H3K4 Di-Methylation Governs Smooth Muscle Lineage Identity and Promotes Vascular Homeostasis by Restraining Plasticity

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Introduction: Dynamic and reversible phenotypic modulation of vascular smooth muscle cells (SMC) between the contractile state and the dedifferentiated state plays a central role in maintaining vascular homeostasis. Yet mechanisms of retaining lineage identity and allowing for reacquisition of the quiescent contractile state during reversible dedifferentiation remain unknown. H3K4 di-methylation (H3K4me2) on SMC contractile genes is a lineage-specific epigenetic signature stably retained in both contractile and dedifferentiated SMC, suggesting it could serve as a lineage memory mechanism. Here we aimed to determine the functional relevance of the stable H3K4me2 signature in mature SMC. **Methods:** We employed the loss-of-function strategy by designing a locus-specific H3K4me2 editing system to selectively demethylate H3K4me2 on SMC contractile gene subset *in vitro* and *in vivo*. We then characterized the biological consequences in H3K4me2-edited SMC by performing a combination of epigenome profiling, transcriptome profiling, functional assays *in vitro*, and SMC fate mapping *in vivo*. **Results:** We discovered that selective removal of H3K4me2 from the SMC contractile genes led to a marked loss of contractility and alteration of SMC-mediated vascular remodeling capacities upon injury due to loss of miR-145 expression. We found that H3K4me2 editing was associated with increased DNA methylation levels caused by impaired recruitment of DNA demethylase TET2 on SMC contractile genes. Mechanistically, we revealed TET2 directly and preferentially interacted with H3K4me2, indicating H3K4me2 served as a lineage-specific footprint for the dynamic TET2 recruitment in SMC. Finally, H3K4me2 editing induced a profound alteration of SMC lineage identity by redistributing H3K4me2 towards genes associated with stemness and developmental programs, thus exacerbating plasticity as characterized by the greater ability of H3K4me2-edited SMC to transdifferentiate into other lineages upon stimulation. **Conclusions:** We identified H3K4me2-TET2 as a central epigenetic mechanism controlling SMC lineage identity and specialized functions, whose alteration could contribute to various pathophysiological processes.