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Review Article

Lipid signaling in the endothelium

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Introduction

The unique position of endothelial cells (ECs), between the intravascular and extravascular spaces, has given them a wide variety of functions—the ability to sense, monitor and transfer molecules from plasma to surrounding tissues, and vice versa. Loss of these physiological functions is a critical step in the etiology of various clinical syndromes, such as atherosclerosis, thrombosis and disruption of the Blood Brain Barrier. In spite of being continuously exposed to circulating lipids and, in some cases to lipids that have accumulated in sub-endothelial regions, ECs were long thought to function as an inert barrier through which lipid exchange between the plasma and surrounding tissues occurs. An accumulating body of evidence however, indicates that lipids act directly on ECs, and activate intracellular signaling cascades [1]. This review will examine the receptors as well as signaling pathways activated in ECs by different lipid classes. It is useful to discuss these topics in terms of *in vitro* vs. *in vivo* findings, with particular emphasis on recent work, taking advantage of small animal models used to study lipid signaling in the endothelium.

Lipoproteins

All cells require a sufficient amount of lipids for the synthesis of the plasma membrane. Multicellular organisms have developed a specialized transport system for distributing lipids throughout the body. This system is based on lipoprotein particles that travel through the vasculature, reaching all organs. Lipoproteins are lipid-protein complexes composed of triacylglycerol lipids, phospholipids, cholesterol lipids, and an apoprotein molecule, which enables their mobilization in the plasma. There are several classes of lipoproteins, which can be classified by the ratio of lipids to apoproteins, their composition, size, density, and function. One traditional classification is based on the density at which lipoproteins float during ultracentrifugation, yielding the following categories: chylomicrons (CM); very low density lipoproteins (VLDL); intermediate density lipoproteins (IDL); low density lipoproteins (LDL); and high density lipoproteins (HDL). The density of lipoprotein particles is inversely related to their size, reflecting the relative contents of low-density, non-polar core lipid, and high-density, surface protein [2]. Additionally, lipoproteins are classified on the basis of their apoprotein content. Proatherogenic lipoproteins generally contain ApoB. Among this group, LDLs are a well-known risk factor for the development of cardiovascular disease [3,4]. LDLs deposit cholesterol along the inside of artery walls, causing the formation of a cholesterol plaque, which leads to the onset of the pathology [5,6].

Lipoprotein receptors

Two receptor families are utilized by cells to bind and/or internalize native and modified lipoproteins from the extracellular fluid:

LDL receptor (LDLR) family

This family is composed of a large group of endocytic receptors that participate in cholesterol homeostasis, lipid metabolism and signal transduction [7]. All family members share common structural motifs unique to this family, including a single transmembrane domain and a short cytoplasmic tail [7,8]. Compared to "classical" signaling receptors, which often contain large intracellular domains with kinase activity, the cytoplasmic tails of LDLR family members are relatively short, with no kinase domains [9]. However, they contain critical elements that enable their interaction with a set of

