Reprogramming

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H3K36 methylation is a reprogramming barrier

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Reprogramming of somatic cells is an inherently inefficient process. A new study has now identified histone H3K36 methylation as a crucial reprogramming barrier that operates downstream of TGFβ signalling. Global inhibition of H3K36 methylation induced PRC2-dependent silencing of mesenchymal genes and dramatically increased reprogramming efficiency.

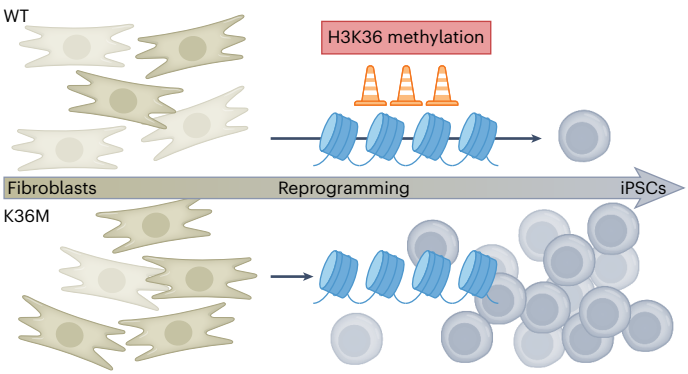

Fig. 1 | H3K36 methylation is a reprogramming barrier. Fibroblasts that express a wild-type (WT) copy of H3.3 reprogram slowly and inefficiently into iPSCs (top). Fibroblasts that express mutant H3.3K36M show global inhibition of H3K36 methylation, as well as dramatically improved and accelerated reprogramming (bottom).

Pluripotency, stem-cell differentiation and early development are largely governed by an intricate web of chromatin-related mechanisms[1]. Composed of DNA, histone proteins and other associated factors, chromatin is the fundamental unit of cellular life. As an elementary unit of chromatin, each of the four core histones, H2A, H2B, H3 and H4, is encoded by multiple genes, which provides backup redundancy, robustness and the necessary mass required during replication. It was therefore very unexpected when large-scale sequencing efforts of multiple cancer types began to reveal an increasing number of mutations in histone genes, dubbed 'oncohistones'[2]. Although in many cases these mutations were found in the replication-independent histone gene variants, similar mutations were also observed in genes encoding core histones.

A notable example is a lysine-to-methionine substitution (K36M) in the histone variant H3.3 (or H3 itself), found in a striking 95% of chondroblastoma cases, as well as in other types of cancer[2]. K36M causes a vast and global reduction in H3K36 methylation (Fig. 1) by inhibiting the H3K36 methyltransferases[3], and it has been shown to impair the differentiation of mesenchymal progenitor cells, inducing the formation of undifferentiated sarcomas[3]. Mesenchymal cells are plastic and migratory, and a major event in cancer progression is the process of epithelial-to-mesenchymal transition (EMT)[4]. One of the key upstream regulators of EMT is the secreted cytokine transforming growth factor-β (TGFβ), which, after binding to its receptor, induces the expression of mesenchymal genes, including the gene encoding the transcription factor Zeb1[5]. In addition to being involved in cancer, EMT has also been shown to play a part during the early stages of the reprogramming of induced pluripotent stem cells (iPSCs). Although the main event during the reprogramming of iPSCs is the reverse pathway of mesenchymal-to-epithelial transition (MET), early activation of EMT immediately before MET increases reprogramming efficiency by over fivefold[6]. Nonetheless, blocking MET via TGFβ expression significantly decreases iPSC reprogramming efficiency[7], which highlights a role for a sequential EMT–MET mechanism during reprogramming. In this issue of *Nature Cell Biology*, Hoetker et al. reveal a striking effect of H3K36M on reprogramming through the promotion of MET[8].

Reprogramming by Oct4, Sox2, Klf4 and cMyc is a relatively slow (with the first colonies appearing after about 1 week) and inefficient process, with most of the cells failing to complete its course. By separately inducing the expression of four different H3.3 lysine-to-methionine substitutions (K4M, K9M, K27M and K36M) during reprogramming, Hoetker et al. showed that ectopic expression of H3.3K36M resulted in a dramatic effect, increasing reprogramming efficiency by up to 100-fold, with colonies appearing significantly earlier[8].

