Review Article

Critical Role of the HP1-Histone Methyltransferase Pathways in Cancer Epigenetics

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Abstract

Posttranslational modifications to histone tails (histone marks) serve as the underlying basis for epigenetic signaling in the activation and repression of gene activity. The specific temporal and spatial context of these modifications is interpreted by nonhistone chromatin proteins called histone mark readers. The best-known example of reader proteins, heterochromatin protein 1 (HP1), recognizes epigenetic marks generated by histone methyltransferases (HMTs), SUV39H1 and G9a/GLP. Changes in the levels of HP1 and its associated HMTs have been associated with the development of several cancers. Here, we review the role of the HP1-HMT pathways in cancer-associated processes as well as the pharmacological targeting of this important epigenetic player for the therapy of malignant diseases.

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The Heterochromatin Protein 1 Family of Proteins

Heterochromatin protein 1 (HP1) was first discovered in *Drosophila* as a regulator of higher-order chromatin structures involved in position-effect variegation, a phenomenon by which the dynamic formation of heterochromatin-euchromatin boundaries creates a mosaic pattern of gene expression [1, 2]. Today, we know that several HP1 isoforms, which are products of different genes known as CBX (chromobox), form a family of nonhistone chromosomal proteins, which is evolutionarily conserved with members in almost all eukaryotic

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organisms. For instance, three distinct isoforms form the mammalian HP1 protein family, each of which is encoded by its own gene. In humans, HP1 α is encoded by the chromobox homolog 5 (*CBX5*) gene, and the *CBX1* and *CBX3* genes encode the genes for HP1 β and HP1 γ , respectively. Interestingly, the genomic structures of HP1-encoding genes are conserved from *Drosophila* to humans [3]. Thus, HP1 genes and proteins appear to have undergone an exquisitely strong evolutionary pressure for their conservation, which reflects the need of all eukaryotes for their function.

Structurally, the HP1s are part of a larger superfamily of proteins containing chromatin organization modifier (chromo) domains. The chromodomain is an evolutionarily conserved region in the N-terminal half of HP1 proteins. Proteins containing the chromodomain mediate the functional aggregation of nucleosomes into heterochromatin [4]. Moreover, HP1 proteins form their own family within the chromodomain superfamily as they are characterized by the presence of a second conserved domain at their C-terminus known as the chromoshadow domain, which is structurally similar yet functionally distinct to the chromodomain [5]. Thus, the presence of both the chromodomain and the chromoshadow domain, joined by a flexible linker peptide, is the major consideration used to group HP1 proteins into a single structural classification and is key for understanding the function of these proteins. Consistent with the histone code hypothesis, the chromodomain binds to di- and trimethylated lysine 9 (K9) of histone H3, which is an epigenetic mark that signals the formation of heterochromatin [6, 7]. On the other hand, the chromoshadow domain is responsible for the homo- and/or heterodimerization between HP1 molecules as well as their interaction with members of other protein families involved in various epigenetic functions [8]. The linker region that separates these domains is more divergent in sequence, but always contains a nuclear localization signal, which is highly amenable to posttranslational modifications [9–12]. Thus, the current model for the function of HP1s recognizes them as epigenetic adaptor proteins, which help to dynamically crosslink key epigenetic regulators to histone marks in a process that is highly regulated by signaling-induced modifications of the linker.

The localization of HP1 to different regions of the chromatin and its interaction with other molecules that aid in this process has been well studied. The isoforms HP1 α and HP1 β are mainly heterochromatic, while HP1 γ is present in both compartments. However, the localization of HP1 γ to either of the chromatin compartments is known to be regulated by posttranslational modifications. For instance, phosphorylation of Ser83 defines a subpopulation of HP1 γ specifically located within euchromatin [13]. Notably, a large number of posttranslational modifications in the three HP1 isoforms, which, analogous to those in HP1 γ , can regulate their function, have been described to form an HP1-mediated subcode within the histone code [9]. Therefore, careful studies on the structure-function relationship of HP1 have expanded our understanding of the function and regulation of this important family of epigenetic players.

