From Hairballs to an Understanding of Transendothelial Migration of Monocytes in Atherosclerosis

Mete Civelek, Aldons J. Lusis

In the current issue of ATVB, Shang et al provide compelling evidence for the involvement of LIM domain binding 2 (LDB2) in the transendothelial migration of monocytes in atherosclerosis.1 The article is also of interest because of the systems analyses that led to its identification as a strong candidate.

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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 2-way clustering analysis3 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 2 previously identified clusters from the lesioned arterial wall and visceral fat. Together, the 3 subnetworks were used to generate a union subnetwork that was significantly enriched for the transendothelial migration of leukocyte activity, independent of lipoprotein levels, responsible for larger lesions in Ldb2−/− mice. To explore the role of Ldb2 in transendothelial migration further, the authors used 2 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 seems 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−/− mice exhibited increased adhesion to aortic arches with Oil Red O and the macrophage marker CDS8 but decreased staining with the smooth muscle cell marker SM22α. These results pointed to an increased transendothelial migration of leukocyte activity, independent of lipoprotein levels, responsible for larger lesions in Ldb2−/− mice. To explore the role of Ldb2 in transendothelial migration further, the authors used 2 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.

In the current study, the authors validated and characterized the role of LDB2 in the transendothelial migration of leukocytes by breeding a targeted Ldb2 gene mutation (Ldb2+/−) onto the Ldlr+/− ApoB100/100 background. Ldlr+/− ApoB100/100 mice have a human-like low-density lipoprotein-cholesterol profile and develop large atherosclerotic lesions on chow, as well as high cholesterol diets. Ldb2 deficiency resulted in ≈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 CDS8 but decreased staining with the smooth muscle cell marker SM22α. These results pointed to an increased transendothelial migration of leukocyte activity, independent of lipoprotein levels, responsible for larger lesions in Ldb2−/− mice.

Ldb2−/− mice exhibited increased migration in vitro in response to monocyte chemotactic protein 1 stimulation. This suggested that Ldb2 deficiency in leukocytes is responsible, in part, for the increased transmigration. However, expression profiling of aortas in younger mice, before the development of atherosclerotic lesions, identified 40 genes differentially expressed between Ldb2−/− mice and wild-type littermates, a gene set enriched for cell adhesion and the transendothelial migration of leukocyte 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 tumor necrosis factor-α stimulation) and in vivo, in lesions of Ldlr+/− ApoB100/100 mice.

Although LDB2 has not been shown to be associated with coronary artery disease 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 coronary artery disease cases when compared with healthy controls. The same allele was also associated with a lower stenosis score, a smaller plaque

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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 several 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, although transmigration contributes importantly to the development of early lesions in mice, macrophage proliferation is primarily responsible for the growth of more advanced lesions. Because 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. These factors bind directly to the microtubule-associated Ste20 kinase, SLK, important in cell migration, and seem 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 a key in revealing new pathways and mechanisms contributing to the disease, the most notable example being familial hypercholesterolemia. But although 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 article provide validation of the value of the approach, as well as the role of the gene.

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References

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