Sphingosine-1-Phosphate and the Leading Edg-1 of Vascular Smooth Muscle Cells

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The migration of vascular smooth muscle cells (VSMCs) from the media to the intima in concert with their subsequent massive proliferation within the intima characterizes restenosis after angioplasty, occlusion of saphenous vein grafts, transplant organ failure, and the progression of atherosclerosis. The identification of molecules and mechanisms that regulate VSMC migration and proliferation is therefore of considerable clinical relevance. A key discovery that led Ross and Glomset to propose the “response to injury” hypothesis of atherosclerosis was the release of platelet-derived growth factor (PDGF), a potent chemoattractant and mitogen for VSMC upon platelet activation. Subsequent studies showed that PDGF is also produced by endothelial cells, macrophages, and SMCs themselves. A role for PDGF signaling in migration and proliferation of intima VSMCs and in the pathogenesis of plaque development and restenosis is now well established.

Studies disrupting the PDGF-B gene or the PDGF-β receptor show that the PDGF-B pathway is required for normal blood vessel formation during embryonic development. Very recently, another class of mediator and another receptor essential for VSMC migration and vascular maturation has been described that involve the lipid sphingosine-1-phosphate (S1P) and its receptor Edg-1.

S1P and the structurally related sister molecule lysophosphatic acid (LPA) are potent bioactive lipids that have multiple biologic actions on vascular cells and blood cells. As is seen with PDGF, S1P is released in large quantities from activated platelets (through a mechanism, which has yet to be described). It is also constitutively produced and secreted by other blood cells (mononuclear cells, neutrophils, and red blood cells) albeit in small quantities. S1P binds with high affinity to the receptor Edg-1, which couples to the G protein pathway. This receptor, the first of S1P and LPA receptors characterized, was identified as a gene induced during human endothelial cell differentiation, and termed Edg-1 for endothelial cell differentiation gene 1. Analogous to the results of the targeted disruption of PDGF-B and its receptor, the disruption of the edg-1 gene in mice was found to lead to incomplete vascular maturation, embryonic hemorrhage, and intrauterine death. Whereas in PDGF-deficient mice, VSMCs and pericytes (VSMCs surrounding capillaries) are absent, in edg-1–deficient animals VSMCs are formed, but not recruited properly into layers surrounding the endothelial cells of vessels. Edg-1 appears to be essential for an S1P-mediated migration response recruiting VSMCs to the vessel wall.

The study by Kluk and Hla in this issue of *Circulation Research* extends these findings for normal blood vessel development to the pathogenesis of vascular diseases. The authors used VSMC cell lines cultured from 2-week-old rat pups that had an epithelioid morphology and similar properties as seen with cultured rat neointimal cells, typically isolated 2 weeks after arterial injury. Pup-intimal cells and neointimal cells also share the overexpression of certain genes such as PDGF-B. The authors now show that Edg-1 expression by pup-intimal VSMCs mediated the migration and proliferation toward S1P. Adult rat medial VSMCs barely expressed Edg-1 and did not migrate in response to S1P. However, Edg-1 transfection of these cells conferred ability to migrate to S1P. These observations by Kluk and Hla suggest a potential role of the S1P/Edg-1 pathway in the progression of atherosclerosis and neointima formation in restenosis. Most importantly, these in vitro results are supported by a recent study by Zohlnhöfer et al demonstrating that Edg-1 is induced in neointimal lesions of human in-stent restenosis. Based upon these studies, it appears that Edg-1 expression may function analogous to an alarm clock, awakening dormant medial VSMCs and inducing them to move into the intima of the injured vessel wall, where they replicate in an Edg-1–dependent manner.

The migration of VSMCs induced by S1P stimulation of Edg-1 is dependent on the G protein pathway and involves the activation of the small GTP-binding protein Rac. Activation of Rac induces localized and dynamic changes of the actin cytoskeleton such as lamellipodia and ruffle formation at the leading edge of the cell causing forward migration. The results of this study, and a recent second study showing enhanced VSMC migration in response to S1P, are in apparent contrast to earlier reports showing inhibition of VSMC migration induced by S1P. S1P has been shown to bind to four additional Edg receptors with high affinity (Edg-3, -5, -6, and -8), and the predominant expression of Edg-5 in rat medial SMCs has been associated with inhibition of migration in response to S1P. It is possible that the promigratory or antimigratory response of VSMCs toward S1P may depend on the expression pattern of individual S1P receptors. A similar picture has recently emerged for LPA, which stimulates or inhibits T lymphocytes depending on the relative expression levels of the various.
Edg-1 activation as central switch for the migration and proliferation of intimal VSMCs. Sph indicates sphingosine; S1P, sphingosine-1-phosphate; PDGF, platelet-derived growth factor; and SMC, smooth muscle cells.

LPA receptors by these cells. Thus, S1P on its own might not be a villain that provokes cardiovascular disease as previously proposed. The beneficial or harmful action of S1P appears to be dependent on the expression profile of S1P receptors.

