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A protective smooth muscle cell transition in atherosclerosis

Huize Pan¹, Muredach P. Reilly^{1,2,*}

¹Division of Cardiology, Department of Medicine, Columbia University Irving Medical Center, New York, NY, USA

²Irving Institute for Clinical and Translational Research, Columbia University, New York, NY, USA.

Abstract

TCF21, a gene associated with coronary heart disease, promotes plaque stability and reduces clinical events by enhancing smooth muscle cell phenotype modulation into “fibromyocytes” in atherosclerosis.

Atherosclerotic coronary heart disease (CHD) is a complex process driven by cholesterol accumulation coupled to inflammatory and fibrotic responses in the artery wall, and it remains the leading cause of death worldwide. These lesions, typically called atherosclerotic plaques, form in the intimal space of mid- to large-sized arteries, between the luminal endothelial cell layer and the medial (middle) layer of contractile artery smooth muscle cells (SMCs). Plaques typically start early in life and expand slowly over decades through uptake of circulating lipoproteins, recruitment of inflammatory cells from blood, migration of de-differentiated medial SMCs into lesions to form a protective fibrous cap over the lesion, and secretion of matrix from SMCs. Such plaques can become unstable if they are inflammatory and develop thin fibrous caps, making them prone to rupture acutely into the circulating blood, leading to arterial thrombosis and acute CHD events such as myocardial infarction (“heart attack”). Recent genome-wide association studies (GWAS) have rekindled interest in the causal roles and therapeutic potential of arterial SMCs in atherosclerosis and CHD events¹. Yet it remains unclear whether SMC phenotype transitions in atherosclerosis are protective or increase the risk of CHD.

The current paradigm is that in response to atherogenic risk factors and early lesion formation, contractile SMCs in the medial layer of the artery can de-differentiate, migrate into lesions and transdifferentiate into distinct lineages that may be harmful or protective. On one extreme, SMC transition to macrophage-like inflammatory cells is proposed to promote plaque rupture and acute CHD events, whereas at the other extreme, SMC modulation to matrix-secreting fibroblast-like cells may strengthen the protective fibrous cap and reduce CHD events². Now, Wirka et al. report that *TCF21* promotes SMC transition to fibroblast-like cells in atherosclerotic lesions, enhancing the formation of protective fibrous caps³. Two partially overlapping conclusions are drawn: first, that *TCF21* is specifically

* mpr2144@cumc.columbia.edu.

Competing interests

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atheroprotective, and second, that the process of SMC phenotypic transition in atherosclerosis more broadly is dependably atheroprotective.

TCF21, a transcription factor belonging to the basic helix-loop-helix family, is required for cardiac fibroblast development but is downregulated in cells that develop into coronary artery SMCs⁴. Reactivation of *TCF21* expression by atherogenic stressors in adult coronary SMCs induces transition of SMCs towards fibroblast-like cells. The authors of this current work previously discovered, through a large-scale collaborative GWAS, a CHD locus near *TCF21*⁵ and have focused on *TCF21* as the likely causal gene in the locus. Here, they extend their work in vivo by genetically labeling SMCs with tdTomato, a type of red fluorescent protein, in mice, which results in a specific and permanent expression of the fluorescence protein in SMCs and its progenitor cells and facilitates tracking of SMCs during atherogenesis. By coupling this with single-cell RNA sequencing (scRNA-seq) of mouse atherosclerotic lesions, they identify one major SMC-derived population resembling fibroblasts, which they term “fibromyocytes.” Deletion of *Tcf21* in the hyperlipidemic apolipoprotein E knockout (*ApoE*^{-/-}) atherosclerosis-prone mouse model markedly reduced SMC transition to fibromyocytes, resulting in thinner fibrous caps. These data suggest that *Tcf21* serves an atheroprotective role by modulating SMC transition to fibromyocytes and by enhancing the formation of protective fibrous caps on atherosclerotic lesions (Fig. 1).

To confirm their findings in humans, the authors performed scRNA-seq of human atherosclerotic lesions and integrated analysis of the mouse and human data to confirm the presence of *TCF21*-expressing fibromyocytes in human lesions. Importantly, by integrating large CHD GWAS datasets with RNA expression profiling of both human coronary artery SMCs and intact coronary arteries, the authors found that increased CHD events are observed in carriers of genetic variants (SNPs) associated with lower *TCF21* expression in SMCs in culture and in coronary arteries ex vivo. This is the first study to show that a GWAS-identified CHD-related gene can fundamentally alter the process of SMC phenotypic modulation in vivo and to establish that *TCF21* and its regulation of SMC phenotype in atherosclerosis are causally protective for CHD.