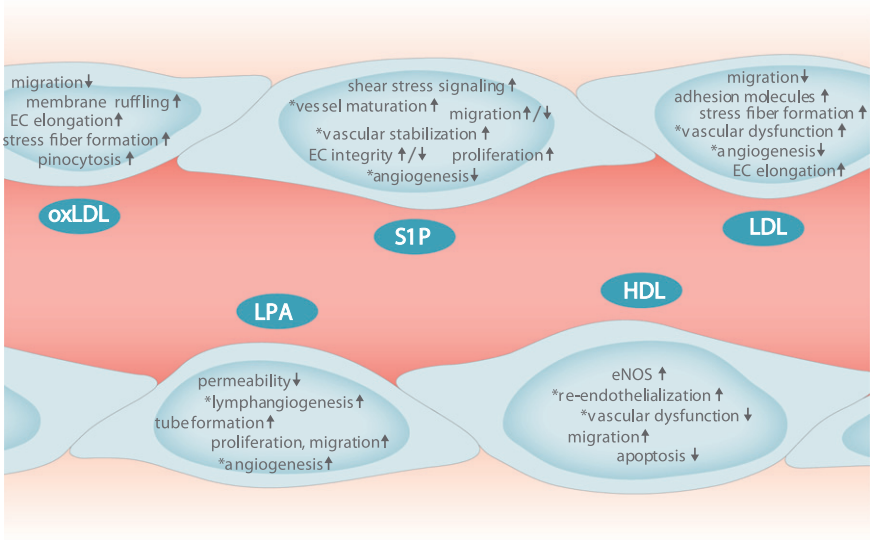

Fig. 1 – Lipid signaling regulates EC behavior. Different lipid classes are indicated in blue, while their effects on EC behavior are listed within the cell illustration. ↑, induction; ↓, inhibition of the EC response. ↑/↓, induction and inhibition are elicited by the same factor (e.g. via activation of different receptors). ∗, EC responses that were observed *in vivo*, under physiological conditions.

cytoplasmic adapter and scaffold proteins, thereby mediating signal transduction. For instance, activation of the ERK cascade in primary microglia and BV2 cells was observed upon stimulation of VLDLR and LRP1 [10], and LRP6 is a critical component in the Wnt signaling cascade [11–13].

The expression pattern of LDLRs is diverse, involving many cells in the body. *In vitro* and *ex vivo* assays have shown that some of these receptors are expressed in ECs as well [14–17]. However, the physiological relevance of these findings remains unclear Fig. 1.

The scavenger receptor (SR) family

The SR family of molecules is large, and structurally diverse. Collectively, the various receptors recognize many different ligands. The common trait characterizing SRs is their ability to bind modified LDLs, such as acetylated LDL (acLDL) and oxidized LDL (oxLDL) [18]. SR receptors are known to be expressed mainly by myeloid cells, ECs, and epithelial cells [19]. Furthermore, they participate in a range of physiological and pathological processes such as atherosclerosis [20–22] and Alzheimer's disease [23].

Lipoprotein signaling in endothelial cells

A large body of data accumulated during the past years, has revealed new roles for lipoproteins as signaling mediators in various cell types, operating at different levels and through various classic and non-classic mechanisms. For instance, in human and rat smooth muscle cells, as well as in human skin fibroblasts [24], LDL was shown to signal through a wide variety of signal transduction pathways (reviewed in [25]). In addition, lipoproteins are able to activate cellular processes by delivering precursors of intracellular steroid hormones [26,27], as well as by presenting morphogens to cells, as in the case of lipophorin, a member of the lipoprotein family, which is required for the long-range signaling activity of both wingless (Wg) and hedgehog (Hh) in Drosophila [28]. While the mechanisms of lipoprotein-mediated signaling have been extensively characterized in neurons, muscle cells and during embryonic development, the effects of lipoproteins on intracellular signal transduction in ECs have only been studied to a limited extent.

Low density lipoproteins-LDL

The link between Low density lipoproteins (LDLs), triglycerides (TGs) in particular, and coronary heart disease (CHD) was already established as long ago as the 1950s, when a few reports showed that fasting TGs (should be TG) levels were increased among patients with CHD [29–31]. Since these initial observations, intensive efforts have been geared towards understanding the nature of the interactions between lipoproteins and the endothelium. Early *in vitro* studies, dating from three decades ago, concentrated mainly on the metabolic aspects of this interaction, examining the ability of cultured ECs to metabolically modify LDL particles [32–34]. Only in recent years has the research focus shifted towards identifying individual signaling pathways within ECs, regulated by LDL. Here, we summarize data describing the effects of lipoproteins on ECs, with a special emphasis on more recent work on emerging *in vivo* animal models, enabling physiological examination of these interactions.

