UnTEThering (Smooth Muscle) Cell Plasticity

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Cellular plasticity has become the subject of intense research, with perhaps the greatest example provided by the recent Nobel Prize winners Drs Yamanaka and Gurdon and the demonstration that terminally differentiated fibroblasts can be coaxed into assuming an embryonic stem cell-like fate. Emerging evidence suggests that plasticity inherent in cells may be hijacked in the progression of disease and cancer. However, the mechanisms by which cells acquire and regulate this plasticity remain incompletely understood. It is appreciated that broad programs must be unleashed during these switches, and, therefore, control at the epigenetic level in regulating these processes has garnered significant interest.

In this issue of Circulation, Liu et al1 provide evidence implicating the epigenetic factor termed ten-eleven translocation-2 (TET2) in control of vascular smooth muscle cell (VSMC) plasticity and development of vascular disease.

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Under homeostatic conditions, the principal function of the VSMC is regulation of vascular tone through the expression of unique contractile proteins, agonist receptors, and ion channels.2 The differentiated contractile phenotype is characterized by the expression of cytoskeletal marker proteins, including smooth muscle actin (ACTA2 [for actin, α2, smooth muscle, aorta]) and smooth muscle-myosin heavy chain (MYH11 [for myosin, heavy chain 11, smooth muscle]). However, unlike other terminally differentiated muscle cell types (skeletal or cardiac), VSMCs exhibit a high degree of phenotype plasticity. In response to injury or damage, VSMCs assume a dedifferentiated synthetic phenotype characterized by a high proliferative index, loss of contractile properties and proteins, and production of extracellular matrix products. This phenotype plasticity, first described by Chamley-Campbell et al3>3 decades ago, is observed both in vitro and in vivo in response to various environmental cues.2 Although this characteristic response is likely adaptive, an exaggerated response can contribute to the development/progression of vascular disease states, such as stent restenosis or atherosclerosis.4,5 Thus, VSMCs are an excellent cell type to study mechanisms underlying plasticity of cells and how this intersects with disease progression.

Previous studies have linked various growth factors, signaling pathways, and transcription factors to the control of VSMC plasticity in health and disease. In particular, a large body of work has focused on deoxyribonucleic acid (DNA)-binding transcription factors and their coregulators. For example, the transcription factor serum response factor (SRF) controls differentiation of VSMCs through the regulation of contractile marker proteins (eg, ACTA2, MYH11, transgelin, and calponin) that contain multiple SRF binding sites termed CArG [CC(AT)6GG] motifs in their regulatory regions. SRF transcriptional activity is modulated by SRF expression itself, posttranslational modification, nuclear localization, and transcriptional coactivators, including Myocardin.6,7 Transcriptional regulation of these markers of differentiation is correlated to the phenotypic state of the VSMC—high expression of differentiation proteins correlating to a contractile phenotype—and proliferating VSMCs are characterized by reduced levels of these differentiation proteins.8 In the current issue of Circulation, Liu et al1 advance our understanding and demonstrate that reversible VSMC differentiation is orchestrated through a mechanism involving the epigenetic DNA-modifying enzyme TET2.

