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Clarifying the distinct roles of smooth muscle cell-derived vs macrophage foam cells and the implications in atherosclerosis

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During atherosclerotic lesion development, low density lipoproteins (LDL) accumulate in the subendothelial intimal layer of the injured vessel wall, and once modified are thought to be taken up by monocyte-derived macrophages or smooth muscle cell (SMC)-derived macrophage-like cells to form lipid-laden foam cells.[1] As previously shown by the Francis lab using flow cytometry and microscopic analysis, up to 70% of foam cells in mouse atherosclerotic lesions and 50% in human lesions are actually derived from resident SMC rather than circulating monocytes.[2, 3] Wang *et al.* further demonstrated that these SMC-derived macrophage-like cells are ineffective in clearing lipid and apoptotic cells from the lesion microenvironment, and this may represent a major contributing factor to lesion progression.[2] Key remaining questions are the mechanisms of the functionally distinct properties of SMC-derived macrophage-like foam cells compared to the “real” macrophage foam cells and the implications in atherosclerosis.

In this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, *Dubland et al* showed that in SMC-derived foam cells, the inherently low expression of the *LIPA* gene and the encoded lysosomal acid lipase (LAL) protein relative to macrophages account for how SMC-derived foam cells are different from macrophage foam cells in intracellular lipid metabolism.[4] It has been well-established that in lipid-loaded macrophages, lysosomal LAL mediates hydrolysis of cholesteryl ester derived from internalized lipoproteins.[5] Increased flux of hydrolyzed cholesterol to the cytoplasmic compartment subsequently suppresses *de novo* cholesterol synthesis and stimulates cytoplasmic lipid droplet formation.[5] The free cholesterol released from the lysosome also provides substrates for 27-hydroxycholesterol synthesis, which enhances liver X receptor-mediated upregulation of ABCA1 (ATP-binding cassette transporter A1) and cholesterol efflux.[5, 6] The authors have now demonstrated that in lipid-loaded SMC, low LAL leads to insufficient capacity to hydrolyze neutral lipids

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Disclosures

None

delivered to the lysosome, thus causing lysosomal lipid sequestration and reduced flux of free cholesterol out of the lysosome. This reduced flux of cholesterol results in failure to suppress *de novo* cholesterol synthesis and upregulate ABCA1 expression and cholesterol efflux.[4] To establish causality, the authors showed that in SMC,[4] supplementation of recombinant LAL and treatment of macrophage-conditioned media containing abundant LAL rescued lysosomal lipid sequestration and led to a shift to increased cytoplasmic neutral lipid formation that resembles lipid-loaded macrophages. Lysosomal lipid sequestration in SMC was not associated with impaired lysosomal acidification, in contrast to lipid-loaded macrophages showing impaired lysosomal acidification and proteolytic capacity. The impaired lysosomal function in macrophages is likely due to increased hydrolyzed free cholesterol accumulating in the lysosomal membrane, disrupting the pH maintenance function of V-ATPase.[4] The authors have also attempted to establish *in vivo* relevance of these *in vitro* findings by confirming lower LIPA expression in CD45- than in CD45+ plaque cells in both human and murine atherosclerotic plaques.[4]

