Arteriogenesis: A focus on signal transduction cascades and transcription factors

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Summary
In recent years intensive investigations have been performed to unravel the molecular mechanisms of collateral artery growth (arteriogenesis), a process designed by nature to compensate the devastating consequences of major arterial occlusions. Currently, a variety of gene products as well as signal transduction pathways involved in arteriogenesis have been identified. However, it is still not clear how the progression of cellular signals evoked by an increased blood flow and therefore mechanical stress proceeds. Literature research identified the transcription factors early growth response-1 (Egr-1) as well as serum response factor (SRF) and myocardin-related transcription factors (MRTFs) as liaisons connecting the key pathways of arteriogenesis, i.e. the Rho-kinase pathway and the MEK/ERK pathway, with each other as well as with downstream genes.

Keywords
Arteriogenesis, Rho-kinase, MEK/ERK, serum response factor, Egr-1

Introduction
About 10 years ago Schaper et al. defined the term arteriogenesis describing the growth of pre-existing arteriolar anastomoses to form large collateral conductance arteries (1). Since then a variety of studies have been performed to identify the responsible molecular mechanisms aiming to enable therapeutic induction of collateral growth in patients with coronary or peripheral artery disease. Already in the mid-1960s Fulton demonstrated that pre-existing collateral arterioles enlarge in order to compensate for the reduction in organ blood supply caused by arterial obstructions (2). Only a few years later it was shown by Schaper et al. that the underlying process is not a simple vasodilatation but involves a massive proliferation of vascular endothelial cells (ECs) and smooth muscle cells (SMCs) (3). Several years elapsed until the techniques of molecular biology found their way into cardiovascular research re-flourishing the work on collateral artery growth. Meanwhile, several animal models on arteriogenesis exist (e.g. [4–10]) and a multitude of factors and signal transduction cascades involved in collateral artery growth have been identified. However, no updated work is available putting individual findings into a framework of interrelated signaling pathways, which is the purpose of this review.

Hypoxia and hypoxia-related processes
It was shown very early that collateral artery growth is not necessarily associated with local ischemia or hypoxia (11, 12). Furthermore, data on different mouse strains evidenced that mice developing only minor ischemia distal from the occlusion site (C57BL/6) had a very good reperfusion recovery, whereas mice with severe ischemia (BALB/c) showed a very poor recovery (13, 14). Results on mice treated with VEGF-TRAPRRR blocking angiogenesis occurring distal from the arteriogenic process evidenced no negative influence on collateral artery growth (15). These data altogether indicate that neither hypoxia or ischemia nor hypoxia-associated processes like angiogenesis are necessary or a prerequisite for proper arteriogenesis. Therefore, it was likely that physical forces like pressure-related stresses (cicumferential, radial and longitudinal) and/or fluid shear stress experienced by collateral arteries due to increased blood flow are triggering arterial growth (16–18).
Mechanical forces, signal transduction pathways and transcription factors

Recently it was shown very elegantly by Eitemenmueller et al. using an arterio-venous shunt model that chronically increased blood flow significantly enhanced reperfusion recovery and led to complete compensation and even overcompensation of maximal vascular conductance confirming the previously only assumed role of fluid shear stress as initial signal and driving force for arteriogenesis (19). Furthermore, Eitemenmueller et al. found that collateral growth was associated with an upregulation of members of the Rho-kinase pathway confirming previous results (19, 20). Since shear stress has been shown to cause a reorganization of the endothelial cytoskeleton (21), it is of special interest to know that the Rho-kinase pathway is involved in regulating actin dynamics by controlling the activity of cofilin (22, 23), a protein which has been shown to be differentially expressed in ECs and SMCs of growing collaterals (17, 20). Actin dynamics play an important role in controlling serum response factor (SRF) activity. STARS, a muscle-specific actin binding protein and activator of RhoA and SRF (24), has recently been identified by the group around Schaper rowing collateral arteries (25). The striated muscle activator of Rho signaling stimulates nuclear translocation of myocardin-related transcription factors (MRTFs) comprising a family of transcriptional coactivators that stimulate SRF-dependent transcription (26).

SRF is a transcription factor that controls a wide range of genes involved in cell proliferation and differentiation. It binds to the consensus-sequence CC(A/T)GG, known as CArG box, located in the promoter of numerous serum-inducible and muscle-specific genes. A variety of these genes have been found to be differentially expressed during arteriogenesis.

The most prominent one for arteriogenesis is Egr-1 (early growth response-1). Egr-1 is an immediate early gene encoding a nuclear transcription factor of the zink-finger class (27, 28) that regulates the expression of a variety of genes, among them cyclin D1 (28, 29). Therefore, the transcription factor represents a critical component in cell cycle progression. Its eminent role in migration and proliferation of vascular cells has previously been described (30–35). Own results evidenced that Egr-1, which is expressed in ECs and SMCs is upregulated in growing collateral arteries (36). In agreement with this observation arteriogenesis was found to be reduced in Egr-1-deficient mice (37) whereas it was enhanced in wild-type mice following adenoviral overexpression (38).