The HP1-Associated Histone Methyltransferases

As mentioned above, at the detailed molecular level, distinct histone marks are the effectors of the HP1-histone methyltransferase (HMT) complex. N-terminal tails of histones protrude from the nucleosome core and are susceptible to a variety of posttranslational modifications. Whole-genome analysis using ChIP-Seq has revealed monomethylation of histones to be associated with transcriptional activation, namely at H3K27, H3K9, H4K20, H3K79, and H2BK5. Alternatively, trimethylation at H3K27, H3K9, and H3K79 is associated with transcriptional repression [14]. Patterns of histone methylation are proposed to be one of the major epigenetic marks that extend the genetic code through heritable regulation of

chromatin structure. HP1 proteins recognize methylated histone marks, which associate with the state (hetero- vs. euchromatin), organization, and function of chromatin, having a major impact on the expression of gene networks that define normal and diseased phenotypes [15]. HP1-specific HMTs interact with modified histone H3 and HP1 [16]. In this regard, it is important to consider that proteins containing the highly conserved SET domain function as HMTs, which are classified into subgroups based on their specific substrates [17]. The human HP1-interacting HMT SUV39H1, which contains the characteristic SET domain, methylates the K9 residue on histone 3 (meH3K9). This forms a binding site for the chromodomain of HP1 and leaves the chromoshadow domain available for interaction with additional SUV39H1 molecules. An HP1 dimer is used to bring together two adjacent nucleosomes that contain the 3meH3K9 mark. Thus, through repeated cycles of binding to meH3K9 marks and HMT recruitment to nucleosomes, HP1 provides one of the best-characterized molecular mechanisms for heterochromatin formation and spreading [15]. Notably, however, HP1 can also recruit HMTs that are distinct from SUV39H1. For instance, G9a (EuHMTase-2), which interacts with the three HP1 isoforms, is specific for generating the 2meH3K9 mark [18] that mediates nucleosome remodeling in euchromatin regions. In summary, while both HMTs recognize H3K9, SUV39H1 contributes to trimethylation in heterochromatin, while G9a primarily regulates mono- and dimethylation within euchromatin [17]. The G9a-related HMT GLP has been shown to interact with G9a in transcriptional silencing complexes. However, how the functional interaction between these two highly related HMTs with HP1 are initiated and maintained as well as the complete functional consequences of HP1 remains to be fully characterized [18].

Function of the HP1-HMT Pathways in Cancer-Associated Processes

The remodeling of chromatin underlies most of the DNA-related biological processes such as DNA repair and replication, chromosome segregation and condensation, and nuclear organization [19, 20]. Given its involvement in a wide range of biological processes, disruptions in the remodeling of chromatin are found to be directly associated with the development and progression of cancer. The critical participation of chromatin remodeling in cancer is largely due to the fact that it causes and perpetuates alteration in the structure and expression of multiple loci, which are critical for establishing malignant phenotypes [20]. For instance, changes in chromatin that correlate with several cancers include either an increase or a decrease in the levels of HP1 proteins [21]. In turn, these alterations of HP1 proteins and the function of the associated HMTs have an impact on gene expression. For instance, the mechanisms by which HP1 regulates cancer invasion and metastasis is postulated to be through its alteration of the expression of genes that participate in cell motility and matrix degradation [21]. Decreased expression levels of HP1 α and HP1 β have been associated with increased metastatic breast tumor development and melanoma, respectively, while inhibition of HP1 α increases cancer cell invasion without affecting growth [20]. Interestingly, in spite of these important observations, no somatic mutations have been found in HP1 that can explain their cancer-specific behavior [22]. Therefore, many efforts have been made to expand the understanding of nongenetic mechanisms involved in transcriptional regulation via HP1-HMT pathways [21]. Emerging data from these studies have described the sequence-specific recruitment of HP1-HMT pathways to transcription factors to silence cancer genes (fig. 1). The Krüppel-like transcription factor 11 (KLF11), a tumor suppressor protein for several cancers [23], contains a signature PXVXL domain at its C-terminus, which mediates its interaction with the HP1 α chromoshadow domain in vivo [24]. KLF11 also recruits SUV39H1 along with HP1 α to promoters in order to limit KLF11-mediated activation of gene networks