***In vitro* studies**. Research carried out over the years has identified MEK–ERK [35], PKC [36], and G-protein coupled receptors (GPCR) [35] as direct targets of LDL in cultured ECs (reviewed in [25]). Studies performed on human umbilical vein endothelial cells (HUVECs) have demonstrated that LDL activates the transcription factor activator protein-1 (AP1) via regulation of the JNK-c-Jun and p-38-ATF-2 signaling pathways [37–39]. In addition, LDL was shown to induce expression of vascular adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1) and E-selectin in human ECs [40,41]. These molecules play a key role in adhesion of monocytes to the vascular endothelium, accelerating the progression of atherosclerosis [42].

Many studies have demonstrated that oxidation of lipoproteins occurs in vertebrates, including humans [43,44]. While it has been reported that both LDLs and modified LDLs interact with ECs [45,46], it is not clear from *in vitro* results, whether they activate distinct signaling pathways, resulting in different cellular outcomes. For example both oxidized-LDL (ox-LDL) and native LDL, were shown to inhibit the wound-healing response of vascular ECs *in vitro* under different experimental settings [47,48]. Conversely, both native, and ox-LDL promote EC elongation and stress fiber formation, but only ox-LDL induces ECs membrane ruffling and promotes pinocytosis as well [49]. The fact that extensive oxidation of LDL results in the generation of novel epitopes on the ox-LDL particle, which are recognized by specific receptors such as LOX1 and the macrophage SRs, may account for the differences in the response of cultured ECs to native vs. ox-LDL [50]. Interestingly enough, both ox-LDL and LDL were shown to activate receptors that lie outside the "classic" LDL-binding receptors families, such as the EGF receptor [51] and VEGF receptor 1 [46]. These results further support a tight connection between lipoprotein metabolism and signaling in ECs.

Since initial observations decades ago [30,31], a large body of literature has accumulated describing the effects of lipoproteins on cultured ECs. While these *in vitro* findings have helped establish the notion that LDL-dependent signaling pathways activated within ECs may lead to the endothelial dysfunction that precedes the onset of atherosclerosis, their *in vivo* relevance still remains controversial. The need to extend these findings to model organisms, in which lipoprotein and ECs biology can be evaluated simultaneously, *in vivo* is especially important in the case of coronary heart disease in which multiple risk factors seem to be implicated. Specifically, the stoichiometry and concentration of LDL particles in blood vessels represents a critical issue, which is difficult to accurately recapitulate in culture.

***In vivo* studies**. The field of lipoprotein metabolism and atherosclerosis has dramatically expanded since the early 1990s, when several strains of genetically modified mice were developed as models for experimental hyperlipidemia [52]. The most typical example is the ApoE-deficient mouse, in which massive hyperlipidemia is accompanied by the development of severe atherosclerotic plaques at the aortic root, and throughout the aortic tree [53]. With the creation of additional mouse models of dyslipidemia (e.g. LDL receptor-, ApoB- and Mtp- knockout mice (reviewed in [54])), a solid body of evidence has emerged on the metabolic effects of lipids on the vasculature, and the progression of atherosclerotic lesion formation. Nevertheless, only a small fraction of these studies addressed the signaling pathways activated within endothelial cells, upon interaction with

lipoproteins. Among them, the regulation of cell adhesion molecules (i.e. VCAM-1, ICAM-1) in the endothelium at atherosclerosis lesion-prone sites [55], received much attention, due to their direct role in macrophage accumulation.

