HUMAN GENETICS '99: THE CARDIOVASCULAR SYSTEM
Nitric Oxide in Endothelial Dysfunction and Vascular Remodeling: Clinical Correlates and Experimental Links

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The patency of the vascular system is essential to maintain normal tissue function. Fortunately for us, the genetic program of vascular cells (endothelial cells, smooth muscle cells, and fibroblasts) endows them with the capacity to respond to physiological and pathophysiological stimuli and to rearrange their architecture to maintain adequate blood flow according to the metabolic demand of the tissue. Rapid calibration of lumen diameter can occur through vasomotor control governed by the sympathetic nervous sytem and vasoactive factors. Long-term structural adaptation, perhaps reflecting the summation of many short-term vasomotor events, occurs through a process called "vascular remodeling."

Vascular remodeling is the ability of the vessel wall to reorganize its cellular and extracellular components in response to a chronic stimulus (Gibbons and Dzau 1994). Experimental studies have shown that increases in blood flow due to an arteriovenous shunt will increase vessel diameter; conversely, reductions in blood flow will reduce vascular diameter. On the basis of these findings, it is believed that chronic reduction in blood flow initiates a signaling cascade that leads blood vessels to contract acutely and remodel themselves, creating a smaller vascular lumen. This remodeling reduces the shear stress and the circumferential wall strain imposed by the lower flow rates (Kamiya and Togawa 1980; Langille and O'Donnell 1986). The layered structure of the vascular system facilitates information transfer from the lumen of the vessel into the underlying smooth muscle and then to surrounding tissue. Likewise, the vessel responds to neural input and tissue factors to influence the smooth muscle and the endothelium. The position of the vascular endothelium at the interface of blood is ideally situated to serve as a tranducer, to relay hemodynamic and biochemical changes into molecular events in the smooth muscle layer (Davies 1995). Smooth muscle cells in the medial layer respond to endothelial cell mediators and/or extracellular matrix (ECM) molecules, allowing expansion or retraction of smooth muscle mass. The outermost layer, the adventitia, consists mostly of fibroblasts and produces growth factors, ECM, and proteases/protease inhibitors that can regulate proliferation and migration of smooth muscle cells. Similar remodeling of the endothelium must also occur during normal angiogenesis, and there is ample evidence showing that increases or decreases in blood flow can influence capillary densities in the heart and skeletal muscle. This plasticity of blood vessel architecture is essential for macro- and microvascular remodeling.

An increasing volume of evidence indicates that abnormal vascular remodeling contributes to such cardiovascular diseases as hypertension, atherosclerosis, transplant arteriosclerosis, and restenosis. In the past decade, many molecules essential for vasculogenesis, angiogenesis, and vessel structure have been identified that, when deleted, cause embryonic lethality due to vascular defects. Although these genes must play fundamental roles in vascular development or remodeling, we have little knowledge of the hierarchy of events underlying physiological or pathological remodeling in the mature vessel wall. We argue, in the present article, that signals transmitted bidirectionally across the endothelium direct the remodeling of blood vessel architecture in the adult. We hypothesize that the inability of the endothelium to couple hemodynamic events efficiently to the production of the gaseous second messenger, nitric oxide (NO), contributes to abnormal vascular remodeling in many diseases.

Impaired Vascular Remodeling in Hypertension and Atherosclerosis

Both human essential hypertension and animal models of this condition show enhanced peripheral vascular re-

Received January 15, 1999; accepted for publication January 19, 1999; electronically published February 15, 1999.

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This article represents the opinion of the authors and has not been peer reviewed.

0002-9297/99/6403-0002$02.00

sistance due to impaired remodeling of resistance arterioles. The increase in resistance arises in part because of impaired structural adaptation to elevated blood pressure and in part because of excess vasoconstriction. In histologic analysis, this change is measured as an increase in the cross-sectional area of the media, relative to the area of the lumen of specialized "resistance" arterioles. The increase in media/lumen ratio can result from any of the following: (1) abnormal lumenal remodeling resulting in a narrower lumen, (2) an increase in muscle mass (wall thickness) with no change in lumen diameter, or (3) a decrease in external diameter of the vessel, leading to encroachment of the lumen (Heagerty et al. 1993). Similarly, in human atherosclerosis there is ample evidence for active remodeling during the early stages of disease prior to significant lumenal stenosis. Compensatory enlargement occurs in peripheral vessels and in coronary arteries with lesions and represents an adaptive cellular response to preserve lumen area and blood flow. This "remodeled" vessel may revert to a more normal structure if the lesion regresses, but persistent or worsening lesions may provoke an abnormal remodeling response, thus contributing to occlusive disease, plaque rupture, and thrombosis (see Birnbaum et al. 1997 for a comprehensive review).

