Review

PIM1 -dependent phosphorylation of histone H3 at serine 10 is required of MYC-dependent transcriptional activation and oncogenic

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Chromatin structure plays s a crucial function in eukaryotic gene transcription. Several transcriptional activators has been shown to utilize chromatin-modifying activities, that are mediated either by covalent modifications of histones or by ATP-dependent mobilization of nucleosomes. Covalent modifications of histones including acetylation, methylation, phosphorylation and ubiquitination have been associated with gene regulation [1,2]. Phosphorylation of histone H3 at Ser 10 (H3S10) has been connected with transcriptional activation in different organisms and with chromosome condensation during mitosis [3,4].

This study, analyzes the function of PIM1, a constitutively active serine/threonine kinase that is essential for the cell cycle progression of human umbilical-vein endothelial cells (HUVECs) and for the differentiation of endothelial precursors *in vitro*[5]. PIM1 and c-Myc (MYC hereafter) cooperation in cell growth and transformation has also been shown in transgenic mice[6-9]. However the nature of this cooperation between MYC and PIM1 in cell transformation is still unknown. MYC codes for a basic helix-loop-helix leucine zipper transcription factor that binds to the E box (CACGTG) when dimerized with MAX (MYC-associated factor X) and regulates the transcription of distinct genes involved in cell cycle progression, apoptosis, cell growth, and differentiation[10-16]. The DNA binding domain of MYC is at the carboxy terminus. The MBII (MYC BoxII) domain from the amino terminal region is essential for MYC-dependent cell transformation and has been shown to recruit multiprotein enzymatic complexes to the MYC-activated genes[17].

To understand the function of PIM1, this study investigates the molecular connection between PIM1 and MYC. The authors propose that the recruitment of PIM1 to the chromatin by MYC contributes to the transcription activation of target genes for MYC by phosphorylating H3S10 at the E-box element and it is also suggested that this cooperation is relevant for MYC dependent tumor formation.

This study defines a new role for the serine/threonine kinase PIM1 as a MYC-dependent modifier of chromatin. On the basis of biochemical and functional evidence, the study shows that after stimulation with growth factor, a MYC-MAX-PIM1 complex forms in the nucleus and moves to the chromatin where it phosphorylates histone H3 at Ser 10. This PIM1-dependent nucleosome phosphorylation is required for the transcriptional activation of a subset of MYC-target genes and MYC-dependent cell transformation. According to the current view, phosphorylation at the N-terminal domain of H3 could be required either to loosen the interaction between DNA and nucleosome and/or to generate a platform to recruit additional regulatory factors[1]. PIM1-dependent phosphorylation at the FOSL1 and ID2 genes (MYC target genes) is specific for its recruitment sites and does not spread over the genes, suggesting that this phosphorylation is necessary to activate the enhancer.

FOSL1 shows two regions of different phosphorylation kinetics of H3S10: a phosphorylation site at the upstream SRE (serum response element) that occurs within 15-30 minutes as a result of MSK1/MSK2 (stress activated protein kinase) action, and phosphorylation site at the enhancer that coincides with the peak

transcriptional activation of the gene mediated by PIM1. These two regions behaved differently with regards to the acetylation of H3 at Lys 9 and Lys 14. Phosphorylation and acetylation of FOSL1 promotor H3 are tightly coupled, as shown previously for other IE promoters [3,18-20], phosphorylation of FOSL1 enhancer at H3S10 takes place in a region highly acetylated at H3 before the binding of MYC and PIM1. This is in agreement with the finding that for recognition by MYC, its target sites must be highly acetylated in H3 as well as methylated in H3K4/K79. On the FOSL1 enhancer, the phosphorylation and acetylation of H3 might have distinct functional roles because PIM1 –dependent phosphorylation of H3S10 is transient and is limited to the E-box element whereas acetylation at Lys 9 and Lys 14 is more stable and distributed along the gene, suggesting that phosphorylation at these elements is a critical step in transcriptional activation. The fast turnover of H3 phosphorylation observed in these experiments, and its temporal coincidence with transcriptional activation, might be a general phenomenon because H3 phosphorylation was previously detected during transcriptional activation of heat shock genes in *Drosophila* and during induction of the RAR-β2 gene by retinoic agonists in murine P19 embryonal cells whereas acetylation was constitutive at these loci[21, 22].

It has been estimated that about 11% of cellular genes have a functional E box with which MYC can associate on the genome [23]. A gene expression profile analysis of genes regulated by MYC and PIM1 in cells treated with serum at 120 minutes reveal that 207 transcripts corresponding to 20% of MYC-regulated genes also require PIM1 for their regulation. The co-regulation includes genes involved in cell metabolism, protein synthesis, cycle progression and oncogenesis. A large number of these genes are transcriptional factors, which suggests that PIM1 participates in MYC-dependent regulatory networks. This data is in agreement with data from cell cycle analysis of PIM1 knockdown cells that show a slow entry into S phase. Cell transformation analysis also show a more marked PIM1 effect since PIM1 silencing strongly inhibited the formation of MYC-dependent transformed colonies in soft agar. Data presented in this study strongly suggests that PIM1-dependent phosphorylation of H3 at MYC-target genes is necessary to regulate key genes required for MYC-dependent oncogenic transformation. Fusion of PIM1 to the transformation-defective MYCΔ MBII could rescue its transforming potential as well as H3 phosphorylation at MYC-binding sites, whereas PIM1 coexpression with MYCΔMBII did not lead to rescue.

This study provides a molecular mechanism for MYC and PIM1 cooperation in gene regulation and oncogenic transformation. Because mutation that alter MYC expression are among the most common found in human and animal cancers[24], it is conceivable that inhibiting MYC association with PIM1 and/or inhibiting PIM1 kinase activity by specific drugs might represent a method for the treatment of cancers in humans.

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