While the "artificial" hyperlipidemia induced in mice and rabbits enabled modeling of many aspects of human atherosclerosis, extrapolation of these results to understand the interaction between lipids and the endothelium under normal, physiological conditions, is often challenging. These limitations highlight the need for animal models permitting detailed analysis of lipid signaling in an intact organism. Recent work has begun to make use of small animal models (e.g. worms, flies, and zebrafish) to examine lipid homeostasis *in vivo* [56]. In particular, the high degree of conservation of the lipid transport machinery [57] makes genetic studies in zebrafish very attractive for uncovering novel mechanisms of lipoprotein metabolism and signaling in endothelial cells [47,57]. A recent study analyzing a zebrafish mutant defective in ApoB-lipoprotein assembly and secretion, uncovered a deleterious role for ApoB-lipoproteins as direct inhibitors of developmental angiogenesis [47]. This study demonstrated that ApoB-lipoproteins regulate the expression of VEGF receptor 1, leading to excessive or impaired angiogenesis. This kind of studies hold promise for identifying novel players involved in lipid signaling to the vasculature during embryonic development, which might be reactivated under pathological conditions. The zebrafish is also emerging as an advantageous model for the study of atherosclerosis. Zebrafish *larvae* fed a high fat diet show a remarkably high accumulation of lipids in the vascular wall, accompanied by morphological abnormalities in the endothelial monolayer [58,59] similar to those observed at the onset of atherosclerosis in higher vertebrates.

High density lipoproteins-HDL

High density lipoproteins (HDLs) undergo continuous remodeling in plasma and therefore constitute a heterogeneous population, which varies in density, size, shape, composition and surface charge (reviewed in [60]). The major apolipoprotein of HDL is ApoAI, which facilitates its many biological functions; namely lipid binding, cholesterol removal from peripheral cells, and the recognition of receptors in the liver and steroidogenic tissues. HDL carries a wide variety of biologically active lipids, among them lysosphingolipids (e.g., sphingosine-1-phosphate (S1P), lysophosphatidic acid, and sphingophosphorylcholine, (reviewed in [61]). In addition, HDL serves as a transporter of several enzymes involved in plasma lipid metabolism, such as cholesteryl ester transfer protein, lecithin cholesterol acyl transferase, and phospholipid transfer protein [62].

Clinical and epidemiological studies have shown that in contrast to LDLs, HDL particles possess atheroprotective effects. One of the mechanisms by which HDLs are thought to protect against atherosclerosis is reverse cholesterol transport [63]. This pathway involves the removal and transport of excess cholesterol from cells to the liver for bile acid biosynthesis, biliary excretion, and recycling into new lipoproteins, or to steroidogenic tissues for steroid hormone production (reviewed in [64,65]).

***In vitro* studies**. HDLs possess anti-inflammatory, anti-thrombotic and anti-oxidant properties, all of which promote endothelial repair [61]. Several of these properties are mediated through the activation of the Scavenger Receptor class B type 1 (SR-B1) signaling pathway within ECs (reviewed in [66]). One of the main targets of HDL within the endothelium is nitric oxide synthase (eNOS) activity [67]. SR-BI and ApoAI play important roles in HDL-induced activation of eNOS [68], as demonstrated by the fact that antibodies against SR-BI or ApoAI completely abolish HDL effects in ECs [69]. HDL-induced eNOS stimulation is mediated through activation of the Src- signaling pathway [70]. HDL is also capable of triggering rearrangements of the actin cytoskeleton, and of inducing migration of ECs [71]. Interestingly, this activity is mediated by SR-BI activation of Rac GTPase, and is independent of nitric oxide synthase activation [68]. Finally, HDL exerts a protective effect against apoptosis in ECs. On the one hand, HDL can prevent TNF α-induced apoptosis of HUVECs, by decreasing CPP32-like protease activity [72]. On the other hand, HDL induces Akt phosphorylation, and subsequent phosphorylation and dissociation of BAD from BCL-XL, which then becomes available to inhibit the mitochondrial apoptotic pathway [73]. Of note, most of the functions of HDL uncovered using cultured ECs, were recapitulated *in vivo*.

