, these bivalent domains are thought to maintain cell fate determining genes in a transcriptionally repressed state that is consistent with proliferation and pluripotency, but is also compatible with activation in appropriate cell lineages (Figure 3). The preferential methylation in aging and cancer of genes carrying these bivalent chromatin marks in stem cells, suggests that stable gene silencing in aged and cancer cells may reflect, in part, an instructive - but erroneous - process that is directed by epigenetic stem cell memory. This erroneous hypermethylation process appears to occur during normal aging, thereby contributing to the age-associated decline in tissue function and predisposition to cancer (Figure 3).

These remarkable observations raise several important questions. In which cells are these genes hypermethylated? What is the trigger for DNA methylation? What is the mechanism

by which these genes are hypermethylated? Since hypermethylated genes harbor a specific chromatin structure in ES cells and adult stem cells, it seems likely that they are methylated at some point during *in vivo* aging of an adult stem or progenitor cell. Once methylation has occurred, it is epigenetically inherited in cells descended from that stem or progenitor cell. The trigger for hypermethylation might depend on accumulation of cellular damage with age, dietary factors or other environmental exposures. There is some overlap between genes hypermethylated in physiological aging and genes hypermethylated after extended culture *in vitro*, suggesting that proliferative exhaustion and onset of cell senescence might be one cause of hypermethylation [64]. Various factors have been proposed to drive the methylation process. EZH2, the enzyme responsible for deposition of H3K27Me3, can recruit DNA Methyltransferases (DNMTs) [65]. Conceivably, upregulation of EZH2, as found in some cancers [19], might promote DNA methylation. However, it seems unlikely that the mere presence of EZH2 is sufficient for hypermethylation, and accumulation of another mark, H3K9Me3, has been suggested to be a contributing intermediate towards DNA methylation [58]. Clearly, additional studies are required to better define the link between bivalent chromatin and DNA methylation.

Conclusions

In this review, we have seen how programmed changes in chromatin structure can arrest the development of cancer in cells that acquire genetic or chromatin damage, due to environmental insults and stochastic processes associated with aging. This mode is well illustrated by the EZH2-p16INK4a-senescence pathway. However, accumulating evidence also indicates that unprogrammed, inappropriate age-associated chromatin changes may themselves be a cause of cancer. This unfolding story promises to help clarify the still murky relationship between aging and cancer.

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Figure 1. The role of programmed and unprogrammed chromatin changes in suppression and induction of cancer

Insults, toxins and carcinogens in the environment and stochastic events that accumulate with age (e.g. DNA replication errors) lead to unprogrammed genetic and epigenetic alterations. Some of these lead to loss of cell proliferation control and cell and tissue homeostasis, and so can be cancer-promoting. Such changes activate programmed tumor suppressor mechanisms, which depend on regulation of chromatin structure, to block the development of cancer. However, over time, genetic and epigenetic alterations accumulate and overwhelm and/or inactivate tumor suppressor mechanisms (dashed lines), resulting in cancer. For simplicity, inherited triggers of cancer (e.g. germline inheritance of mutations in tumor suppressor genes) are not shown.

Figure 2. A model for regulation of p16INK4a by chromatin structure

In proliferating non-senescent cells or transformed cells, p16INK4a is repressed through the action of polycomb proteins (PRC) and H3K27Me3. In senescent cells, polycomb-mediated repression is relieved and replaced by activators of transcription, e.g. H3K4Me3; chromatin remodeller, hSNF5, also activates transcription; H2AZ contributes to a chromatin boundary that prevents incursion of gene-silencing heterochromatin.

Figure 3. Age-associated DNA methylation of bivalent genes contributes to tissue dysfunction and cancer

In ES cells, genes involved in cell differentiation are typically silenced, but bivalently marked with histone modifications characteristic of transcriptionally silent and active chromatin, H3K27Me3 and H3K4Me3 respectively. Some of these marks persist into adult tissue stem and progenitor cells. At this stage, CpG islands are unmethylated. Removal of repressive histone marks, in a tissue specific manner, facilitates gene activation and cell differentiation. As tissues age, these bivalent genes are predisposed to DNA methylation which tends to stably and heritably silence transcription. This blocks tissue specific differentiation, leading to tissue dysfunction with age; and also predisposes these cells to neoplastic transformation, because the proliferation arrest typically associated with irreversible cell differentiation contributes to tumor suppression.