What of the authors’ broader inference that SMC phenotype modulation is atheroprotective? If true, this concept could accelerate the development of therapeutics because it would alleviate previous concerns that targeting SMCs might have both pro- and anti-atherogenic effects. This broader conclusion is based on the authors’ single-cell profiling, which suggests that SMCs transition almost exclusively to fibromyocytes, but not at all to macrophage-like cells, during 16 weeks of disease progression in the *ApoE*^{-/-} mouse model. Indeed, Wirka et al. find that trajectories of gene expression in SMC-derived cells move further away from lesion macrophages during diseases progression. This is an unexpected result, as multiple recent studies have reported identifying SMC-derived macrophage-like cells using SMC fate-mapping strategies, flow cytometry and immunohistochemistry⁶⁻⁸. In those studies, SMC-derived macrophages have been reported to constitute a sizeable proportion of all SMC-derived lesion cells in both advanced mouse and human atherosclerosis. Such inference, however, was based largely on single cell-surface protein markers. The current analyses combining scRNA-seq with simultaneous profiling of

multiple cell surface markers reveal that previously proposed cell-specific protein markers (e.g., Lgals3) are expressed on multiple cell types in mouse and human disease.

However, the concept that SMC phenotype modulation is dependably atheroprotective needs further investigation. First, it should be noted that Wirka et al. use only one atherosclerosis mouse model (*ApoE*^{-/-}) and one type of genetic SMCs tracer (tdTomato), and that the duration of the atherogenic diet (16 weeks) was less than ideal for modeling advanced human-like lesions prone to rupture⁹. Second, lesion digestion protocols for the preparation of single cells may introduce bias, with selective loss of more fragile populations including macrophages¹⁰. Furthermore, other emerging studies of fate mapping and single-cell profiling of mouse atherosclerosis lesions suggest that lesions do indeed contain SMC-derived macrophage-like cells¹¹. In the future, analysis of cell-type-specific epigenetic marks¹² combined with single-cell profiling should also reveal whether SMC-derived macrophage-like cells exist in human lesions. Importantly, the effect of other major regulators of SMC fate will reveal whether they control more diverse SMC-derived cell states, relative to the role of *Tcf21* in specifying fibroblast differentiation.

Finally, recent fate-mapping studies using a mouse model known as the Confetti mouse suggest that SMC phenotype transition in mouse atherosclerosis is monoclonal or oligoclonal in lesions⁸: i.e., a multitude of lesion SMC-derived cells in a given atherosclerotic plaque region express the same fluorescent protein and have arisen from one medial progenitor SMC. In this context, it remains to be established whether each SMC clone behaves similarly or differs in its progeny depending on genetic and epigenetic characteristics or environmental cues for each clone. Whether fibromyocytes in the current study have arisen from single or multiple clones and how *Tcf21* affects lesion clonality remain to be determined. More broadly, it remains to be seen what the precise identity and function of fibromyocytes are or whether they encompass a continuum of cell states in transition to various terminal phenotypes and lesion distributions.

This work demonstrates that *TCF21* alters SMC phenotypic modulation in vivo and builds on prior studies demonstrating that *TCF21* and its modulation of SMC phenotype to fibroblast-like cells protect against CHD. The combined application of advanced genomic technologies in mouse and humans, as elegantly illustrated by Wirka et al., provides a roadmap to reveal the complexity, diversity and causal roles of specific cell populations and their master regulators in atherosclerotic CHD. Such data will transform our understanding as a step toward novel, mechanism-based translational advances in clinical practice.

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Fig. 1 |. The role of *TCF21* in atherosclerosis.

The SMC transdifferentiation model to “fibromyocytes” proposed by Wirka et al. SMCs mainly transdifferentiate into “fibromyocytes,” a fibroblast-like cell type. Deficiency of *TCF21* attenuates the SMC phenotypic switch to fibromyocytes and may result in thin fibrous caps, less stable atherosclerotic lesions and higher rates of plaque rupture and clinical CHD complications.