



Krüppel-like factor 4-mediated smooth muscle cell phenotype switching to a galectin-3 positive subclass is a detrimental event in the pathogenesis of atherosclerotic cardiovascular disease

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Cardiovascular diseases such as coronary artery disease, myocardial infarction and stroke are considered the major cause of death worldwide. Atherosclerosis, narrowing of the arterial lumen due to accumulation of cholesterol within the vessel wall, represents the primary underlying pathology of cardiovascular diseases (1). Cholesterol lowering treatments are currently the first choice therapy for human subjects at risk of developing cardiovascular disease given that hypercholesterolemia is an established risk factor for the development lipid-laden macrophage foam cells, a decisive step in the formation of atherosclerotic lesions. Unfortunately, statin-induced lipid lowering is only able to achieve a 15–25% risk reduction in cardiovascular disease event rate (2,3). As such, there remains a clear need to uncover additional means to battle the occurrence of atherosclerotic cardiovascular disease.

Although cholesterol accumulation in macrophages is a hallmark of atherosclerosis, many other cell types have also been suggested to play a role in the initial development and progression towards more advanced atherosclerotic lesions. These include cells from the innate and adaptive immune system, i.e., T and B cells, neutrophils, and mast cells as well as smooth muscle cells (1). However, the relative contribution of each individual cell type to the atherosclerosis pathology and overall cardiovascular disease outcome is not well understood.

In the paper by Alencar *et al.* published in the journal *Circulation* in July 2020 (4), the authors have tried to

provide novel insight in the specific role of smooth muscle cells in atherosclerosis. In detail, through combining novel and earlier established gene editing approaches in mice with state-of-the-art single cell RNA sequencing and plaque visualization methods, the authors have tested the hypothesis that smooth muscle cells can obtain different phenotypes within the atherosclerotic lesion context and that the different smooth muscle cell subtypes also differentially impact on the pathology.

Using unbiased RNA sequencing it was found by Alencar *et al.* that 14 different cell clusters are present in advanced atherosclerotic lesions from both mice and humans and that the smooth muscle cell clusters overlap significantly between the two species with regard to their functional classification (4). Recently published RNA sequencing studies by Depuydt *et al.* have similarly identified 14 distinct cell populations in human carotid atherosclerotic plaques. They also observed that—besides smooth muscle cells—human atherosclerotic lesions indeed contain endothelial cells, mast cells, B cells, myeloid cells (monocytes/macrophages), and T cells (5).

An interesting aspect of the studies by Alencar *et al.* was that they could trace back the cellular origin of individual cell clusters through the use of lineage-specific fluorescent reporters. More specifically, it was revealed that 7 out of the total 14 cell populations in murine atherosclerotic plaques are actually derived from smooth muscle cells (4). Only 3 of these 7 specific cell clusters—however—displayed a relative

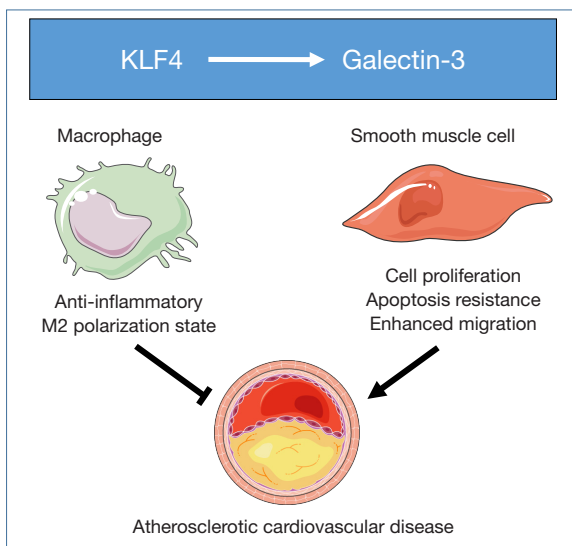


Figure 1 Krüppel-like factor 4 (KLF4) mediated upregulation of galectin-3 expression executes a cell type-dependent impact on atherosclerotic cardiovascular disease outcome. KLF4 activity in macrophages stimulates polarization to the anti-inflammatory M2 subtype, whilst a shift towards a pro-atherogenic migrating and proliferating smooth muscle cell phenotype is induced upon activation of the KLF4-galectin-3 axis.

