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AUTHOR'S VIEW

Linking H1 with chromatin and growth control

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ABSTRACT

The retinoblastoma protein (pRb) is considered to be one of the key regulators of cell proliferation. Here we describe our recent findings that linker histone H1.2 is an interaction partner for pRb and impacts upon the genome-wide chromatin binding properties of pRb. Consequently, H1.2 influences transcriptional repression and cell cycle control.

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The tumor suppressor retinoblastoma protein (pRb) is widely considered to be a master regulator of the mammalian cell cycle and mutation in the *RB1* gene and the upstream pathways that regulate its activity represent one of the most frequent oncogenic events in human cancer, contributing to cancer initiation and progression.[1] pRb belongs to a protein family termed "pocket proteins," which also includes the proteins p107 and p130. These proteins harbour a common central domain termed the "pocket" which serves as a binding site for several cellular proteins and viral oncoproteins.[1] In the classical model, inhibition of cell proliferation by pRb is principally achieved by binding to and inactivating the E2F family of transcription factors.[1] The E2F family drives the expression of genes required to promote cell proliferation. In addition to orchestrating the transcription of genes required for cell cycle progression, E2F family members are also responsible for activation of genes required for a wide variety of other processes including replication, DNA repair, differentiation, apoptosis, stress response and metabolism.[2] During the cell cycle, pRb has the capacity to arrest cells in G1, by not only inhibiting E2F activity, but also facilitating the recruitment of additional co-repressors to E2F target genes promoters [1]Phosphorylation of pRb, through the sequential action of cyclin/cyclin dependent kinase (Cdk) complexes during the cell cycle, triggers the release of E2F1 from pRb resulting in transcriptional activation of genes that are necessary for cell proliferation.[1] Control of pRb activity is not limited to regulation by cyclin/Cdk, because in excess of 300 proteins have been identified as potential pRb regulatory interactions.[1] Additionally, different post-translational modifications, including acetylation, methylation and sumoylation, have been identified that can fine-tune the functions of pRb.[3,4]

We recently extended our understanding of the mechanisms which underpin pRb growth control through studies on the pRb interactome which identified linker histones as a significant interaction partner for pRb.[5] The linker histone H1 family comprises 7 somatic subtypes in human cells and are generally thought to play a role in gene repression through chromatin compaction.[6] Pursuing the functional significance of the interaction with the H1.2 subtype revealed the presence of pRb and H1.2 as a chromatin-bound complex on the promoters of many E2F target genes. Interestingly, upon depletion of H1.2, a quantitative reduction in the level of chromatin-bound pRb was observed on E2F target genes, which was accompanied by enhanced expression of E2F target genes, thus suggesting that H1.2 is important for pRb-mediated transcriptional inactivation and chromatin binding. Because the network of genes regulated by E2F is extensive and expands to a plethora of genes linked with many different cellular outcomes, we used ChIP-seq to interrogate the global influence of H1.2 on the chromatin binding properties of pRb. In line with our previous observations, there was a decreased association of pRb with E2F targets upon H1.2 depletion. Furthermore, gene ontology analysis revealed that the group of genes most affected by H1.2 loss was linked to cell cycle control. In support of this observation, we were able to show that the pRb/H1.2 chromatin interaction was more prevalent in growth-arrested cells, which is compatible with the biological cell cycle arrest function of pRb which is executed at the G1 to S phase transition of the cell cycle. Further, the physiological consequence of inactivating H1.2 in cancer cell lines was increased proliferation rate, and a distinct growth advantage over wild-type cells. Moreover, cells that lack H1.2 were overall less responsive to pRb-dependent growth control. Collectively, these observations indicate that H1.2 is functionally important in mediating the growth-regulatory effects of pRb (Fig. 1).