***In vivo* studies**. The role of HDL and SR-B1 *in vivo* was primarily examined in different mouse models of atherosclerosis. Expression of SR-B1 resulted in decreased atherosclerosis in LDLR and apoE knockout mice [74,75], thereby reproducing the atheroprotective role of HDL and SR-B1 observed in cultured ECs. Furthermore, re-endothelialization of carotid arteries after injury was impaired in SR-BI null mice, compared to SR-BI-expressing controls [71]. In similar fashion, HDL-dependent activation of eNOS within ECs conferred a protective effect against hypercholesterolemia-related vascular dysfunction, observed in ApoE-/-; eNOS-/- knockout mice [76].

While the physiological relevance of *in vitro* studies assessing the effects of LDL on ECs remains uncertain, the opposite seems to be the case with HDL. This might be due to the fact that while HDL signaling to the endothelium is mediated almost solely by the SR-B1 receptor, LDL appears to signal through a wide variety of receptors that might be differentially expressed in different vascular beds. This high variability makes it difficult to draw solid conclusions from *in vitro* studies and highlights the need for the use of *in vivo* animal models.

In essence, the physiological role of lipoproteins as signaling mediators in ECs is only now beginning to be appreciated. Future studies combining the use of different animal models with the application of new technologies such as live imaging, lipidomic, and metabolomic analyses, are likely to shed light on the molecular pathways underlying lipoprotein-endothelial interactions. Furthermore, the resulting findings will be instrumental in clarifying the physiological relevance of the signaling pathways proven to operate in cultured ECs.

Lysophospholipids

Lysophospholipids are derivatives of glycero- or sphingophospholipids. The major bioactive forms of lysophospholipid are lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P). LPA and S1P circulate in blood, where they are bound to plasma proteins such as albumin, or embedded within lipoprotein particles [77]. They induce a wide variety of biological effects, typically through activation of cell-surface G-protein coupled receptors. LPA is produced mainly by platelets, through

hydrolysis of lysophosphatidylcholine (LPC) by the secreted lysopholipase D (lysoPLD) autotaxin [78]. S1P, on the other hand, appears to be produced in all cells, with the major sources in the vascular system being hematopoietic cells (i.e., erythrocytes, platelets, mast cells, and leukocytes), as well as ECs [79–81].

Lysophospholipid receptors

LPA receptors

Five mammalian LPA receptors have been identified so far (LPA1-LPA5) [82]. LPA receptors activate at least four distinct G-protein families defined by their alpha subunits ($G\alpha_i$, $G\alpha_{12/13}$, $G\alpha_{q/11}$ and $G\alpha_s$) which, in turn, lead to the activation of multiple effector systems such as phospholipase C (PLC), the RAS–MAPK cascade, phosphatidylinositol 3-kinase (PI3K), and small GTPase RhoA, as well as to the inhibition of cyclic AMP accumulation (reviewed in [82]). Specifically in ECs, MAPK kinase/ERK, NF-kB, and calcium influx-dependent pathways were found to be activated in response to LPA treatment [83,84]. Individual LPA receptors (LPA1–3) appear to be dispensable for mouse embryonic vascular development, suggesting functional redundancy among these receptors [82].