The work fills a major gap in understanding the difference between SMC-derived foam cells and macrophage foam cells. Yet, the findings also raise further questions. (Figure 1) **First,** in addition to aggregated-LDL, how other modified-lipoproteins and toxic lipid species resembling the atherogenic environment may affect the uptake, processing, and downstream signaling in SMC-derived foam cells remain to be further confirmed. **Second,** in the presence of apolipoprotein-AI, LAL supplementation in SMC enhanced lysosomal neutral lipid hydrolysis and promoted ABCA1-dependent cholesterol efflux, therefore reducing lipid accumulation. However, in an atherogenic environment with compromised apolipoprotein-AI availability, will increased intracellular cholesterol flux lead to increased cytoplasmic lipid accumulation therefore further promoting SMC-derived foam cell formation? Will prolonged LAL supplementation in SMC increase lysosomal free cholesterol accumulation and subsequently lysosomal dysfunction? **Third,** in light of the human genome-wide association studies and functional genomic findings, risk alleles of CAD (coronary artery disease) variants in the *LIPA* locus are associated with higher *LIPA* mRNA and LAL activity in human monocytes,[7] but not healthy arteries,[8] supporting a hypothesis that gain-of-function of myeloid *LIPA* may confer increased CAD risk. Also, there are currently no datasets determining how CAD *LIPA* alleles may affect *LIPA* expression in SMC-derived foam cells. The authors showed that in cultured macrophages, recombinant LAL treatment did not further enhance macrophage LAL activity in cell lysates, nor alter macrophage lysosomal function and lipid accumulation upon lipid loading. Yet, it is imperative to establish the *in vivo* relevance and disease causality by modeling the role of gain-of-function of *LIPA* in macrophages and/or SMC in experimental atherosclerosis as implicated by human functional genomic findings.

More broadly, the true identity of SMC-derived cells in atherosclerosis remains an unresolved question. Given that previous flow cytometry-based studies relied on candidate markers (e.g. ACTA2 for SMC, CD68 for macrophages and CD45 for monocytes),[2, 3] these markers could be non-specific and thus could confound the interpretation of foam cell origins. More recent single-cell RNA-seq studies coupled with single or dual-lineage tracing in mouse atherosclerotic lesions have identified a more complex intermediate SMC-derived

cell type, which could give rise to either fibroblast-like or macrophage-like cells.[9–11] Importantly, these transitions were confirmed in human atherosclerotic lesions and involve genome-wide associated risk loci for CAD (e.g. *TCF21*) and altered retinoic acid signaling to these target genes.[9, 10] It is worth noting that standard approaches to identify cell clusters during single-cell RNA-seq analysis involve manual labeling steps, which may introduce substantial biases from the literature.[12] Thus a combination of manual and automated learning based cell assignment approaches may provide a more balanced assessment of SMC-derived macrophage identity in these different lesions.[13] Further, inherent to all of these methods is the destructive nature to cell-cell contacts and interactions when liberating individual cells from the lipid and extracellular matrix-rich lesion microenvironment. Pseudotemporal trajectory analyses may provide some mechanistic insights into cellular transitions, and mitochondrial lineage tracing could support these inferences in human tissues.[14] However, spatial transcriptomics and multiplexed protein imaging analyses,[15, 16] which may preserve important cellular communication pathways, may be required to fully interpret expression/functional changes and transition states of foam cells during atherosclerosis.

In summary, *Dubland* and colleagues have moved an important step forward toward fully understanding how SMC-derived foam cells are different from macrophage foam cells. With the evolving human functional genomic data in conjunction with novel mouse models and single cell technologies, the genetic contribution of SMC and macrophage *LIPA* and the spatio-temporally resolved identity of SMC-derived cells in atherosclerosis are yet to be fully established.

