Published in final edited form as:
Arterioscler Thromb Vasc Biol. 2014 September ; 34(9): 1809–1810. doi:10.1161/ATVBAHA.114.304151.

From hairballs to an understanding of transendothelial migration of monocytes in atherosclerosis

Mete Civelek[1] and **Aldons J. Lusis**[1,2,3]

[1]Department of Medicine, University of California, Los Angeles

[2]Department of Microbiology, Immunology and Molecular Genetics, University of California, Los Angeles

[3]Department of Human Genetics, University of California, Los Angeles

In the current issue of ATVB, Johan Bjorkegren and colleagues provide compelling evidence for the involvement of Lim Domain Binding 2 (*LDB2*) in the trans-endothelial migration of monocytes in atherosclerosis[1]. The paper is also of interest because of the systems analyses that led to its identification as a strong candidate.

LDB2 was identified earlier as a "key driver" of atherosclerosis based on studies of gene expression profiles of tissues obtained from patients[2]. Using samples from the Stockholm Atherosclerosis Gene Expression (STAGE) study, the authors profiled gene expression of 5 atherosclerosis-relevant tissues from 114 patients undergoing coronary artery bypass grafting. The tissues collected were distal internal mammary artery, wall of the ascending aorta at the aortic root, anterior hepatic edge, skeletal muscle, and visceral fat. A total of 278 gene expression profiles were used in a coupled two-way clustering analysis[3] to identify 60 gene subnetworks in these tissues. Two of the gene clusters, one in atherosclerotic arterial wall (49 genes) and the other in visceral fat (59 genes), segregated the patients according to the extent of atherosclerosis as measured by quantitative coronary angiography. The authors further validated their findings using expression data obtained from carotid lesions isolated from patients undergoing carotid stenosis surgery. Clustering of data identified 8 gene subnetworks in carotid lesions, one of which segregated the patients according to the extent of atherosclerosis as measured by ultrasound-measured intima-media thickness. This cluster significantly overlapped with the two previously identified clusters from the lesioned arterial wall and visceral fat. Together, the three subnetworks were used to generate a union subnetwork that was significantly enriched for the transendothelial migration of leukocyte (TEML) KEGG pathway. *LDB2*, a transcriptional regulator, was present in all three subnetworks and had the greatest number of interactions with genes in the union subnetwork. Analysis of gene expression changes in *LDB2* in late and early atherosclerotic lesions in mice further suggested a role of this gene in endothelium or macrophages. Based on these findings, the authors hypothesized that *LDB2* is a driver of transendothelial migration in atherosclerosis.

Correspondence: Aldons J. Lusis, Department of Medicine/Division of Cardiology, A2-237 CHS, UCLA, Los Angeles, CA 90095-1679.

Disclosures - None

In the current study, the authors validated and characterized the role of *LDB2* in the TEML pathway by breeding a targeted *Ldb2* gene mutation (*Ldb2*$^{-/-}$) onto the *Ldlr*$^{-/-}$*Apob*$^{100/100}$ background. *Ldlr*$^{-/-}$*Apob*$^{100/100}$ mice have a human-like LDL-cholesterol profile and develop large atherosclerotic lesions on chow as well as high cholesterol diets. *Ldb2* deficiency resulted in an approximately 2 fold increase in aortic lesion area in mice fed a 30-week chow or 25-week high-fat diet. This was not accompanied by any discernible effects on the plasma cholesterol, triglyceride or glucose levels. Lesions from the *Ldb2*$^{-/-}$ mice exhibited increased staining with Oil Red O and the macrophage marker CD68 but decreased staining with the smooth muscle cell marker SM22α. These results pointed to an increased TEML activity, independent of lipoprotein levels, responsible for larger lesions in *Ldb2*$^{-/-}$ mice. To further explore the role of *Ldb2* in transendothelial migration, the authors utilized two separate transmigration models, the dorsal air pouch and the retinal vasculature. On a hyperlipidemic background, the *Ldb2*$^{-/-}$ mice exhibited increased leukocyte migration in both models.

The effect of *Ldb2* deficiency on lesion development appears to result from multiple pathways. The authors provide evidence that *Ldb2* deficiency acts in part by directly altering the properties of macrophage or other leukocytes. Using labeling with latex beads, the authors showed that leukocytes from *Ldb2* deficient mice exhibited increased adhesion to aortic arches in *Ldlr*$^{-/-}$*Apob*$^{100/100}$ mice. Moreover, leukocytes from *Ldb2* knockout mice exhibited increased migration *in vitro* in response to monocyte chemotactic protein 1 stimulation. This suggested that *Ldb2* in leukocytes is responsible in part for the increased transmigration. On the other hand, expression profiling of aortas in younger mice, before the development of atherosclerotic lesions, identified forty genes differentially expressed between *Ldb2* deficient mice and wild-type littermates, a gene set enriched for cell adhesion and the TEML pathway. Notably, the adhesion protein *Vcam1* was increased in both vessel wall and macrophages at the mRNA and protein levels. In addition to effects on transmigration, the proliferation of *Ldb2*$^{-/-}$ macrophages, as measured by levels of the proliferation marker Ki67, was increased both *in vitro* (in response to TNFα stimulation) and *in vivo*, in lesions of *Ldlr*$^{-/-}$*Apob*$^{100/100}$ mice.

