Toward functional genomics of flow-induced outward remodeling of resistance arteries

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De Mey, Jo G. R., Paul M. Schiffers, Rob H. P. Hilgers, and Marijke M. W. Sanders. Toward functional genomics of flow-induced outward remodeling of resistance arteries. Am J Physiol Heart Circ Physiol 288: H1022–H1027, 2005; doi:10.1152/ajpheart.00800.2004.—In resistance-sized arteries, a chronic increase in blood flow leads to increases in arterial structural luminal diameter and arterial wall mass. In this review, we summarize recent evidence that outward remodeling of resistance arteries 1) can help maintain restored tissue perfusion, 2) is not intimately related to flow-induced vasodilatation, 3) involves transient dedifferentiation and turnover of arterial smooth muscle cells, and 4) is preceded by increased expression of matricellular proteins, which have been shown to promote disassembly of focal adhesion sites. Studies of experimental and physiological resistance artery remodeling involving differential gene expression analyses and the use of knockout and transgenic mouse models can help unravel the mechanisms of outward remodeling.

flow-induced vasodilatation; matricellular protein; gene expression microarray analysis

IN ARTERIES, TRANSMURAL PRESSURE and blood flow influence wall thickness and structural luminal diameter (13, 16, 28, 29, 49). Mathematical modeling, cell and molecular biological approaches, and experiments in genetically modified animals are beginning to shed light on the mechanisms that underlie these arterial remodeling responses. Here, we summarize recent observations concerning flow-induced outward remodeling of resistance arteries. Exploration of this type of arterial structural response might lead to pharmacological treatments to 1) improve collateral perfusion and 2) reverse the inward arterial remodeling in essential hypertension.

TERMINOLOGY

Remodeling is frequently used to describe any alteration of arterial structure. In agreement with others (4, 38), we find it useful to note the differences among several types of arterial remodeling: 1) neointima formation, 2) alteration of the arterial structural luminal diameter (outward or inward), and 3) alteration of tunica media mass (hypertrophic, eutrophic, and hypotrophic). Although this classification is helpful, it also highlights gaps in current knowledge, such as the manner in which media, adventitia, and ultrastructural wall components, such as extracellular matrix, focal adhesion sites, and cytoskeleton, determine the diameter. Arterial structural diameter refers to the maximally dilated diameter and results from the elastic properties of the arterial wall. The diameter of an arterial segment in vivo obviously depends on this structural diameter and on the degree of vasomotor tone or smooth muscle contractile activity.

Resistance arteries are small (300–100 μm diameter) muscular arteries that are situated between large elastic conduit arteries and arterioles. In contrast to the large arteries, resistance arteries 1) not only respond to, but also influence, local mean arterial pressure and blood flow, 2) rarely, if ever, develop a neointima, and 3) do not display hypertrophy but, rather, exhibit inward eutrophic remodeling during chronic hypertension (37). In resistance arteries, circumferential wall stress and wall shear stress are maintained at higher values than in arterioles (43), and the number of feeding resistance arteries in a vascular bed is less susceptible to chronic modulation than the number of arterioles and capillaries (42). An exception is the upgrading of preexisting collateral arterioles into resistance arteries after occlusion of a major feed artery. This arteriogenesis (20) represents outward hypertrophic remodeling.

DRIVING FORCES OF REMODELING

Anatomic analyses and experimental animal studies suggest that wall shear stress and circumferential wall stress are maintained constant throughout the systemic arterial tree (16, 28), with the exception of the true microcirculation, where these parameters change progressively toward venous values (43). Angioadaptation, representing arterial responses to the interacting influences of altered wall shear stress and circumferential wall stress (43), can be brought about acutely by altered vasomotor tone and chronically by structural autoregulation or arterial remodeling. Circumferential wall stress and wall shear stress are considered major driving forces during morphogenesis and remodeling of arteries. They do, however, not suffice to predict the architecture of arterial beds or the existence of arcading collateral arteries, which are abundant in vascular beds that undergo marked fluctuations in blood flow (e.g., the female urogenital system and the gastrointestinal tract). Therefore, additional influences, such as metabolic
signals conducted upstream along the arterial tree (for review see Ref. 43), must be considered. The relation between arterial vaso- 
motor and structural responses to stress changes is the subject of intense research.

