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Review

Chromatin, cancer and drug therapies

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ABSTRACT

The structure and organization of chromatin have attracted a great deal of attention recently because of their implications for the field of epigenetics. DNA methylation and the post-translational modifications that occur on histones can specify transcriptional competency. During cancer development, tumor suppressor genes become silenced by DNA hypermethylation and chromatin modifiers no longer perform in their usual manner. Current epigenetic therapy has been able to take advantage of the reversibility of these epimutations. Progress has been made in the treatment of hematological malignancies and some solid tumors. As the knowledge of how chromatin regulates gene expression is enhanced, improvements in the treatment of cancer can be made.

Contents

1. Introduction to chromatin structure

The organization of chromatin has been intensely studied and now the focus in the field of epigenetics has shifted to understanding the biological relevance and function of chromatin structure. Epigenetics, which is defined as the study of heritable changes in gene expression that occur without a change in the DNA sequence, provides insight into the extent by which chromatin structure exerts control on transcriptional regulation. Interpreting the patterns of post-translational histone modifications as well as DNA methylation and how these epigenetic mechanisms contribute to gene expression in a normal state and in cancer are key to developing drugs that can reverse abnormalities that occur during tumorigenesis.

Chromatin is comprised of DNA, histone proteins and non-histone proteins. The fundamental repeating unit of chromatin is the nucleosome, which consists an octamer of histones with two each of the four small and highly basic histones (H3, H4, H2A, and H2B) [1]. Approximately, 146 bp of DNA are wrapped twice around each histone core providing a means for higher order packaging of DNA in the nucleus. The histone amino terminal tails that project out of the nucleosome core are subject to many post-translational modifications such as phosphorylation, ubiquitination, sumoylation, acetylation and methylation on specific amino acid residues [2,3]. This review will highlight the most highly studied modifications, acetylation and methylation of histones H3 and H4 (Fig. 1).

Acetylation by histone acetyl transferases (HATs) occurs on the lysine residues of histone tails and is strongly correlated with active gene expression. The basic charges of the histone tails become

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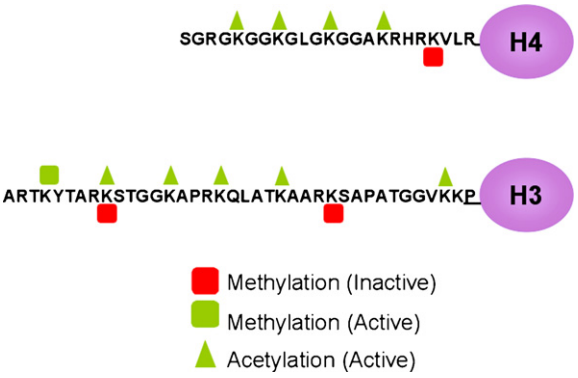

Fig. 1. Post-translational histone modifications on histone tails. Modifications made on the N-terminal tails of histones are important in establishing the activity state of chromatin. Many modifications are possible, however, only acetylation and methylation of a subset of lysine residues are depicted here. Active acetylation or methylation marks (green triangles or squares), can act to "loosen" chromatin to allow for access of transcriptional machinery while also serving as docking points for nucleosome remodeling complexes. Conversely, inactive marks such as methylation of specific residues can cause an inactive conformation of chromatin and can recruit repressive complexes.

neutralized upon acetylation. This causes increased accessibility for further modifications or access to the DNA for binding factors and transcriptional machinery [4–6]. Unlike acetylation, methylation of histones does not change the charge of the histone tails [7]. Lysine residues can accept up to three methyl groups, which are added by various histone methyltransferases (HMTs). The degree of methylation is informative for both the state of gene activity as well as which proteins/complexes might bind and read the message displayed by those marks [8,9]. Methylated lysine residues may constitute either active or inactive marks. Active marks include histone H3 lysine 4 (H3K4), lysine 36 (H3K36) and lysine 79 (H3K79) [10–12]. The methylation marks on lysines 9 and 27 on histone H3 and lysine 20 of histone H4 are associated with an inactive chromatin state [13–15]. Interestingly, there is cross regulation between different marks such as the competition for lysine 9 on histone H3 between an inactive methyl mark and acetylation [16,17]. The distinct patterns of post-translational modifications make up the "histone code" and the precise combinations determine how the chromatin is read [18].