S1P receptors

The existence of extracellular S1P receptors was postulated in the early 1990s [85]. Since the identification of the first S1P receptor, EDG-1 (S1P1) in 1998 [86], four other G-protein coupled receptors that specifically bind S1P (S1P2–5) have been identified in mammals [77]. S1P receptors were found to be expressed in many tissues and cell types, and considered to be involved in many physiological and pathophysiological processes. S1P1–3 are expressed in ECs and vascular smooth muscle cells, whereas the expression of S1P4–5 within the vascular system is still controversial [77]. S1P1 activation was shown to take place via G_i, which activates Ras-MAP kinase, the phosphoinositide (PI) 3-kinase-Akt, and phospholipase C pathways. S1P2 and S1P3, on the other hand, are coupled to multiple G proteins: G_q and $G_{12/13}$ all of which can activate the phospholipase C and Rho pathways, in addition to the G_i-dependent pathways [87]. S1P1 knockout as well as endothelial-specific S1P1 knockout, lead to embryonic lethality due to pronounced defects in vascular maturation [88,89] (see below). S1P2 knockout mice are characterized by vascular dysfunction, whereas S1P3 knockout mice display no vascular phenotype [90]. Interestingly, the S1P receptors differ in their expression patterns. S1P1 and S1P3 are widely expressed in ECs, as opposed to S1P2, which is detected only in ECs of certain vascular beds, such as the retina and the ear [91]. The phenotypic abnormalities observed in these knockout mice provided important biological insights into the specific roles of the different S1P receptors in controlling important vascular functions such as vessel formation, vascular integrity and EC proliferation. Interestingly, while activation of S1P1 and S1P3 induces stimulatory signals, activation of S1P2, triggers mostly inhibitory signaling pathways [92]. This ability of S1P to induce diverse cellular outputs through activation of different receptors can serve as a mechanism to fine-tune blood vessel growth and behavior. For instance, S1P can induce EC migration through activation of S1P1, but can also inhibit it by activating S1P2 when EC migration is no longer required. This mode of action is also supported by the differential expression pattern of the receptors, which as a whole enables a wide combinatorial range of cellular outputs.

Lysophospholipid signaling in endothelial cells

Lysophosphatidic acid (LPA)

***In vitro* studies**. LPA has been implicated in the proliferation and migration of ECs *in vitro* [93]. LPA-induced migration of bovine pulmonary artery endothelial (BPAE) cells was found to be mediated by the recruitment of hydrogen peroxide inducible clone 5 (Hic-5), a paxillin family member, to the focal adhesions of the ECs, via MEK activation of ERK [83]. In addition, LPA-induced migration of EAhy926 human ECs was traced to increased matrix metalloproteinase-2 (MMP-2) expression with concomitant activation of the MAPK kinase/ERK, NF-kB, and calcium influx-dependent pathways [84]. LPA was also shown to decrease HUVEC permeability, by a mechanism that requires activation of RhoA and Rho kinase [94,95]. It is important to note that a tight connection was established between LPA and the VEGF signaling pathway. Ptaszynska et al. [96] demonstrated that VEGF stimulates ATX expression and consequent LPA production, as well as LPA1 signaling in HUVECs, through VEGFR2 binding [77]. Knockdown of ATX in HUVECs, on the other hand, resulted in reduced expression of LPA1, LPA2 and VEGFR2 as well.

The ability of ATX to stimulate motility in human coronary artery smooth muscle cells *in vitro*, and to induce tube formation in HUVECs [97], provided initial mechanistic insights into ATX-induced tumor angiogenesis *in vivo*.

***In vivo* studies**. Although LPA receptor (LPA1-3) knockout mice show only moderate vascular defects such as occasional frontal hematomas, the link between LPA and vascular development is demonstrated by the phenotype of autotaxin (ATX/ Enpp-2)-deficient mice [98]. ATX is necessary for hydrolysis of lysophosphatidylcholine (LPC), to generate lysophosphatidic acid (LPA). ATX-deficient mice do not survive beyond E9.5, and display profound vascular defects in the yolk sac and embryo that were attributed to the loss of LPA signaling through $G\alpha_{13}$ [98]. Autotaxin (ATX) was shown to modulate the behavior of normal ECs and VSMC, and to promote tumor vascularization [97]. In an *in vivo* Matrigel plug assay, injection of ATX-transfected NIH3T3 cells into athymic nude mice resulted in increased blood vessel formation, as compared to control cells [78,97].

In addition to its role in blood vessel growth, the LPA pathway was also shown to be involved in developmental lymphangiogenesis. LPA1 downregulation in zebrafish resulted in lymphatic defects, such as the absence of the thoracic duct, and pericardial edema [99]. This lymphangiogenic role of LPA was further supported by *in vitro* experiments, in which LPA induced the expression of vascular endothelial growth factor-C (VEGF-C) in HUVECs [100]. VEGF-C upregulation by LPA was shown to be NF-κB-dependent, and partially regulated by COX-2. Many pro-inflammatory cytokines such as IL-1β and TNF-α were also shown to upregulate VEGF-C expression in HUVECs. These findings may suggest that under certain conditions (NF-κB and COX-2 activation), LPA can act as a pro-inflammatory cytokine, to promote lymphangiogenesis.