FLOW-INDUCED VASODILATATION AND FLOW-INDUCED REMODELING OF RESISTANCE ARTERIES

A chronic increase in blood flow has been reported by several groups to result in outward hypertrophic remodeling of resistance arteries (8, 9, 41, 52, 54, 55, 57). A widely used experimental animal model involves ligation of feed arteries in the mesenteric bed of rodents, whereby flow is shunted through adjacent parallel arcades. The increase in flow that is imposed might mimic 1) the local resistance arterial response in a collateral circuit during occlusion of a major artery and 2) the response of resistance arteries supplying a tissue with enhanced metabolism as a result of growth or increased activity. The putative mechanism of outward hypertrophic remodeling is summarized in Fig. 1. Buildup of vasodilator metabolites causes arteriolar dilatation, which reduces their resistance. Local arteriolar and ascending vasodilatation results in an increased blood flow through the upstream resistance arteries. The resulting increase in wall shear stress triggers release of endothelium-derived vasodilators, such as nitric oxide (NO), prostacyclin, bradykinin, and endothelium-derived hyperpolarizing factor (22, 24). The increase in luminal diameter tends to restore wall shear stress and leads, together with the increase in transmural pressure, to an elevation of circumferential wall stress and of the strain on the resistance arterial smooth muscle cells (SMC). These outcomes can stimulate the expression of oncogenes and the production of growth factors in the arterial wall (4, 56) and, thereby, contribute to the hypertrophy of resistance arteries chronically exposed to increased blood flow. Surprisingly, tissue angiotensin-converting enzyme does not seem to be required for flow-induced resistance artery hypertrophy (23). It should be pointed out that the interaction between shear stress and wall stress effects was shown by mathematical modeling to predict the eutrophic inward remodeling (not hypertrophy) of arterioles during chronic elevation of cardiac output at the initiation of essential hypertension (43). The explanation for these differences may reside in influences of metabolic and growth signals, but this remains to be demonstrated.

Much less understood are the mechanisms whereby arterial diameter increases not only through vasodilatation but also through an increase in the structural (maximally dilated) diameter. Several possibilities that are not mutually exclusive may be considered. 1) Long-term vasodilatation leads to outward remodeling, for instance, by shifting the load borne by the contractile apparatus to the cytoskeleton and focal adhesion sites of the arterial SMC. 2) The endothelium-derived vasodilators trigger outward remodeling when high concentrations are chronically maintained. 3) The arterial hypertrophy resulting from the elevated circumferential wall stress may lead to outward remodeling. 4) Shear-induced endothelial responses include vasodilators as well as modulators of the extracellular matrix such as transforming growth factor-β or the recruitment of monocyte/macrophages through increased expression of monocyte chemoattractant protein-1 (25). 5) Vasodilatation may not be sufficient, and an alternative slow structural process is engaged to normalize wall shear stress.