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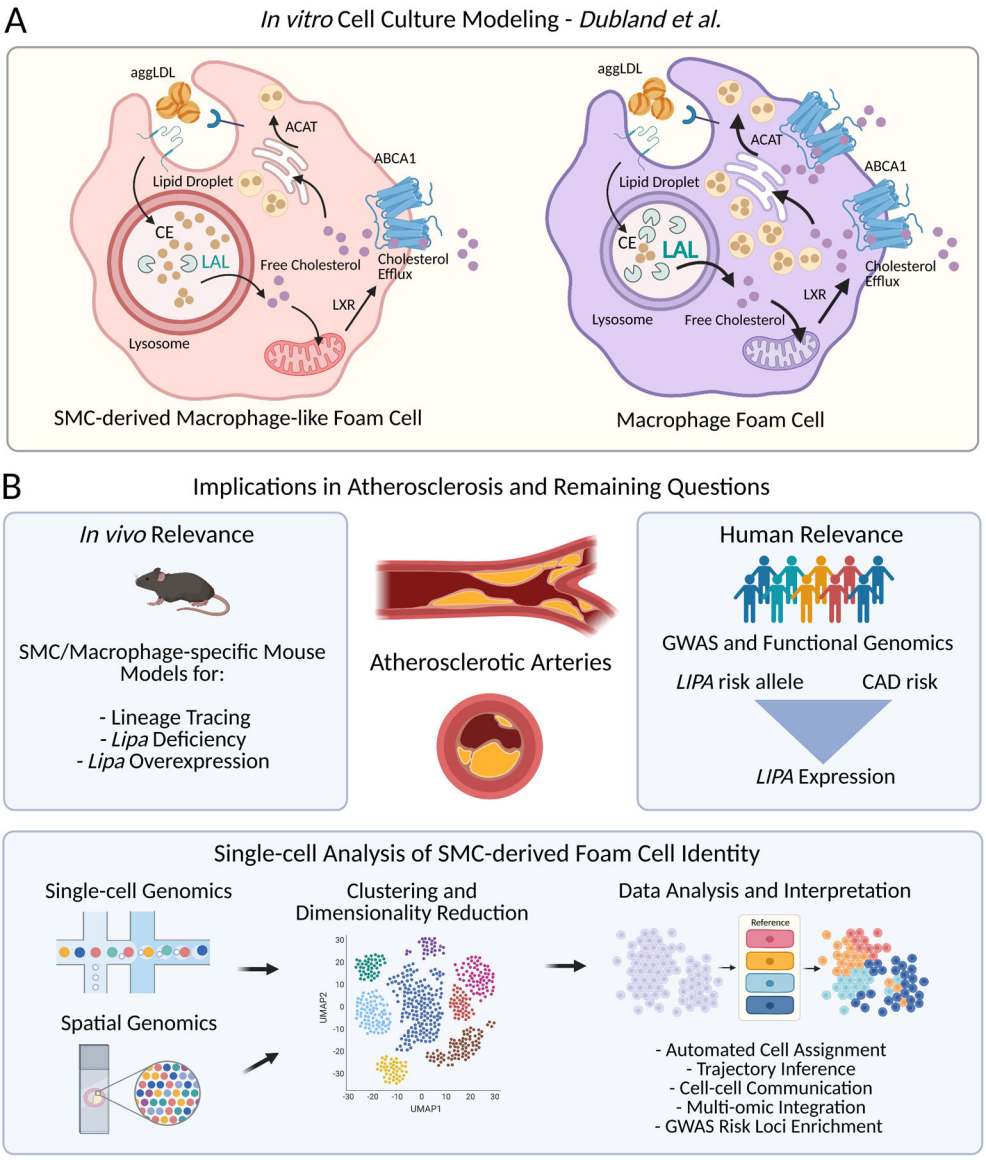

Figure 1. The differences between SMC-derived foam cells and macrophage foam cells in atherosclerosis: what are known and what remain to be determined.

(A) *Dubland et al* showed *in vitro* that low lysosomal acid lipase (*LIPA or* LAL) expression by SMC relative to macrophages account for the key mechanisms explaining their differences in intracellular lipid metabolisms.[4] **(B)** Questions remaining to be addressed are: 1) whether and how increased LAL, as implicated by human functional genomic findings to be associated with increased CAD risks, may affect SMC-derived foam cell and macrophage function *in vivo* in experimental and human atherosclerosis; and 2) the identity and contribution of SMC-derived foam cells in atherosclerosis with greater temporal and spatial resolution at single cell levels. aggLDL, aggregated-low density lipoprotein; ABCA1, ATP-binding cassette transporter A1; ACAT, Acyl-coenzyme A:cholesterol acyltransferases; CAD, coronary artery diseases; CE, cholesteryl ester; LXR, liver X receptor; SMC, smooth muscle cells. (The figure was created with BioRender.com.)