Although the causes of these diseases are different, in both cases endothelial dysfunction occurs concomitantly with changes in vascular structure—that is, with vascular wall thickening in hypertension (Girerd et al. 1994; Ghiadoni et al. 1998) and with compensatory vessel wall enlargement in atherosclerosis (Birnbaum et al. 1997). Endothelial dysfunction, defined here as an impairment in endothelium-dependent relaxation of blood vessels (Harrison 1997), is a reversible condition, as evidenced by the recent clinical trials with drugs such as angiotensin-converting enzyme inhibitors (Mancini et al. 1996) and cholesterol-lowering agents (Treasure et al. 1995). These functional responses are largely mediated by the endogenous vasodilator NO, an unusual signaling molecule that has been identified as the endothelium-derived relaxing factor—as originally characterized by Furchgott and Zawadski in 1980.

Mutations in the gene for endothelial isoform of NO synthase (eNOS) seemed to provide an attractive explanation for these abnormal responses. However, linkage studies show little evidence that eNOS is a candidate gene in vascular disease. In patients with essential hypertension (Bonnardeaux et al. 1995; Hunt et al. 1996) and in genetic models of hypertension in rats (Deng and Rapp 1997), polymorphisms of the eNOS gene (*NOS3*) do not segregate with high blood pressure. This lack of linkage occurs despite evidence that whole-body basal NO production in patients with essential hypertension is diminished (Wang et al. 1997) and that offspring of hypertensive patients have endothelial dysfunction (Taddei et al. 1996). A recent study in a Japanese population of patients with essential hypertension has shown that a missense variant of eNOS (Glu298Asp) is significantly associated with the disease; however, the functional relevance of this variant is not yet known (Miyamoto et al. 1998). This same mutation is associated with coronary vasospasm (Yoshimura et al. 1998) and myocardial infarction (Shimasaki et al. 1998) in this population. A recent study in France (Lacolley et al. 1998) showed a higher prevalence of the Glu298 Asp mutation in hypertensives compared with normotensive patients, but no association between this allele and blood pressure or aortic stiffness was noted. The only linkage study correlating mutations in the eNOS gene positively with endothelial dysfunction is a study showing a positive association between an intronic polymorphism and patients who were smokers or former smokers with a history of myocardial infarction (Wang et al. 1996). If abnormal function or expression of eNOS is involved in the disease processes associated with endothelial dysfunction, it is unlikely to be a primary determinant of disease in the populations studied thus far.

The Endothelium as a Sensor for Remodeling

A major breakthrough in understanding the importance of the endothelium in the remodeling process was the development of surgical models of flow-dependent remodeling. Thus, high-flow states, similar to those seen after long-term exercise training, can be studied by introducing an arterial anastomosis, or flow can be reduced in the common carotid artery by ligation of the external carotid. Using the latter model, which mimics critical stenosis, Langille and O'Donnell (1986) elegantly demonstrated that the endothelium, or a substance produced by the endothelium, was essential for remodeling toward a smaller lumen after a long-term flow reduction. Results of this study suggested that the endothelium sensed the hemodynamic changes and initiated the reorganization of the preexisting cellular and extracellular components. This remodeling requires coordinated changes in cellular proliferation, apoptosis, migration, cell organization, and matrix-integrin interactions throughout the layered structure of the vessel. Because of the complexity of the process, it is not surprising that it can be perturbed by persistently elevated blood pressures, local inflammation, high cholesterol, or the deposition of oxidized lipids.

NO as a Critical Regulator of Remodeling

Pharmacologic studies using inhibitors of NOS have implicated NO as an important endothelial mediator of flow- or pressure- induced arterial remodeling. Long-term treatment of rats with the NOS inhibitor, nitro-L-

arginine methyl ester (L-NAME), causes a sustained increase in systemic blood pressure accompanied by marked microvascular remodeling, as judged by an increase in wall-to-lumen ratios of resistance coronary arteries (Numaguchi et al. 1995). Of interest, in this study, coadministration of hydralazine to offset the pressor effects of L-NAME did not affect the remodeling of coronary vessels, suggesting that the elevated pressure, per se, could not account for pathological remodeling. Indeed, NO is a potent vasodilator, but it may influence vascular remodeling because of its nonhemodynamic actions. This signaling molecule has profound effects on many cell types—inhibiting platelet and leukocyte adherence to the endothelium, promoting endothelial and preventing smooth muscle–cell migration and proliferation, influencing extracellular matrix synthesis and degradation, and regulating gene expression. In addition, NO acts as a second messenger for the actions of many growth factors, peptides, coagulation factors, and hormones. Luvara et al. (1998) showed that long-term blockade of whole-body NOS leads to a proinflammatory vascular phenotype associated with the up-regulation of cellular adhesion molecules ICAM-1 and VCAM-1 and inflammatory cell infiltration. In a study examining the importance of NO in high-flow remodeling of the carotid artery, rabbits with surgically created arteriovenous fistulas were given nonpressor doses of L-NAME (Tronc et al. 1996), and remodeling was examined. As predicted, L-NAME blocked the flow-induced increase in luminal remodeling, supporting the idea that activation of eNOS by changes in flow promotes NO release and the orchestration of events leading to remodeling. These results have been corroborated in rats (Guzman et al. 1997).