The position of nucleosomes on the DNA further adds to the complexity of chromatin structure. Nucleosome positioning and occupancy can also play a key role in regulating gene expression and the presence of a nucleosome at the transcription start site is commonly seen in inactive genes [19]. Studies have shown evidence of the loss of a nucleosome directly upstream of the transcription start site upon gene activation. This may allow greater access for binding of transcription complexes or factors [20]. It has been shown that a promoter of a gene with a basal level of transcription can already be depleted of nucleosomes which allows for quick induction upon stimulation [21]. Also, the reactivation of a completely silenced gene is associated with nucleosome loss [22]. These studies demonstrate the importance of nucleosomes in gene regulation.

DNA methylation influences gene regulation in concert with histone modifications and nucleosome positioning [23]. DNA methylation at the transcriptional start sites of genes is associated with inactivity and is important in imprinting, X inactivation and the silencing of retrotransposons. The five carbon on the cytosine ring in DNA can be modified by the placement of a methyl group by DNA methyltransferases (DNMTs). DNMT1 is referred to as the "maintenance" methylase due to its preference for hemimethylated CpG sites in DNA [24]. DNMT3a and DNMT3b are considered to be *de novo* methylases because they can methylate unmethylated DNA [24,25]. However, all three DNMTs have been shown to act cooperatively and the functional differences between the methylases may to a large extent be due to the genomic regions that they act upon [26,27]. Methylation occurs in the context of CpG dinucleotides, which are underrepresented in the genome possibly due to evolutionary depletion [28]. Regions of high CpG content are termed "CpG islands" and are found at the promoters of more than 50% of genes in the genome. CpG islands are often located at the promoter regions of housekeeping genes in an unmethylated state [29]. The DNA methylation mark can act both directly and indirectly to silence a gene by either inhibiting the binding of transcription factors or by possibly recruiting methyl-binding domain proteins (MBDs), which further recruit histone deacetylases (HDACs) [30].

MicroRNAs (miRs) are another mechanism used by the cell to regulate the expression of genes involved in differentiation, cell proliferation and apoptosis [31]. They are short RNAs 19–24 nucleotides in length that often bind to the 3′UTR of their target mRNA to either inhibit that mRNA's translation or cause its degradation [32]. MiR expression profiles differ depending on cell type and like DNA methylation, they help to establish the cells identity. Currently, more than 400 human miRs have been experimentally identified and are proposed to regulate more than 30% of all mRNAs post-transcriptionally [32,33].

2. Epigenetic changes in cancer

Epimutations in cancer can result in the activation of oncogenes, the silencing of tumor suppressors, and ultimately in the cell's ability to proliferate uncontrollably. These changes are often linked to the presence of altered levels of chromatin modifying enzymes and a shift in the genome-wide distribution of DNA methylation. Changes in histone marks work together with DNA methylation or independently to silence gene expression depending on the region of chromatin and the type of gene. Advances in our understanding of how these abnormalities occur will help in designing and improving drugs to target the factors that cause these changes during tumorigenesis.

Altered activity of the histone lysine methyltransferases can contribute to the deviant histone methylation patterns found in cancer. For example, histone lysine methylation on histone H3K9 and H3K27 are normally present at transcriptionally inactive or heterochromatic regions, yet they can be found at genes that are aberrantly repressed in cancer cells [34,35]. The methyltransferase MLL, which methylates H3K4, is involved in translocations that lead to the inappropriate expression of various homeotic (Hox) genes, which contributes to leukemic progression [36]. Methyltransferases within complexes well known for their suppressive activities are also up-regulated in cancer.

The Polycomb group (PcG) complexes are chromatin modifiers that are crucial to development, and have been implicated in the development of cancer [37]. These negative regulators of gene expression are very important in sustaining the repressive state of their target genes through the cell cycle [38]. Two of the PcG repressive complexes (PRC1 and PRC2) have both been shown to be involved in various cancers. Enhancer of zeste homologue 2 (EZH2), a component of PRC2 with H3K27 methyltransferase activity, is upregulated in mantle cell lymphoma, breast and prostate cancer [39–41]. RING1, a component of PRC1 that aids in the ubiquitylation of histone H2A lysine 119, is upregulated in prostate cancer [42].

The demethylation of histones is important in transcriptional regulation. Histone lysine methylation had been previously thought to be a very stable mark. However, the discovery of LSD1, a demethylase of mono- and dimethylated histone H3K4, showed that these chromatin marks are reversible [43]. LSD1's mechanism