Although the endothelium senses and responds to shear stress (11, 14) and flow-induced outward remodeling has been shown to be endothelium dependent (29, 50), there is no strong proof for any of the above-mentioned strategies. Rather, several observations argue against possibilities 1–3. Only a few examples are provided here. Chronic vasodilator treatment does not uniformly lead to outward remodeling of resistance arteries (36). Inward remodeling can be demonstrated during organ culture of resistance arteries in the presence of vasoconstrictor stimuli, but chronic vasodilatation does not result in outward remodeling in this setting (1, 2, 33). In rats, transgenic expression of renin or chronic infusion of angiotensin II leads to resistance arterial hypertrophy but not to outward arterial remodeling. In spontaneously hypertensive rats (SHR) and NOS-nitro-l-arginine methyl ester-treated rats, circumferential wall stress seems to be normalized by inward remodeling. Furthermore, chronic inhibition of NO synthase (NOS) (9) and several observations in knockout mice indicate that adaptive flow-induced outward hypertrophic remodeling of resistance arteries can proceed despite reduced flow-induced vasodilatation (Table 1). In contrast, others found that, after arteriovenous shunting in large arteries and growth factor-induced arteriogenesis, NO seemed to play an obligatory role (51, 58). Consistent with the possibility that vasodilatation may not be sufficient and an alternative slow structural process is engaged to normalize wall shear stress, are observations that rat first-order mesenteric artery side branches, which display outward remodeling in response to a doubling of blood flow in vivo (9), fail to dilate in response to drug application in situ. In view of
the large number of unknowns, curiosity-driven research may help build testable hypotheses about the mechanisms of outward remodeling.

STRUCTURAL CHANGES DURING FLOW-INDUCED REMODELING

In the rodent mesenteric artery model of flow-induced outward resistance artery remodeling, signs of SMC turnover and dedifferentiation have been reported. In arteries exposed in vivo to twice the normal blood flow, increased bromodeoxyuridine labeling, increased immunoreactivity for proliferating cell nuclear antigen, and a significant increase in arterial SMC number indicate stimulated arterial SMC proliferation (8, 54). Signs of arterial SMC apoptosis, such as DNA laddering and increased TdT-mediated dUTP nick end labeling, were equally evident in chronically hyperperfused arteries (twice normal blood flow) undergoing outward hypertrophic remodeling and in chronically hyperperfused arteries (0.1 times normal blood flow) undergoing hypertrophic inward remodeling (8). Apoptosis and proliferation of SMC in different layers of the media may help the wall expand, but this remains to be established. Along with increased arterial SMC turnover, a drop in the immunoreactivity of the arterial wall for desmin and caldesmon was documented, indicating (transient) dedifferentiation of arterial SMC (8). Dedifferentiated synthetic arterial SMC are more prone to proliferate, migrate, and synthesize and degrade their matrix (4, 40). Matrix alterations during resistance artery remodeling have remained elusive, partly because of the low density of this material in the wall. Work in large arteries, such as the mouse common carotid artery, strongly suggests that marked flow-related inward or outward arterial remodeling can proceed without significant modifications in the arterial collagen and elastin contents (23). It may be hoped that vital molecular imaging of arterial wall structure will reveal the ultrastructural basis of the arterial structural luminal diameter. Cells, nuclei, collagen, and elastin fibers can easily be visualized, and other matrix components such as fibronectin can be traced with the appropriate antibodies. Multiphoton lifetime laser scanning confocal microscopy (33, 59) has the necessary penetration and spatial resolution to approach these issues in intact resistance arteries in relation to transmural pressure and blood flow.