### Intimate Interplay Between PDGF and S1P

The relevance of the study by Kluk and Hla for the pathogenesis of atherosclerotic lesion and neointimal formation has received further impetus from the recent study by Sarah Spiegel’s group, demonstrating that VSMC migration toward PDGF is also dependent of Edg-1 expression. Together with previous reports from this group, their recent study shows that the PDGF and S1P signal transduction pathways are intimately linked: PDGF receptor activation stimulates sphingosine kinase, which leads to increased intracellular levels of S1P, Edg-1 receptor activation, Rac stimulation, and cell migration (Figure). Inhibition of sphingosine kinase and Edg-1 deletion suppressed Rac activation and chemotaxis toward PDGF. Although S1P could not be detected in the medium of PDGF-stimulated cells, the authors presented experiments suggesting that endogenous S1P activates Edg-1 in an autocrine or a paracrine manner. Thus, it is conceivable that S1P acting on VSMCs in the neointimal lesions is not only derived from activated platelets but also by PDGF-activated cells. This study establishes a central role of Edg-1 in VSMC migration induced not only by S1P but also by PDGF.

Concerning the proliferative response of pup-intimal cells to S1P, Kluk and Hla delineated a signal transduction pathway that is also dependent on Edg-1 and Gs activation and involves p70 S6 kinase activation and cyclin D1 expression (Figure). Edg-1–stimulated proliferation was not associated with the activation of ERK1/2, phosphatidylinositol 3-kinase, and Akt, protein kinases commonly involved in mitogenic signaling. Like neointimal VSMCs, the pup-intimal cells showed a higher basal rate of proliferation compared with the adult medial cells. Remarkably, this higher proliferation capacity of pup-intimal cells was not due to Edg-1 expression, because pretreatment of the cells with pertussis toxin did not reduce proliferation. The adult medial VSMCs showed also a proliferative response to S1P stimulation that was mediated by Edg-3 and Edg-5 receptor activation and proceeded through a mechanism independent of Gs. The physiological relevance of these in vitro results is unclear because medial VSMCs do not proliferate in vivo.

Extracellular S1P can protect cells from apoptosis. The activation of sphingosine kinase leading to an increase of intracellular S1P has a similar effect. In contrast, the inhibition of sphingosine kinase or enforced expression of S1P phosphatase, which specifically degrades S1P, leads to apoptosis. The signaling pathway involves activation of Edg-1, the heterotrimeric protein Gs, and, notably in endothelial cells, the stimulation of nitric oxide production through protein kinase Akt activation and Akt-mediated phosphorylation of endothelial nitric oxide synthase. If S1P can also promote the survival of VSMCs, this action could further contribute to pathological intimal thickening and neointimal formation.

The factors that regulate edg-1 gene expression in VSMCs are not known. The question of what sets off the alarm clock in the medial VSMCs is of obvious interest. Three binding sites for nuclear factor-κB (NF-κB) are located in the promoter region of edg-1. Multiple stimuli can activate NF-κB in VSMCs, among them are inflammatory cytokines (tumor necrosis factor-α [TNF-α], interleukin-1β [IL-1β]), oxidized LDL, or growth factors such as PDGF-B. Notably, LPA, which is released from activated platelets, and also a biological active component of mildly oxidized LDL and human atherosclerotic lesions, similarly activates NF-κB in various cells. Intimal VSMCs within human atheroma express a growth factor–independent NF-κB activity, and stimulatory components in serum activate NF-κB in cultured SMCs. Moreover, NF-κB has an antiapoptotic role in VSMCs. It is thus possible that after endothelial injury substances released from activated vascular cells or platelets stimulate NF-κB activity thereby upregulating Edg-1 expression in responsive medial VSMCs. Edg-1 activation could then mediate the antiapoptotic action of NF-κB. Kluk and Hla demonstrate an enhanced expression of Edg-1 with increased cell density. Our group has recently shown that the activity of NF-κB and the induction of apoptosis are regulated by the density of VSMCs. An increasing density of VSMCs in the intima may further reinforce the activity of NF-κB and Edg-1 expression, leading to inhibition of apoptosis and further cell proliferation.

Together, these findings imply that Edg-1 could be expressed in intimal VSMCs of atherosclerotic plaque, as well as in restenotic lesions. These questions can be addressed by analyzing Edg-1 expression in these lesions by immunohistochemistry or in situ hybridization. VSMC proliferation in atherosclerotic lesions, if not excessive, is expected to stabilize the plaque, and Edg-1 expression under these conditions should be beneficial. In contrast, excessive VSMC proliferation in the intima such as after angioplasty is undesirable, and Edg-1 could be an attractive target for the prevention of restenosis.

The Edg-1 receptor is inactive without its ligand S1P. Whether S1P accumulates in certain regions of human atherosclerotic lesions, as it has been previously shown for LPA, is not known. The analysis of the cellular localization of
S1P formation and degradation, and the characterization of expression of various S1P receptors in normal and atherosclerotic vessels, will foster our understanding of the specific role of S1P and its receptors in cardiovascular diseases.

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References

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