While *LDB2* has not been shown to be associated with CAD in large scale genome-wide association studies[4], the authors showed that a minor allele of a single nucleotide polymorphism in *LDB2* (rs10939673) was underrepresented in a small cohort of CAD cases compared to healthy controls. The same allele was also associated with a lower stenosis score, a smaller plaque area and a thinner intima-media in other studies and this association seemed to be independent of other risk factors. In contrast to mouse studies, however, the minor allele was associated with lower expression of *LDB2* in the arterial wall and visceral fat, suggesting the complete ablation of the gene in the mouse may not fully represent the subtle variations in the expression levels of this gene in the humans.

These findings raise a number of questions. Although the results are consistent with leukocyte migration playing an important role in the *Ldb2*$^{-/-}$ phenotype, additional studies such as bone marrow transplantation or tissue-specific knockouts would help clarify the relative importance of macrophages versus endothelium. Also, a recent study provided evidence that, while transmigration contributes importantly to the development of early

lesions in mice, macrophage proliferation is primarily responsible for the growth of more advanced lesions[5]. Since lesional macrophages in the *Ldb2*$^{-/-}$ mice exhibited increased proliferation as judged by Ki67 staining, this aspect could also be important. An earlier study of fibroblasts implicated *Ldb2* and its transcription cofactor, *Ldb1*, in cytoskeletal reorganization[6]. These factors bind directly to the microtubule-associated Ste20 kinase, SLK, important in cell migration, and appear to maintain it in an inactive state before its activation. It will be of interest to understand whether *Ldb2* is acting similarly in leukocytes and endothelial cells.

This study also provides a lesson on how to move forward in understanding atherosclerosis and other complex disorders. Genetic studies have been key in revealing new pathways and mechanisms contributing to the disease, the most notable example being familial hypercholesterolemia. But while Mendelian disorders such as familial hypercholesterolemia can often be addressed using molecular biology approaches, and engineered mouse models, understanding the many genetic and environmental interactions contributing to the common forms of the disease is much more challenging. The systems approach taken by these authors seeks to identify relationships between molecular phenotypes, such as transcript levels, and clinical pathways that occur among populations of patients. The results presented in this paper provide validation of the value of the approach as well as the role of the gene.

Acknowledgments

None

Sources of funding - M.C. is supported by Ruth L. Kirschstein National Research Service Award T32HL69766 and K99HL121172. A.J.L. is supported by the US National Institutes of Health grants HL28481, D094311, HL114437, HL123295 and Transatlantic Networks of Excellence in Cardiovascular Research Program Award from Foundation Leducq.

References

1. Shang M-M, Talukdar HA, Hofmann JJ, et al. Lim domain binding 2—a key driver of transendothelial migration of leukocytes and atherosclerosis. Arterioscler Thromb Vasc Biol. 2014 in press.
2. Hagg S, Skogsberg J, Lundstrom J, et al. Multi-organ expression profiling uncovers a gene module in coronary artery disease involving transendothelial migration of leukocytes and LIM domain binding 2: the Stockholm Atherosclerosis Gene Expression (STAGE) study. PLoS Genet. 2009; 5:e1000754. [PubMed: 19997623]
3. Getz G, Levine E, Domany E. Coupled two-way clustering analysis of gene microarray data. Proc Natl Acad Sci USA. 2000; 97:12079–12084. [PubMed: 11035779]
4. Deloukas P, Kanoni S, et al. Consortium CAD. Large-scale association analysis identifies new risk loci for coronary artery disease. Nat Genet. 2013; 45:25–33. [PubMed: 23202125]
5. Robbins CS, Hilgendorf I, Weber GF, et al. Local proliferation dominates lesional macrophage accumulation in atherosclerosis. Nat Med. 2013; 19:1166–1172. [PubMed: 23933982]
6. Storbeck CJ, Wagner S, O'Reilly P, McKay M, Parks RJ, Westphal H, Sabourin LA. The Ldb1 and Ldb2 transcriptional cofactors interact with the Ste20-like kinase SLK and regulate cell migration. Mol Biol Cell. 2009; 20:4174–4182. [PubMed: 19675209]