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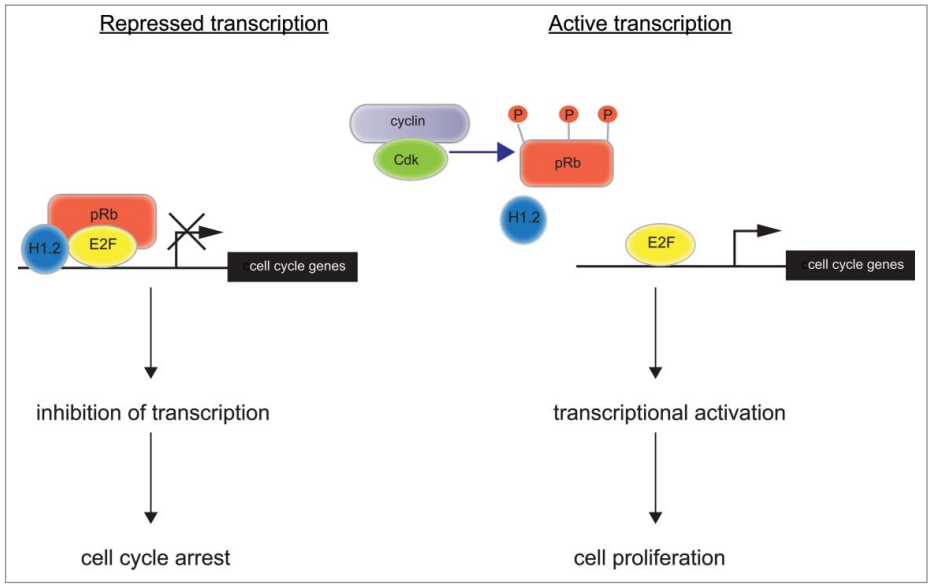

Figure 1. Functional impact of H1.2 on pRb. H1.2 associates with the retinoblastoma protein (pRb) at E2F regulated promoters linked with cell cycle control and augments the ability of pRb to silence transcription, resulting in cell cycle arrest. Under conditions favorable to cell growth, pRb is phosphorylated by cyclin/cyclin dependent kinases (Cdk), resulting in the dissociation of the pRb-H1.2 complex from chromatin and allowing active transcription of cell cycle-associated genes by E2F, leading to cell cycle progression.

It is well established that linker histones mediate general repressive effects on transcription by altering chromatin dynamics.[7] Our recent findings demonstrate that H1 can also be paired with specific binding partners, such as pRb, which preferentially target and regulate key gene networks. In our initial investigations, we identified other H1 subtypes in addition to H1.2 as potential interaction partners for pRb. Whether other H1 subtypes may direct and confer target gene specificity to chromatin bound pRb remains to be explored in further detail, although it is a tempting hypothesis that different H1 subtypes direct pRb to distinct groups of target genes. Further, it will interesting to determine whether fine-tuning of transcriptional repression by linker histones extends to the other members of the pocket protein family, p107 and p130. Another intriguing question to consider is whether the interaction between pRb and H1.2 creates a protein complex that has a structural preference for particular target genes, is there a particular architecture around certain promoters that makes them preferred targets for the pRb/H1.2 complex?

There is increasing evidence that H1 histone genes undergo somatic mutation in malignant disease.[8,9] In diffuse large B cell lymphoma (DLBCL) and follicular lymphoma (FL), somatic mutation in H1 histone genes represents one of the most frequently mutated gene families occurring in FL (over 50% of the samples analyzed).[8,9] Our results raise the possibility that somatic mutation in H1 histone genes could contribute to tumorigenesis and provide a growth advantage by selectively deregulating repression and therefore growth control mediated by pRb.

Understanding the widening network of protein interactors and the post-translational modifications that underpin the complex and diverse biological roles of pRb remains a significant challenge. Our results have unearthed an unexpected interplay between pRb and linker histone H1, which not only illuminates a new and specific role for H1, hitherto believed to be general in its regulatory properties, but deepens our understanding of the mechanisms which influence pRb growth control. Given the increasing evidence that H1 genes are subjected to oncogenic mutation in malignant disease, our results provide a rational explanation and mechanistic understanding for how such mutations could contribute to aberrant growth control.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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