

## Zinc Fingers in the Pizza Pie Aorta

Jonathon W. Homeister, Cam Patterson

The multifactorial etiology of atherosclerosis includes inflammatory and immune processes that involve numerous cell types, including monocytic and lymphocytic leukocytes, endothelial cells, smooth muscle cells, and fibroblasts. It is likely that each of these cell types, as a contributor to the atherosclerotic process, possesses a gene expression profile unique to its role in disease pathogenesis, although perhaps also dependent on the disease stage, as well as on genetic and environmental factors. Modulation of gene expression patterns by various transcription factors will therefore dictate how a particular cell type contributes to the atherosclerotic process. Krüppel-like factors (KLFs) are a subclass of zinc finger transcription factors originally implicated in cell growth and differentiation. KLF2 and KLF4 in particular regulate the expression of certain genes relevant to atherosclerosis in a shear-dependent manner in endothelial cells and monocytes.<sup>1</sup> It is therefore important to understand the mechanisms and consequences of KLF activation in these cell types in vitro and in vivo. Such studies have the potential to uncover previously unknown transcriptional links between shear-dependent monocytic and endothelial processes that may underlie the pathogenesis of atherosclerosis.

The study by Atkins et al<sup>2</sup> in the issue of *Circulation Research* addresses this issue directly using mice with a hemizygous deficiency of KLF2 (KLF2<sup>+/-</sup>) and on the ApoE<sup>-/-</sup> background to examine the role of KLF2 in atherosclerosis. Aortic lesion extent was increased 30% to 35% in KLF2<sup>+/-</sup> mice compared to control littermates. The increase in atherosclerosis in the KLF2<sup>+/-</sup> mice was associated with no alterations in aortic expression of the antiinflammatory genes endothelial nitric oxide synthase (eNOS) and thrombomodulin, or the proinflammatory gene vascular cell adhesion molecule (VCAM)-1. Lesion macrophage content was not significantly increased in KLF2<sup>+/-</sup> mice, but both lipid uptake and expression of a lipid chaperone protein (aP2/FABP4) were increased in macrophages from KLF2<sup>+/-</sup> mice and were concomitantly reduced in a macrophage cell line overexpressing KLF2. These observations suggest that KLF2 expression

is an important modulator of foam cell formation and thereby atherogenesis via regulation of aP2/FABP4 expression.

Atkins et al<sup>2</sup> show for the first time that an approximate 50% reduction of KLF2 results in an increase in the extent of atherosclerosis in vivo. These experiments imply that global expression of KLF2 exerts an atheroprotective effect in vivo, but they do not determine in which cell type gene expression is being regulated by KLF2 to protect against atheroma formation. Interestingly, Das et al<sup>3</sup> recently modulated KLF2 expression to show that KLF2 negatively regulates the expression of proinflammatory genes and processes in monocytes. They also showed that monocytes from patients with severe atherosclerosis and elevated levels of markers for inflammation have an approximate 30% reduction in KLF2 expression. The in vivo data from the KLF2 hemizygous mice therefore correspond closely to KLF2 expression changes in circulating cells from humans with atherosclerosis, highlighting the importance of monocyte/macrophage KLF2 expression levels in the regulation of atherogenesis.

Because of the global nature of the KLF2 deletion, the atheroprotective effects of KLF2 identified in the present study cannot be attributed to a particular cell type. The data of Das et al<sup>3</sup> discussed above implicate KLF2 regulation of gene expression within the monocytic lineage as a likely mediator, at least in part, of the phenotype. However, KLF2 has been implicated in differentiation of additional cell types that participate in atherogenesis (Figure). KLF2 functions as a key molecular switch in endothelial cells, regulating expression of numerous genes that modulate the inflammatory, thrombotic, angiogenic, and vasoactive properties of endothelial cells.<sup>1</sup> KLF2 expression also serves as a regulator of T-cell development and directs their trafficking and recirculation via modulating their chemokine receptor expression patterns.<sup>4,5</sup> Finally, KLF2 is a negative regulator of adipogenesis and modulator of lipid metabolism as a result of its ability to inhibit PPAR $\gamma$  expression in adipocytes.<sup>6</sup> Each of these cell types participates in the pathogenesis of atherosclerosis, either directly or indirectly, and therefore may contribute to the observed phenotype. The atheroprone phenotypes in mice with a global reduction of KLF2 expression are quite possibly a compound effect of consequences in multiple cell types. Thus, cell type-specific deletions of KLF2 will be required to determine the relative contribution to the phenotype of KLF2-mediated processes in each cell type.