DIFFERENTIAL GENE EXPRESSION

The small size of resistance arteries, which might be beneficial for imaging purposes, complicates molecular biological analyses during remodeling. However, with techniques that are standard practice in single cell studies, a sufficiently large mRNA pool can be generated from isolated resistance arteries. Recently, using DNA microarrays after T7 RNA polymerase amplification of the extracted mRNA, we compared gene expression in rat mesenteric resistance arteries exposed for 1–32 days in vivo to normal or twice the normal blood flow in vivo (57). For a large fraction of the genes (>800 of 10,500), the expression was modified by more than twofold during exposure to altered blood flow in vivo. These included 1) several growth factors (receptors), oncogenes, cell cycle progression factors, and modulators of apoptosis, consistent with the above-mentioned increased turnover of arterial SMC, and 2) many cytoskeletal and contractile protein markers of arterial SMC phenotype, consistent with the above-mentioned dedifferentiation. These alterations reached statistical significance after a few days, suggesting that they were involved in the execution, but not in the initiation, of the outward hypertrophy. The number of genes from which the expression was rapidly modified (<24 h) was, however, comparatively small (44 of 10,500, 18 of which were downregulated and 26 upregulated). They included a downregulation of the 82-kDa, but not the 70-kDa, subunit of soluble guanylyl cyclase, which is compatible with chronic activation of the NO-guanylyl cyclase-CGMP-protein kinase G pathway (7). They also included transient upregulation of the expressions of thrombospondin (TSP)-1, but not TSP-2 or TSP-3; tenascin C (TNC), a desintegrin and metalloproteinase with TSP motifs (ADAMTS-1), and membrane type 2 metalloproteinase (MMP)-15, but not MMP-2, MMP-7, or MMP-9 (Fig. 2). In view of the recent literature on matricellular proteins (10, 26, 30, 39), these findings are compatible with the working hypothesis outlined in Fig. 3. TSP-1 stimulates activity of MMP-2, which produces fibrillar collagen, which in turn promotes TNC production. TSP-1 and TNC promote disassembly of focal adhesion sites through mechanisms that require protein kinase G and inhibition of the RhA-Rho kinase pathway. Subsequently, arterial SMC can slide, dedifferentiate, and proliferate more easily. This hypothesis is compatible with the temporal changes in

Table 1. Flow-induced vasodilatation and flow-induced outward remodeling in rodent experimental animal models

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Model</th>
<th>Species</th>
<th>Artery</th>
<th>FID</th>
<th>FIR</th>
<th>Ref.</th>
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<td>Desmin−/−</td>
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<td>Mesenteric</td>
<td>reduced</td>
<td>Normal</td>
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<tr>
<td>Dystrophin−/−</td>
<td>Ligation</td>
<td>Mouse</td>
<td>Mesenteric</td>
<td>Reduced</td>
<td>Reduced</td>
<td>31</td>
</tr>
<tr>
<td>Vimentin−/−</td>
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<td>Mesenteric</td>
<td>Reduced</td>
<td>ND</td>
<td>21</td>
</tr>
<tr>
<td>Vimentin−/−</td>
<td>Ligation</td>
<td>Mouse</td>
<td>Carotid</td>
<td>ND</td>
<td>Increased</td>
<td>47</td>
</tr>
<tr>
<td>TissueACE−/−</td>
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<td>Uterine</td>
<td>Increased</td>
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<td>Rat</td>
<td>Mesenteric</td>
<td>ND</td>
<td>Normal</td>
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<td>Reduced</td>
<td>Normal</td>
<td>19</td>
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</tbody>
</table>

ACE, angiotensin-converting enzyme; t-NAME, chronic treatment with Nω-nitro-L-arginine methyl ester; ND, not determined; FID, flow-induced vasodilation; FIR, flow-induced outward remodeling; eNOS, endothelial nitric oxide synthase; t-kallikrein, tissue kallikrein.
gene expression, dedifferentiation, proliferation, and outward remodeling and with the observation that although pharmacological inhibition of Rho kinase modifies inward remodeling, it does not alter outward remodeling (57). Dedicated drug-intervention, transgenic, knockout, and rescue studies are required to strengthen this hypothesis. Because only a few pharmacological tools modulate the presence and activity of matrix cellular proteins, transfection techniques (6) may be considered for these purposes.

**PHYSIOLOGICAL FLOW-INDUCED RESISTANCE ARTERY REMODELING**

The mesenteric artery ligation model that was frequently used in rats can also be applied, to some extent, to mice. Knockout mice can provide evidence for the involvement (or noninvolvement) of key molecules in flow-induced arterial remodeling (31, 32). Transgenic expression of reporter constructs has recently been applied to visualize effects of blood flow on local gene expression in vivo (48). Other more indirect approaches may be considered to benefit from the growing collection of mouse models.