The TET proteins (TET1–TET3) are DNA demethylases that oxidize 5-methylcytosine and generate 5-hydroxymethylcytosine (5-hmC).9 TET proteins have been implicated in maintaining embryonic stem cell pluripotency, whereas mutations are associated with hematopoietic disorders. However, a role for the TET family of proteins in regulating adult somatic cells, in particular VSMCs, has not been studied extensively. Bearing this in mind, Liu et al1 first established that TET2 was the most highly expressed TET isofrom in both human coronary artery VSMCs and rapamycin-induced differentiated VSMCs. Notably, TET2 expression directly correlated with VSMC differentiation markers, including MYH11 and ACTA2. Furthermore, Tet2 and 5-hmC levels were reduced after VSMC dedifferentiation in experimental models, findings recapitulated in biopsies from human atherosclerotic arteries. The investigators extended these observations and showed that TET2 is both necessary and sufficient to drive smooth muscle synthetic and differentiated phenotypes. In vitro TET2 knockdown resulted in attenuation of both VSMC differentiation gene expression and increased proliferation and synthetic phenotype marker gene expression in human coronary artery cultures. In contrast, in vitro TET2 overexpression enhanced key aspects of the VSMC contractile phenotype in the absence of any differentiation stimuli and, furthermore, resulted in the activation of a VSMC differentiation program in fibroblast cell lines.
Importantly, the investigators demonstrated that altering TET2 in an existing lesion may have important therapeutic consequences. Using a murine model of femoral wire injury, localized gene delivery of TET2 knockdown virus potentiated both intimal hyperplasia and neointimal area. Alternatively, delivery of TET2 overexpression virus at 3-weeks after injury greatly reduced neointimal hyperplasia. These results were correlated with the predicted alterations in the expression pattern of differentiation marker genes, as well as 5-hmC levels.

Mechanistically, their data suggest that TET2 alters the epigenetic landscape of VSMCs and regulates plasticity through a mechanism involving interaction with master regulators of VSMC differentiation. Specifically, TET2 occupied regulatory regions of SRF, Myocardin, and MYH11. Moreover, using innovative quantitative techniques to detect locus-specific 5-hmC expression, Liu et al. demonstrate hypermethylation of SRF, Myocardin, and MYH11 promoters with an accompanied decrease in 5-hmC levels after TET2 knockdown; similar results were observed with TET2 overexpression. Together, the work described by Liu et al suggests a model in which TET2 modulates the phenotype of smooth muscle cells by differentially demethylating DNA at critical loci (Figure).

DNA methylation typically occurs at the C5 position of cytosine (5-methylcytosine) in CpG dinucleotides and historically has been considered a stable epigenetic modification. However, the current work adds to a growing body of evidence that DNA methylation is a critical mechanism for dynamic regulation of gene expression (activation and repression), cellular phenotype, and function. DNA methylation is facilitated by DNA methyltransferases 3a and 3b, whereas DNA methyltransferase 1 has been found to reinforce existing methylation patterns. However, it was discovered recently that the TET family of proteins promotes DNA demethylation through the conversion of 5-methylcytosine to 5-hmC, as well as 5-formylcytosine and 5-carboxylcytosine. TET family proteins have been studied extensively during the early stages of embryonic and germ cell development, as well as during reprogramming of somatic cells into induced pluripotent stem cells. These studies demonstrate a fundamental role of TET proteins in driving successive waves of demethylation across the genome, critical in regulating broad programs of lineage commitment and differentiation. Postnatally, Tet2-null mouse models recapitulate various forms of myeloid neoplasms, and mutations in TET2 have been found in patients with myeloid cancers. Liu et al. add to this literature and advance our understanding by providing evidence of how these factors may regulate plasticity of a somatic cell type and how it relates to disease progression.

In summary, the current work provides evidence that TET2 is instrumental in balancing the contractile/differentiated and synthetic/dedifferentiated smooth muscle phenotype, two phenotypes with distinct molecular states. These provocative findings raise a number of interesting questions for vascular biology and, more broadly, the field of epigenetic control. First, the authors have focused on an aspect of SMC plasticity, namely expression of characteristic proteins. Whether TET2 also regulates other aspects, such as VSMC proliferation, migration, and extracellular matrix production, are important unknowns. Such efforts will be enhanced with the composition of a genomic DNA methylation map in wild-type and Tet2-null VSMCs in an effort to identify novel regulators of smooth muscle biology and plasticity. Second, does TET2 affect the development of other vasculopathies involving synthetic VSMCs, such as aneurysms, stent restenosis, or atherosclerosis? Third, what are the cellular cues that instruct TET2 to affect a subset of cellular genes targets, and, in turn, how does methylation at certain regions of DNA influence assembly of specific transcriptional complexes? Finally, can the epigenetic landscape of VSMCs, perhaps through a TET2–5-hmC axis, be exploited for therapeutic gain?

Disclosures

None.

References


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