SRF is not the only factor regulating the expression of Egr-1. Day (39) as well as Vogel (40) have shown that Egr-1 expression induced by Angiotensin II and fibroblast growth factor 2 (FGF-2), respectively, is mediated in SMCs by the MEK/ERK pathway independently of c-Jun amino-terminal kinase (JNK) activity. The role of the FGF-system for collateral artery growth is well established. Whereas VEGF for example failed to promote peripheral arteriogenesis significantly (12), it has been shown in several in-vivo models that exogenous application of FGF-2 strongly enhanced peripheral as well as myocardial collateral flow (41–46). Recently it was reported that the MEK/ERK signal transduction cascade is activated during arteriogenesis (19) arguing for the FGF/MEK/ERK/Egr-1 pathway in vivo.

Although there are data available indicating that Egr-1 might also be upregulated by the Akt/PI3K pathway (47), this seems not to be the case during arteriogenesis as recently shown by Eitemenmueller (19).

The cardiac ankyrin repeat protein (carp) presents a transcription cofactor that is differentially expressed during collateral artery growth (48). Overexpression of carp in cos cells resulted in increased protein levels of Egr-1 indicating that carp is an upstream modulator of the zink-finger transcription factor. Kanai et al. showed that transforming growth factor-β1 (TGF-β1) activates carp via SMAD signaling (49). Furthermore, it was evidenced by Fan et al. that TGF-β1 facilitates nuclear accumulation of MRTFs (50). During uninnfluenced arteriogenesis, activated TGF-β1 is present in increased amounts (51). Infusion of TGF-β1 in a model of peripheral collateral artery growth did not only result an in increased expression of carp (48) but also in enhanced arteriogenesis (51). A further function of TGF-β1 is to modulate the expression of the FGF receptor-1 (FGFR-1) in a positive manner (52). Increased levels of FGFR-1 have likewise been found during arteriogenesis, and blocking the FGFR-1 strongly interfered with vessel growth (53).

A gene that has been described downstream of Egr-1 as well as of SRF is the urokinase plasminogen activator (uPA). uPA converts the proenzyme plasminogen to its active derivative plasmin (54). Plasmin is a potent and specific chemoattractant for peripheral monocytes (55). During the process of arteriogenesis, monocytes invade into the walls of collateral vessels were they accumulate and mature to macrophages. Independent of its enzymatic activity uPA also induces stimulation of cell chemotaxis (56), adhesion of monocytes and neutrophils (57, 58), as well as expression of matrix metalloproteinases (MMPs) (59). An up-regulation of MMP-2 and MMP-9 during arteriogenesis has previously been shown by Cai (60). Own results evidenced that mice deficient for uPA show a reduction in collateral artery growth caused by a reduced level of perivascular macrophages (61) – the biofactories in arteriogenesis supplying growth promoting factors like FGF-2 (4).

Molecules that are further involved in monocyte recruitment and extravasation are monocyte chemoattractant protein-1 (MCP-1) and intercellular adhesion molecule-1 (ICAM-1), both of which have been shown to be upregulated during the process of arteriogenesis (62) and have been described to be downstream the Rho-kinase pathway (63, 64).

The bottle neck

This synopsis on signal transduction cascades in arteriogenesis highlighted the role of Rho-GTPases as well as the FGF/MEK/ERK pathway. However, it was not discussed how both pathways are activated during arteriogenesis, i.e. how the mechanical stress is translated into the biological response peaking in positive vessel remodeling. Although it is not likely that mechanical forces are transmitted via a single mechanoreceptor (65), integrins might play a central role. Outside-in signaling elicited by the extracellular matrix is primarily mediated by integrins, and virtually all molecules of the matrix are able to communicate with a cell by binding to integrins (66, 67). Recently Fernandez and Broich have shown that αβ3 integrin is selectively increased in ECs and SMCs of growing vessels (68). Since the heterodimer
is an upstream effector of Rho-GTPases, it might play a central role in the induction of arteriogenesis.

The signal transduction cascade associated with the FGF-system has been described to be dependent on the bioavailability of the FGF-1, the expression of which is positively influenced by TGF-β1 \((52, 53)\). The question how latent TGF-β1 is activated during arteriogenesis has also not been elucidated up to now. However, it might be a function of plasmin \((69)\), the product of proteolytic cleavage of plasminogen by uPA.

**References**

23. Toshima J, Toshima JY, Amano T, et al. Cofilin Phosphorylation by protein kinase testicular protein ki-