high expression level of the “traditional” smooth muscle cell lineage gene markers alpha-smooth muscle actin/actin alpha 2 (ACTA2), myosin heavy chain 11 (MYH11), or calponin 1(CNN1). It can thus be suggested that lesional smooth muscle cells exhibit a high degree of plasticity, i.e. many smooth muscle cells located within atherosclerotic plaques have lost the expression of these specific markers as this particular smooth muscle cell population shifted its function towards that of other types of cells. In line with this assumption, Alencar *et al.* detected relatively high expression levels of traditional gene markers for endothelial cells, macrophages, and T cells in certain cell populations that could be traced back to a smooth muscle cell (MYH11-expressing lineage) origin (4). In addition, a significant extent of endothelial to mesenchymal transition seems to occur within plaques as evident from the finding that several endothelial lineage-derived cell populations exhibited relatively high levels of the (vascular) smooth muscle cell markers ACTA2 or vimentin. Notably, these findings corroborate the earlier observation by Allahverdian *et al.* that ~40% of all CD68-positive macrophages within human coronary artery lesions are derived from smooth muscle

cells (6).

The transcription factor Krüppel-like factor 4 (KLF4) has a pro-atherogenic function within smooth muscle cells, since smooth muscle cell-specific gene deletion of KLF4 in mice is associated with a marked reduction in lesion size, and increases in multiple indices of plaque stability (7). The most important insight from the combined imaging, cell isolation and RNA sequencing studies by Alencar *et al.* was that they also provided novel clues to a possible mechanism driving this pro-atherogenic contribution of KLF4 in smooth muscle cells. More specifically, it was shown that KLF4 expression in smooth muscle cell induces a phenotype shift towards a galectin-3-positive state (4). Galectin-3, encoded by the LGALS3 gene, is a prominent member of the beta-galactoside-binding lectin protein family and known to play a role in many cellular functions including cell-cell adhesion, cell-matrix interactions, transcription regulation, and the control of the cell cycle (8). Recent clinical association studies by Aguilar *et al.* have shown that high plasma levels of galectin-3 are a predictive marker for the risk of the development of cardiovascular events (9). In addition, higher levels of galectin-3 in serum of a cohort of patients with acute ischemic stroke were independently associated with increased risk of death or major disability after the stroke onset (10). Furthermore, Lisowska *et al.* have shown that concentrations of galectin-3 are significantly elevated in patients suffering from coronary artery disease than those in non-diseased controls and that people with the highest levels of galectin-3 displayed a significantly increased risk of dying after suffering from a myocardial infarction (11). Galectin-3 is a direct transcriptional target of KLF4 (12). It can therefore be proposed that (pharmacologically) disrupting the interaction of KLF4 with the galectin-3 promoter to inhibit galectin-3 function could be of therapeutic interest to reduce the risk of developing atherosclerotic cardiovascular disease and the associated event mortality. However, it should be acknowledged that blockade of KLF4/galectin-3 function would have to occur in a smooth muscle cell-specific manner, since in macrophages, as opposed to smooth muscle cells, the KLF4-galectin-3 axis rather executes an anti-atherogenic effect (see Figure 1). A selective deficiency in either KLF4 or galectin-3 within macrophages is associated with a more pro-inflammatory state and, as result, a higher susceptibility for the development of atherosclerotic lesions in mice (13,14). In further support of a putative anti-atherogenic effect of galectin-3, an increased (inflammatory) early atherosclerosis burden was detected upon feeding an

atherogenic diet in total body galectin-3 knockout mice as compared to pair-fed wild-type mice (15).

In conclusion, Alencar *et al.* have highlighted that targeting KLF4-driven plasticity of smooth muscle cells within atherosclerotic lesions could be of therapeutic interest. Furthermore, they spotlight the application potential of their combined single cell RNA sequencing and fluorescent cell lineage-tracing technique to not only (1) globally study cell plasticity in many disease settings, including atherosclerotic cardiovascular disease, but also (2) investigate the potential pathogenic role of individual transcriptional regulators within specific cell lineages.

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Footnote

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