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Citrullination Unravels Stem Cells

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Abstract

Maintenance of the pluripotent stem cell state is regulated by the post-translational modification of histones. The discovery that citrullination of the linker histone H1 is critical to this process represents a new role for the protein arginine deiminases (PADs) in development.

The protein arginine deiminases (PADs1,2,3,4,6) convert protein encoded arginines into citrulline through a calcium-dependent reaction coined citrullination or deimination. PAD expression and activity is upregulated in inflammatory diseases and cancer[1,2], and multiple isozymes represent attractive therapeutic targets. Despite their disease relevance, the cellular roles of the PADs remain poorly understood. Their best-characterized role is as histone modifying enzymes that regulate gene transcription. For example, PAD2 and PAD4 citrullinate histones H3 and H4 and these modifications are correlated with either the repression or activation of genes under the control of the estrogen receptor and p53[3–5]. Histone citrullination impacts chromatin structure, as citrullination of histone H3 leads to the expulsion of heterochromatin protein 1α (HP1α) from the chromatin, thereby creating an 'open' state that promotes gene transcription[6]. Additionally, the PAD4 catalyzed citrullination of histones H1 and H3 in neutrophils leads to massive chromatin decondensation and expulsion of DNA to form neutrophil extracellular traps (NETs)[7], a pro-inflammatory form of cell death that is aberrantly increased in numerous inflammatory diseases[2] Adding to the role of the PADs in histone biology, Christophorou et al. report that PAD4 citrullination of histone H1 promotes its dissociation from DNA, thereby creating an open chromatin architecture that is necessary for stem cell pluripotency during early embryogenesis [8].

Pluripotent stem cells are 'master' cells that differentiate into any cell lineage, and can either be isolated as embryonic stem cells (ES cells) or genetically reprogrammed through the reversion of differentiated cells into induced pluripotent cells (iPS cells). Reprogramming of iPS cells is initiated by upregulating pluripotency genes, and key to initiating this process is the generation of an open chromatin structure around these genes. This process involves modifications of the proteins that constitute the core histone octamer as well as Histone H1, which directly binds to nucleosome bound DNA and maintains a properly compacted state (Figure 1).

Given the ability of the PADs to modulate the chromatin architecture in neutrophils, Christophorou et al. questioned whether PAD4 played a role in ES and iPS cells. Initial experiments performed with mouse ES cells (ES Oct4-GIP) and committed neural stem-cells (NSO4G) showed that PAD4 was only expressed in ES cells. Upon reprogramming into iPS

cells, NSO4G cells express PAD4, and remarkably, this expression highly correlates with the levels of Nanog, an essential stem-cell transcription factor, as well as a subset of other known pluripotency genes, including Klf2, Tcl1, Tcfap2c, and Kit. Nanog appears to induce PAD4 activity because in its absence the levels of citrullinated H3 are reduced. The expression of pluripotency genes was also found to be dependent on PAD4 enzymatic activity, as inhibition with the pan-PAD inhibitor Cl-amidine[9] and the PAD4-selective inhibitor TDFA[10] reduced citrullinated H3 (H3cit), which in turn reduced the expression levels of the pluripotency genes Nanog, Tcl1, and Klf5. Inhibition of PAD4 activity also led to increased expression of differentiation genes including Prickle1, Epha1 and Wnt8a, and stem cells treated with TDFA reduced the number of pluripotent cells in early embryogenesis. These results were validated by RNAi knockdown of PAD4.

To further investigate the role of PAD4 in pluripotency, Christophorou et al. identified several citrullinated proteins including AtrX, Dnmt3b, Trim28, and histone H1, all of which help control the pluripotent state. Importantly, histones H1.2, H1.3, H1.4, and H1.5 were citrullinated in the central winged helix DNA binding domain at Arg54 (H1R54Cit) and mutation of this residue (R54A) results in the release of H1 from chromatin. Inhibition of PAD4 expression or activity also decreases histone citrullination and favored a compacted chromatin state, which correlated with the down-regulation of pluripotency genes, and the up-regulation of differentiation genes. Interestingly, there are parallels to NET formation where chromatin decondensation is driven by the PAD4-mediated citrullination of both histone H1 and H3, and the site of H1 citrullination is the same as that observed in pluripotent stem cells[7].

