Editorial

Molecular Phenotypes of Atherosclerosis
Fingering the Perpetrators

Peter F. Davies

The initiation and progression of atherosclerosis appears driven by a combination of genetic and environmental factors. Access to circulating lipoproteins or to molecules shed into the blood from arterial wall cells permits genetic diversity to be evaluated in relation to disease risk; environmental factors are commonly understood to relate to macro-risk such as diet, smoking, etc. However, a more precise and detailed understanding of the genetic and in vivo environment in the immediate vicinity of the predictive, developing, or advanced lesion is desirable. The presence at autopsy of atherosclerotic lesions of varying severity and classifications within the same individual reflects the complexity of this chronic inflammatory process. Furthermore, certain arterial locations are predictive for lesion formation before any detectable pathological change is apparent, whereas other sites appear to be protected. Systematic and serendipitous studies of genetic variants associated with atherogenesis have largely been conducted in lipoproteins and blood cells, yet genetic variation in the arterial wall components is an important contributor to disease susceptibility and progression. The most prevalent gene expression changes may identify the inheritance of multiple gene variants as a collection of single nucleotide polymorphisms (SNPs) that may predict both the susceptibility to environmental risk factors and the characteristics of lesion progression. However, the development of molecular signatures that define lesion predisposition, stage of progression, treatment indications, and predicted outcomes is challenging because of spatial and temporal physio(path)ological diversity, even within single vascular beds such as the aorta.

See page 1922

Transcript profiling of atherosclerotic material by DNA microarray analyses provides a potentially valuable global approach to identify single genes and multi-gene pathways uniquely associated with pathological progression. A number of studies have reported profiles of human atherosclerotic lesions with varying success and comprehensibility; problems include those associated with small sample size obtained from genetically diverse patients, the limited availability of adjacent normal tissue from the same specimens for critical comparison with the diseased state, and a rather low level of rigor in the statistical and bioinformatics analyses. The latter situation is improving dramatically with the more aggressive input of academic bioinformatics centers and the development of commercial analytical software comprehensible to the biologist. Nevertheless, various degrees of subjective or experimental design-imposed bias often limit the conclusions of such studies. Databases of lesion phenotypes contain large amounts of important, but poorly accessible, information. Can the profiling of heterogeneous gene expression in atherosclerosis be turned to better advantage?

In this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Seo et al. report the application of novel statistical techniques to the analysis of the human atherosclerotic aorta. Their work is directly derived from the solution of similar problems in cancer research where the methods have recently been successfully applied to complex databases to diagnose and predict lymph node metastasis in primary breast cancer. The approach is unbiased, exploits the complexity of the datasets, and fully recognizes the multigenicity and dynamics of the disease. The work integrates centers of statistics, cardiology, and surgery. Although a limited study, the analyses reveal gene expression signatures for lesion severity that are statistically robust and have predictive value, including the identification of novel genes not previously associated with atherogenic mechanisms.

The authors isolated fresh human aortas exhibited varying degrees of atherosclerosis. Tissues were segmented into 4 lateral regions symmetrical with respect to the longitudinal midline and 4 proximal-to-distal segments, arch to diaphragm. Sagittal symmetry was assumed, allowing a sample to be evaluated for lesion location (if any) and for severity by Sudan staining and raised lesion mapping. Mirror-image segments of full thickness artery wall were processed for RNA isolation and DNA microarray analysis for expression of 12 563 genes followed by limited validation by quantitative RT-PCR. Fifteen minimally diseased and 16 severely diseased segments were analyzed, and 31 proximal segments were compared with 32 distal segments. The statistical approach uses the “metagene” construction (coexpression of multiple genes acting in concert) and binary prediction tree analysis as applied successfully in previous cancer analyses. The metagene patterns identified were cross-validated and shown to possess high predictive value for blinded aortic samples, with >93% accuracy for both lesion severity and location in the aorta. The value of such predictive capability, however, lies not in the subsequent management of the disease (transmural aortic biopsy is not an option), but in a
better understanding of lesion pathogenesis using 2 principal approaches: first, metagene analyses that reinforce known pathological pathways of atherogenesis, and second, the identification of novel genes not previously linked to atherosclerosis but which, as inclusions in highly significant metagene patterns, are strongly implicated. Prioritization of these will lead to efforts to identify SNPs for large-scale analyses of gene variants including combinations of SNPs. These in turn may lead to new diagnostic and clinical management strategies.

The unbiased and comprehensive nature of the statistical approach is a major strength of this study and a good example of the appropriate use of microarray analysis as a tool to focus attention on the most important biological characteristics of the system. It illustrates the necessity of high-level bioinformatics and statistics in microarray analyses. The power of the metagene approach is apparent in the high predictive accuracy resulting from analysis of RNA derived from the entire thickness of the vessel wall rather than diseased intima alone, and in the deliberate neglect of the relative contributions by different cell types to expression patterns (which usually confounds interpretation). It is also possible that some expression patterns may be more correlative with, and less directly involved in, atherogenesis; however, these will be apparent on subsequent focused analysis. A further interesting result of the study is the high predictability of lesion location, a fact that is well established descriptively but that only recently has become the object of multi-gene quantitative analysis in situ. Because flow characteristics and the associated hemodynamic forces appear to play a major role in regional susceptibility to atherogenesis, statistical approaches such as that reported by Seo et al may provide valuable spatial information about protective and predilection mechanisms in atherosclerosis.

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References
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