Fig. 1. Sequence-specific recruitment of HP1 to gene promoters. Sequence-specific transcription factors such as KLF11 have the ability to bind HP1. In turn, HP1 can recruit the G9a/GLP HMT complex and/or the SUV39H1 HMT to di- or trimethylate, respectively, the lysine 9 residue of histone H3. This methylation permits additional HP1 molecules to bind the promoter, thereby resulting in gene silencing.

that regulate the malignant phenotype. Therefore, these types of studies have provided new insight into the recruitment of the HP1-HMT complexes in a sequence-specific manner rather than through their well-characterized binding to methylated histones. Moreover, HP1 indeed might function as a cofactor for transcription factors responsible for the recruitment of associated HMTs to carry out its tumor-associated functions [24]. Similarly, it has been demonstrated that HMTs play a role in the aberrant silencing of genes encoding tumor suppressor transcription factors [25]. In addition, HMTs can directly act upon other nonhistone proteins through methylation and thereby affect the function of important cancer-associated proteins [25]. For example, the tumor suppressor protein p53 is amenable to a number of posttranslational modifications, one of which being a G9a-specific methylation site at Lys373. Dimethylation at Lys373 is associated with an inactive form of p53, thus disrupting its tumor-suppressive capabilities. G9a and GLP are overexpressed in a number of cancers [25, 26], including pancreatic adenocarcinoma, which are characterized by the altered function of p53, establishing a mechanistic link of significant importance for cancer development. This knowledge is driving efforts to develop small drug inhibitors, which, by targeting the functions of HMTs, may reactivate tumor suppressor genes and reestablish the normal control of cell growth [25]. Thus, collectively, these discoveries are expanding our understanding of the role of HP1-HMT complexes in the suppression of neoplastic cell growth and cancer progression.

Pharmacological Targeting of the HP1-HMT Pathways

The highly reversible nature of cancer-associated epigenetic events, which are mediated by the HP1-HMT complex, makes this pathway a desirable target for rational drug design [27]. In fact, several drugs that target HP1-HMT pathways are currently either in development or being tested in preclinical trials for the treatment of a wide variety of neoplasias. The devel-

opment of these drugs has taken advantage of the knowledge that HMTs transfer methyl groups from the S-adenosylmethionine (SAM) cofactor to histone lysine/arginine residues, generating S-adenosylhomocysteine and a methylated histone. This mechanism of action is analogous to the kinase-catalyzed reaction that generates adenosine diphosphate and a phosphorylated protein [28]. Thus, medicinal chemists have made use of the extensive knowledge generated from developing adenosine triphosphate-competitive kinase inhibitors, either to compete with the SAM cofactor or the H3 peptide substrate [28]. For instance, SAM-competitive inhibitors have been previously developed through random screening to target G9a such as chaetocin and BIX-01338. However, they also inhibit the function of SUV39H1 [29]. On the other hand, substrate-competitive compounds such as BIX-01294 have been identified as selective G9a inhibitors by high-throughput screening, but have subsequently shown toxicity not seemingly related to its HMT inhibitory activity [30]. However, more recently, one structural analogue, UNC0638, was found to have increased potency with reduced cell toxicity [30]. In efforts to continue developing additional and even more selective compounds to target specific epigenetic pathways, the most contemporary SAM-competitive inhibitor BRD4770 was discovered to selectively inhibit G9a and induce senescence in pancreatic adenocarcinoma cells [30]. Furthermore, when combined with the natural product gossypol, BRD4770 displays enhanced cytotoxicity and is suggested to work in a synergistic manner with gossypol to induce autophagy-related cell death in p53-mutant cells [31]. Thus, these novel combinatorial drug treatments may have promise as epigenetic therapies to combat cancer progression.

Concluding Remarks

Research on epigenetics and chromatin dynamics has elucidated the existence of a robust machinery that can modulate and revert cancer-associated processes. Successful investigations in this field are revealing important mechanistic insights into the role of several HP1-HMT pathways in tumor development and progression. The combination of new medicinal chemistry approaches, organic synthesis, and robust testing has provided the proof-of-principle for this pathway being a bonafide target for controlling several aspects of malignant transformation. The excitement brought about by the discoveries in this field is encouraging new investigations with more advanced technology and models. As we see this field developing at an accelerated speed, we anticipate that in a relatively short time, new advances will allow us to manipulate HP1-mediated pathways for treating human cancers with high efficacy.

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