**Development.** During growth and development, local blood flow and arterial size increase. This setting might be instrumental in investigation of the mechanisms whereby tissue size and activity, local blood flow, and arterial size are matched to each other. Two extreme models illustrate the potential of this approach. In growth hormone transgenic mice (12), body weight (>50 g), peripheral organ weight, and resistance artery size are proportionally increased compared with wild-type mice (35 g body wt). The size of the brain and of the carotid arteries is, however, not modified in this model. In mice that lack a hypoxia-responsive element in the promoter sequence of their vascular endothelial growth factor (VEGF) genes (27), blunted angiogenesis probably results in severe growth retardation (<20 g) and small hearts, kidneys, skeletal muscles, and gastrointestinal tract, while the brain is relatively spared (15). In these VEGF<sup>-/-</sup> mice, the luminal diameter and media mass are reduced in mesenteric and saphenous arteries, but not in carotid arteries (unpublished observations).

**Pregnancy.** During mammalian pregnancy, uterine blood flow increases from <1% of cardiac output to >20% of the 1.3-fold increased cardiac output. Angiogenesis, estrogen-induced upregulation of endothelial NOS, vasodilatation, and remodeling of spiral arteries and uterine arteries contribute to the increased blood flow to the growing uterus and fetuses (5). The luminal diameter and media mass of uterine arteries increase markedly in pregnant mice (22, 23). This increase is accompanied by dedifferentiation of the SMC (19). After pregnancy, most of the uterine arterial structural changes are rapidly reversed (23). Maternal deficiency in key modulators of endothelium-dependent flow-induced vasodilatation, such as tissue angiotensin-converting enzyme, tissue kallikrein, and endothelial NOS, has only limited consequences for uterine artery remodeling, fetal growth, and litter size (18, 22, 23). Yet, in middle-aged mice, in which flow-induced vasodilatation reaches an amplitude similar to that in young mice, uterine artery remodeling and fetal survival are simultaneously reduced (19). These observations add to our conclusion that flow-induced vasodilatation and outward remodeling are not directly related (Table 1). The relations between uterine arterial remodeling, uterine blood flow, and fetal growth may help explain the growing body of evidence for a link between intrauterine fetal growth retardation and the risk of developing hyper-

![Fig. 2. Differential gene expression of the 82-kDa subunit of soluble guanylate cyclase (sGC), thrombospondin-1, tenascin C (TNC), and a desintegrin and metalloproteinase with thrombospondin motifs (ADAMTS1) in rat mesenteric arteries exposed to twice the normal blood flow in vivo. Increase in arterial luminal diameter is statistically significant from that 8 days after exposure to increased blood flow. [Data from Wesselman et al. (57).]](image)

![Fig. 3. Proposed mechanism whereby a chronic increase in wall shear stress stimulates expression of thrombospondin (TSP-1) and TNC and expression and translocation of the transcription factor Egr-1 as well as activity of matrix metalloproteinases (MMP) and extracellular signal receptor kinases (ERK) and outward remodeling in resistance arteries. FAS, focal adhesion site; CRT, calreticulin.](image)
tension and coronary artery disease at later stages of life (3, 17, 46).

Perspective

Increases in blood flow or wall shear stress can cause outward remodeling of resistance arteries. The mechanisms do not seem to involve flow-induced vasodilatation. With functional genomics approaches such as differential gene expression analyses and transgenic mouse models applied to imposed and physiological situations of outward remodeling, alternative hypotheses can be formulated and tested. Potential applications in atherosclerotic ischemic disease and in hypertension make this worthwhile. Arteriogenesis or outward remodeling has been proposed to determine the success of proangiogenic therapy with growth factors and endothelial progenitor cells (35). In hypertensive patients, stimulation of outward remodeling may help reverse the inward remodeling of resistance arteries that has been observed to be the most potent predictor of cardiovascular events (44).

GRANTS

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