In summary, this work (Figure 1) adds to our growing understanding of PAD biology in a variety of (patho)physiological processes, and furthermore reiterates the value in developing selective and potent chemical probes to pharmacologically modulate enzymes such as the PADs in cellular systems. These studies also raise a number of important issues that will undoubtedly fuel future studies. First, although the authors focused on PAD4, they also observed increased expression of PADs 1, 2, and 3 in ES and/or iPS cells, which could suggest that multiple PADs play a role in pluripotency or could function at different stages during cellular differentiation. Second, PADs 1-4 are overexpressed in multiple cancers, and it seems likely that their activity may ‘reprogram’ cancer cells into a more stem cell-like state and thereby promote their unrestrained growth. Third, as interest in the PADs as therapeutic targets for cancer and inflammatory diseases continues to increase, some caution is warranted because drugs targeting these enzymes may impact stem cell fate and embryogenesis. Nevertheless, the future of PAD biology is bright, and represents a promising area of growth in our understanding of the delicate balance between health and disease.

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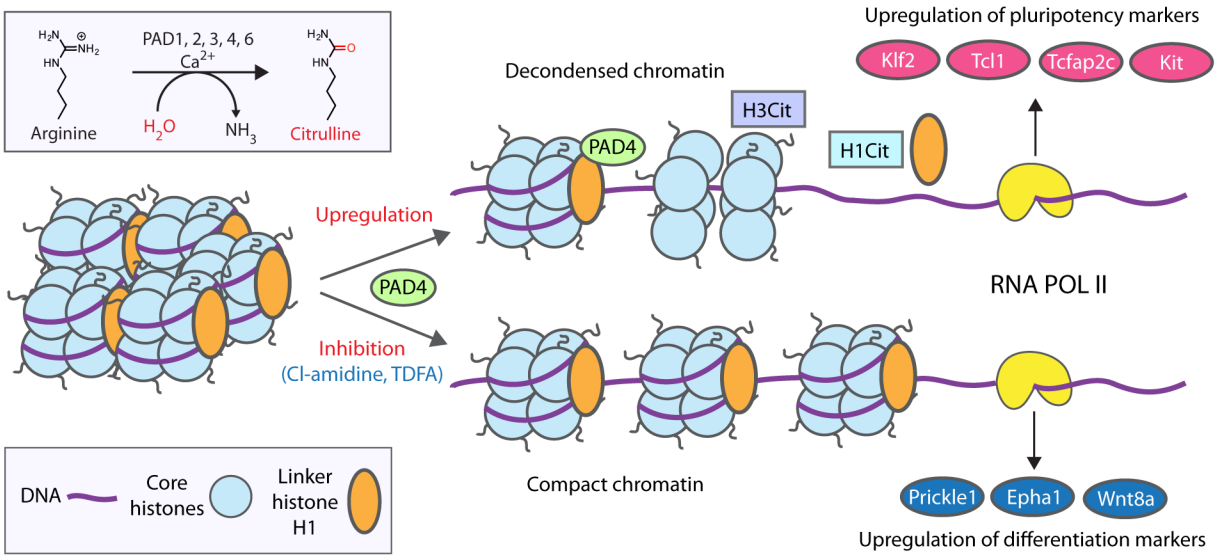

Figure 1.

PAD4 is a regulator of pluripotency gene expression through the conversion of arginine to citrulline in histones. Protein arginine deiminase 4 (PAD4) citrullinates core (H3, H4) and linker (H1) histones, leading to chromatin decondensation and the expression of pluripotency markers in embryonic stem-cells, which can be reversed upon inhibition of PAD activity.