The blood vessels that comprise the circulatory system are extraordinarily sophisticated and complex. The organization of the cells and extracellular matrix that make up the vessel wall define its structural integrity and regulate vascular physiology and homeostasis through biochemical regulation. The endothelial lining of the normal blood vessel is a continuous, selectively permeable and antithrombotic barrier between the circulating blood and the vessel wall.1 The endothelium detects shear and humoral signals and in response generates products that regulate blood flow, vessel tone, platelet activation, and thrombosis.2–3 The endothelium also regulates smooth muscle cell migration and proliferation among its legion of roles.1–6 Endothelial and vascular injury that are associated with invasive procedures such as angioplasty, vessel and organ transplantation, and coronary and peripheral arterial by-pass surgery, as well as noninvasive phenomenon such as atherosclerosis, disrupt the physical barrier provided by the endothelium, interfering with vascular homeostasis.7–11 This sets a series of events in motion that lead to a proliferation and migration of smooth muscle cells. Often these proliferative and migratory events extend beyond the normal healing process and result in obstructive arterial lesions. Depending on the exact pathology and mechanism, this process of restenosis or neointimal hyperplasia leads to the failure of bypass grafts, critical narrowings in up to 50% of patients after angioplasty, and proliferative vascular disease in transplanted hearts. While significant advances have been made in the management of restenosis to prevent or reopen these narrowings and innovative treatments continue to be developed, the most effective measures that remain are management of symptoms rather than intervention of the disease process. For example, pharmacologic and lifestyle changes are the most effective treatment for atherosclerosis, prevention of rejection delays transplant vasculopathy, and stenting is the most effective measure to prevent balloon injury restenosis. While these treatments are effective, and in the case of transplant patients, essential, they illustrate our modest understanding of the underlying biological processes involved in neointimal formation. More recent reports of novel and effective therapies, such as the use of beta-radiation after heart transplantation, eximer laser angioplasty, and drug eluting stents, are promising but are again invasive rather than directly preventive.12–14

The key to effective prevention and treatment of neointimal hyperplasia and restenosis is rooted in a more thorough understanding of the biology of the neointima and how it differs from normal vascular tissue. While recent studies and reports concentrating on single or small numbers of gene products have been informative and important, they have not been able to offer a global view of neointimal biology.15,16 Moreover, subtractive approaches comparing expression before and after balloon injury of normal arteries have led to the assertion that observed changes in gene expression are generic to other forms of intima. The report by Geary et al17 in this issue of Arteriosclerosis, Thrombosis, and Vascular Biology describes the first study to characterize different vascular smooth muscle cells using a more systematic and global approach.

Using cDNA array technology followed by independent verification by in situ hybridization and Northern Blotting, Adams et al17 have identified 147 genes contributing to a unique neointimal smooth muscle cell phenotype compared with normal aorta and vena cava. In general, microarray technologies allow for the efficient and rapid detection of RNA transcript abundance from thousands of genes simultaneously. Although many technologies exist to profile and quantify transcript abundance (and therefore provide a representation of the transcriptional state of the cell), a distinct advantage of microarray technologies is the ability to perform comparative studies among large numbers of samples simultaneously. These types of comparative studies are very difficult if not impossible using techniques such as Differential Display or Serial Analysis of Gene Expression (SAGE) and are comparatively inefficient when using PCR-based methods. In a previous study, the authors first compared the expression profiles of normal media isolated from vena cava and aorta.18 Although very different in structure, only 68 of 4048 genes were differentially expressed between the two vessel types. Interestingly, all identified transcripts were more abundant in the aortic smooth muscle compared with vena cava. In the present study, the authors have extended this comparison and approach to the analysis of gene expression in primate smooth muscle cells isolated from the neointima of synthetic aortic bypass grafts compared with normal aorta and vena cava smooth muscle cells. Focusing on the smooth muscle cells, the authors took special care to avoid gene expression patterns associated solely with inflammation, migration, and proliferation during the healing process. Special attention was also given to removing endothelial cells by gently denuding the endothelium prior to isolating the media of the neointima and normal vessels.

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In the last several years, high-density microarrays have garnered a sort of Jekyll and Hyde reputation. The pleasant Dr Jekyll aspect is the efficiency with which profiles can be performed and data generated. The less pleasant Mr Hyde aspect is the special attention and considerations that must accompany the analysis of microarray data, often over looked in early studies. Appropriate statistical and analysis measures must be taken to avoid significant false identifications of genes and assign a level of statistical significance to the overall results. In the Adams et al study, the authors used a number of analysis techniques to refine and support the gene groupings that are presented in the report. In addition to performing each experiment four times and using only data points valid in all replicates, the authors used a number of statistical analysis and filtering techniques described fully in the manuscript. A detailed discussion of some of the statistical considerations and caveats one encounters during a study of this type are also presented and discussed. Following a rigorous and detailed analysis, the authors present a relatively small number of genes that are able to differentiate neointima from vena cava and aorta. Following the trend in their previous study, the number of genes that differentiate the three tissues are more abundant in the aorta and neointima relative to vena cava. Another advantage inherent to microarrays is that many “expected” or control genes can be observed concurrently with novel or unexpected ones, providing a level of comfort or control in each experiment. Indeed, the study by Adams et al reports genes such as elastin and fibromodulin, among others, to be more abundant in aorta and neointima compared with vena cava. Based on simple physiology, one would expect to see elastin and other matrix proteins expressed at higher levels in arterial structures compared with venous. Additionally, nearly all of the genes that differentiate neointima from aorta were associated with extracellular matrix production, consistent with previous reports implicating overproduction of matrix proteins in other types of neointima.

While the study at hand analyzes a relatively small number of genes (approximately 10% to 12% of the human genome), it illustrates the potential of DNA microarrays in the characterization of the diverse intimal smooth muscle cell populations. The authors have identified some potentially interesting gene targets associated with graft neointima formation and have laid the groundwork for more in-depth studies. Although genes identified in previous studies of neointimal pathology were not identified as differentially expressed in the Adams et al study, the authors suggest this is due to the diversity between intimal smooth muscle cell populations and biological variability in the animal models, and indicate the need for additional study. Further expansion of these studies to include a larger number of genes, more diverse conditions of neointimal formation, and a larger biological sample base will certainly provide a more complete picture of smooth muscle cell biology as it relates to neointimal formation. These types of studies will likely identify novel targets for therapeutic intervention aimed at preventing neointimal hyperplasia and stenosis following peripheral arterial bypass surgery, balloon angioplasty, and transplant vasculopathy. Looking into the future, one could envision expression profiling as a preoperative diagnostic tool to assess the probability of a patient developing certain types of neointimal hyperplasia or restenosis and predict how they will respond to available therapies.

References


Microarray Analysis of Neointima: Flowing Toward a Clear Future
Shawn E. Levy and James A.S. Muldowney, 3rd

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