Evidence in Genetic Models Implying a Role for NO in Vascular Remodeling

Recent studies with eNOS knockout mice confirm that eNOS serves as a major regulator of physiological and pathological remodeling. eNOS knockout mice are viable, with slightly less than Mendelian segregation of the appropriate genotypes (Huang et al. 1995; Shesely et al. 1996; Godecke et al. 1998; Gregg et al. 1998). The mice are moderately hypertensive, have resting bradycardia in vivo (Shesely et al. 1996; Godecke et al. 1998), and do not exhibit either pressor responses to L-NAME or endothelium-dependent relaxations in response to acetylcholine (Huang et al. 1995). One of four studies indicated a trend toward limb-reduction defects in eNOS knockout mice (Gregg et al. 1998), but all studies confirm that there were no gross vascular phenotypes, showing that eNOS-derived NO is not an essential regulator of vasculogenesis, angiogenesis, or remodeling during embryogenesis. Rather, the vascular roles of NO manifest themselves in the adult mouse. Rudic et al. (1998) recently developed a mouse model of physiological remodeling in which ligation of the left external carotid artery for 2 weeks provokes a reduction of lumen diameter and medial cell number of the ipsilateral common carotid artery. This reduction in lumen diameter was triggered by a 30% reduction in blood flow (R. D. Rudic and W. C. Sessa, unpublished data). Surprisingly, eNOS mutant mice were unable to reduce lumen diameter in response to external carotid artery ligation. Recent data (fig. 1) showed a gene-dosage effect of disruption of the eNOS locus on impaired luminal remodeling—that is, loss of one copy of the eNOS gene yields an intermediate phenotype between control and homozygote knockout mice. Equally provocative, the vascular media in abnormally remodeled vessels from eNOS knockout mice were hyperplastic, as shown by marked increases in wall thickness, medial nuclei, and bromodeoxyuridine incorporation. The increase in wall thickness is reminiscent of the arterial thickening seen in human hypertension and atherosclerosis. Similar results were reported by Moroi et al. (1998), using an injury model initiated by a vascular cuff placed around the femoral artery of wild type or eNOS knockout mice (Moroi et al. 1998). This treatment causes marked intimal proliferation for both genotypes, but the response was greatly exaggerated in eNOS knockout mice. Finally, in studies of ischemic angiogenesis and remodeling, eNOS knockout mice did not revascularize to the same extent as did wild type mice after hindlimb ischemia. Moreover, administration of vascular endothelial growth factor (VEGF) increased capillary density in wild type mice, but not in eNOS-deficient mice, supporting

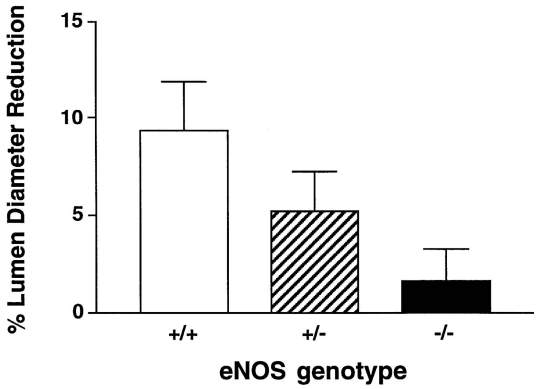

Figure 1 Allele-dependent abnormal luminal remodeling in eNOS knockout mice. Wild type (+/+), heterozygote (+/−), or homozygote (−/−) eNOS knockout mice were exposed to low-flow remodeling as described elsewhere (Rudic et al. 1998), and the percent reduction in lumen diameter of the remodeled left common carotid artery (relative to the contralateral artery) was measured by quantitative morphometry.

the idea that VEGF uses NO as a critical second messenger for endothelial cell phenotypes associated with angiogenesis (Ziche et al. 1994; Papapetropoulos et al. 1997). Collectively, results of these studies indicate that eNOS is critical to coupling luminal hemodynamics to a remodeling response, and they imply that NO is likely the major factor produced by the endothelium required for physiological remodeling as originally described by Langille and O'Donnell (1986).

Conclusions and Future Directions

The strong relationship between endothelial dysfunction and abnormal vascular remodeling is a prominent feature of many cardiovascular diseases. Endothelium-derived NO is a powerful regulator of vascular function, and it appears that abnormalities in the production or actions of NO lead to endothelial dysfunction and abnormal vascular remodeling. The lack of linkage between eNOS and the disease phenotypes analyzed thus far argues against a simple model in which eNOS mutations directly cause heritable vascular disease, but it is possible that further analysis of identified polymorphisms may show significant vascular phenotypes.

The critical issues yet to be addressed are the molecular mechanisms by which NO orchestrates the remodeling response and the link between cardiovascular diseases and suppressed NO production or bioactivity. Our ability to engineer mice with desired genotypes should soon make such questions tractable. For example, breeding eNOS knockout mice to *ApoE* knockout mice will allow us to test the importance of NO in vessel adaptation and the progression of atherosclerotic lesions. Similar methods may show genetic and environmental effects that alter eNOS signaling by influencing ECM molecules, growth factors, or the fibrinolytic system.

Acknowledgments

This work was supported by National Institutes of Health grants HL57665 and HL 51948. W.C.S. is an Established Investigator of the American Heart Association. The authors wish to thank Dr. Steven Segal for his advice during these studies.

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