The present study also documents the important observation that the KLFs may exhibit some amount of redundancy. KLF4 expression was increased by 40% in the KLF2<sup>+/-</sup> mice. Such compensatory changes may serve to maintain homeostasis, at least in endothelial cells, in which both KLF2

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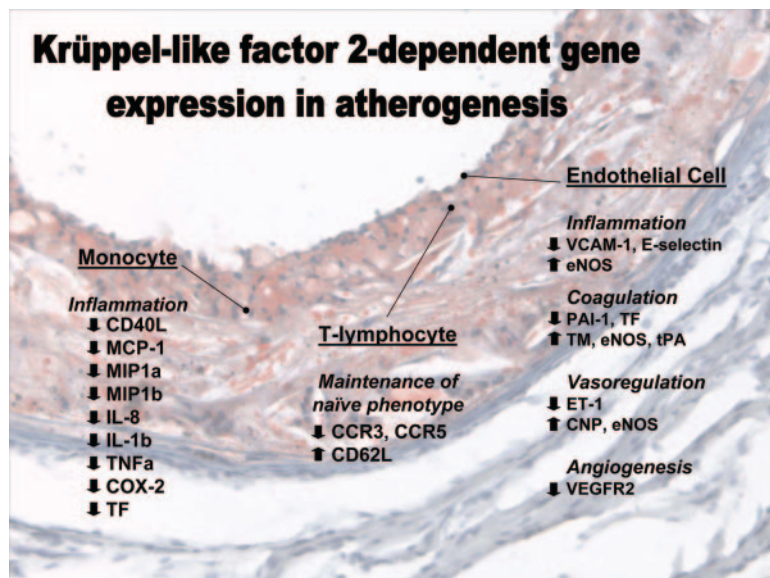
From the Carolina Cardiovascular Biology Center, Departments of Pathology (J.W.H.) and Medicine (C.P.), School of Medicine, University of North Carolina at Chapel Hill.

Correspondence to Cam Patterson, MD, Director, Division of Cardiology and Carolina Cardiovascular Biology Center, University of North Carolina, 8200 Medical Biomolecular Research Building, Chapel Hill, NC 27599-7126. E-mail cpatterson@med.unc.edu

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**Figure.** Schematic diagram showing for certain cells involved in atherogenesis the known genes whose expression is regulated by KLF2. Text is superimposed on a frozen section micrograph of a low-density lipoprotein receptor-deficient mouse aorta stained with oil red O and hematoxylin. CD 40L indicates CD40 ligand; MCP-1, monocyte chemotactic protein-1; MIP, macrophage inflammatory protein; IL, interleukin; TNF $\alpha$ , tumor necrosis factor  $\alpha$ ; COX-2, cyclooxygenase 2; TF, tissue factor; CCR, C-C type chemokine receptor; CD62L, L-selectin; PAI-1, plasminogen activator inhibitor-1; TM, thrombomodulin; tPA, tissue plasminogen activator; ET-1, endothelin-1; CNP, C natriuretic peptide; VEGFR2, vascular endothelial growth factor receptor 2.

and -4 are both known to regulate eNOS and VCAM-1 expression in a similar manner.<sup>7</sup> The possibility of redundancy is supported in part by phylogenetic studies showing that in man, mouse,<sup>8</sup> xenopus,<sup>9</sup> and zebrafish,<sup>10</sup> KLF2 and KLF4 are closely related members of the larger KLF family, suggesting the possibility of a gene duplication event. Functional redundancy of these 2 family members is also suggested by the observation of both increased eNOS expression and decreased VCAM-1 expression in endothelial cells.<sup>7</sup> However, it is not clear whether this overlap in gene expression can be generalized to other KLF2-regulated genes such as E-selectin, tissue factor, endothelin-1, vascular endothelial growth factor receptor 2, or others.<sup>1</sup> It is also not clear whether functional similarities between KLF2 and KLF4 are present in other cell types, such as monocytic cells, or T-lymphocytes. It is possible that further investigation will reveal that the functional relationship between KLF2 and KLF4 is similar to that between PPAR $\alpha$ , PPAR $\beta/\delta$ , and PPAR $\gamma$ . Initial studies demonstrated that these transcription factors can each regulate a common set of genes. However, more detailed studies have demonstrated differences in their gene regulatory actions based on cell-specific activity modulation by numerous coactivator and corepressor molecules.<sup>11</sup> The extent and functional consequences of the proposed redundancy of the KLFs will require additional studies of the actions of KLF2 and especially KLF4 in endothelial cells as well as other cell types. Such studies will likely require the use of compound loss-of-function mutations for both KLFs, as well as tissue-specific loss-of-function mutations for 1 or both KLFs.

Given the remarkable observations of Atkins et al,<sup>2</sup> it is intriguing to consider the possibility that pharmacological manipulation of KLF2 expression may be an effective therapeutic strategy for the treatment of atherosclerosis and/or other cardiovascular disease processes. In fact, there is evidence suggesting that a common therapeutic treatment for atherosclerosis works, in part, by modulating KLF2 expression. Several groups have shown that hy-

droxymethylglutaryl-coenzyme A reductase inhibitors (commonly referred to as statins) increase KLF2 expression in monocytic cells<sup>12</sup> and in endothelial cells.<sup>13</sup> To the extent that the atheroprotective effects of statins exceed what is expected because of their lipid-lowering effects in humans, the additional protective effects of statins independent of lipid effects may arise as a result of increased KLF2 expression. Future experiments to determine whether the beneficial effects of statins are mediated, in part, by KLF2 will be extremely interesting and important to our understanding of the mechanisms of atherosclerotic disease and to the application of this knowledge to treat the most common cause of death and disability in our society.

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## Disclosures

None.

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