Aiming to identify potential reprogramming barriers resolved by K36M, the authors analysed gene-expression changes during reprogramming, comparing K36M-expressing cells with control cells expressing wild-type H3.3. The authors observed that although expression of mesenchymal genes, including the aforementioned *Zeb1*, remained relatively stable in cells that eventually failed to reprogram, their expression was significantly lower in the K36M cells at day 4 of reprogramming than in control cells, which would suggest Zeb1 as a potential reprogramming barrier. In support of this, inducing *Zeb1* expression in the K36M cells delayed reprogramming. The authors next investigated whether TGFβ signalling, the inducer of mesenchymal gene expression, acts upstream or downstream of K36M. They found that although inhibition of TGFβ enhanced reprogramming in control cells, it had no effect on K36M cells, which places K36M downstream of TGFβ signalling.

How does K36M, or inhibition of K36 methylation, lead to suppression of mesenchymal genes? A likely candidate would be polycomb repressive complex 2 (PRC2), which methylates H3K27, suppressing gene expression. As H3K36 methylation has been shown to limit the spread of H3K27 methylation[9], inhibition of K36 should lead to increased H3K27 methylation. To test this idea, the authors inhibited PRC2 in K36M and control cells and assessed the consequences. They found that inhibition of PRC2 reduced reprogramming efficiency in both control cells and K36M cells, and enhanced the expression of mesenchymal genes, including *Zeb1*, in the K36M cells. Together, these results suggest that H3K36 methylation serves a role in the cellular

response to TGFβ signalling by opposing PRC2-mediated silencing and regulating mesenchymal gene expression.

The authors next turned their attention to DNA methylation, which acts to suppress gene expression, as H3K36 methylation was previously shown to recruit DNA methyltransferases[10]. In the K36M cells, Hoetker et al. observed overactivation of ten-eleven translocation enzymes[8], which are responsible for DNA demethylation. In contrast to the observed PRC2-mediated silencing of mesenchymal genes in the K36M cells, epithelial and pluripotency genes, including Sox2 targets, lost H3K27 methylation, gained active histone marks and displayed increased gene activity. Remarkably, in the K36M cells, Sox2 switched from promiscuous binding to specific binding to pluripotency and epithelial target genes, promoting their expression. How exactly H3K36 methylation mediates this dual function of activation of epithelial and pluripotency genes on the one hand and silencing of mesenchymal genes on the other remains to be further explored.

Beyond their important insights into reprogramming, the authors' experiments support a model of impaired differentiation in H3K36M-related cancers. EMT occurs naturally during gastrulation, enabling the migration of epithelial cells during development[11]. Hoetker et al. report that gastruloids derived from embryonic stem cells expressing K36M failed to transition from a spherical structure to a tube-like structure[8], which suggests that K36M limits EMT. This further supports the proposal of a requirement for H3K36 methylation during the exit from pluripotency. Interestingly, inspecting K36M action in various differentiation trajectories, the authors reported suppressed differentiation into myotubes, but enhanced differentiation towards neurons. This is in contrast to H3K27M, which has been shown to support stemness and impair neuronal differentiation in embryonic stem cells[12]. As H3K36 methylation antagonizes H3K27 methylation, this suggests an opposing interaction between H3K27 methylation and H3K36 methylation on the establishment of cell fate.

Although iPSC reprogramming is not a natural phenomenon, there are important implications in the current study for understanding of the potential actions of K36M substitutions in cancer. If inhibition of H3K36 methylation leads to such a strong tendency of fibroblasts to differentiate into iPSCs, it is no surprise that in vivo, this mutation drives tumorigenesis, as many other cell types may react similarly. Moreover, the identification of the involvement of TGFβ signalling operating upstream of K36M, and of PRC2 and DNA methylation operating downstream of K36M, provides potential therapeutic opportunities for K36M-related cancers in the form of targeted inhibition of PRC2 with or without combined DNA re-methylation, although tinkering with EMT may prove dangerous. These directions will be the subject of future research.

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Competing interests

The authors declare no competing interests.