Sphingosine-1-phosphate (S1P)

***In vitro* studies**. Initial *in vitro* studies focused on the role of S1P in the regulation of EC integrity [77]. S1P was shown to

maintain EC barrier integrity primarily by activation of S1P1, which in turn strengthened EC junctions [101]. Activation of S1P2, on the other hand, resulted in disruption of adherens junctions, and increased paracellular permeability [91]. The role of S1P3 in endothelial barrier function remains under debate [77]. Using cultured mouse allantois explants, Argraves et al. [102] uncovered an additional role for S1P in EC migration. In this case, S1P1/3 receptors were found to induce EC migration through activation of p38 mitogen-activated protein kinase (p38 MAP Kinase) [103], G_i, and Rho signaling [104]. Conversely, S1P2 activation was shown to inhibit EC migration via PTEN phosphatase, in a Rho GTPase-dependent manner [105]. Finally, activation of S1P1/3 resulted in enhanced EC proliferation, through activation of ERK [103].

In contrast to other lipid classes, the identification of S1P receptors specifically expressed in ECs has enabled extensive research on S1P signaling to the endothelium *in vivo*. Through the use of both mouse and zebrafish, the intricate functions and interactions of S1P signaling within ECs are beginning to be elucidated.

***In vivo* studies**. *S1P1*-null embryos die *in utero* between E12.5 and E14.5, as a result of severe bleeding. Initial formation of the vascular network, as well as angiogenesis in these mice, appeared normal. Therefore, the vascular abnormalities observed in these animals were originally attributed to a defect in the association of mural cells with ECs [88,89]. Recently however, three independent reports [81,106,107] described novel EC-autonomous roles for S1P1. Using S1P1 knockout mice, mice bearing endothelial specific deletion of S1P1, and zebrafish, Shoham et al. [81] demonstrated that S1P1 negatively regulates sprouting angiogenesis during vascular development. This function of S1P1 was independent of the presence of pericytes, as both severe aberrations in vessel size and excessive sprouting were found in limbs of *S1P1*-null mouse embryos, prior to vessel maturation. Further analysis of *S1P1-Vegfa*, and *S1P1-Hif1a* double knockout embryos at E11.5 [81] indicated a genetic interaction between *S1P1* and VEGF-A, and suggested that these factors may function in concert to regulate vascular development: VEGF-A promotes sprouting, whereas S1P1 inhibits it, to prevent excessive sprouting and fusion of neo-vessels. In a broader sense, because the S1P ligand is blood-borne, these findings suggest a new mode of regulation of angiogenesis, whereby blood flow closes a negative feedback loop that inhibits sprouting angiogenesis, once the vascular bed is established and functional. This role of S1P1 was further supported by experiments carried out using a mouse retina model [106]. S1P1 was also shown to be essential for fluid shear stress signaling in ECs. Of note, this function of S1P1 was activated not only by S1P but also by laminar shear stress in a ligand-independent manner, to transduce flow-mediated signaling in ECs. Additional insights into the mechanism of action of S1P1 were provided by Gaengel et al. [107], who showed that S1P1 and VEGF-A play opposing roles in VE-cadherin localization at endothelial junctions: While VEGF triggers a decrease in junctional VE-cadherin, S1P stimulation leads to increased junctional VE-cadherin, with concomitant inhibition of angiogenesis and stabilization of the vasculature. According to this study, S1P1 signaling acts as a vascular-intrinsic stabilization mechanism, protecting developing blood vessels against aberrant angiogenic responses via two critical routes: stabilization of junctional VE-cadherin, and inhibition of VEGFR2 phosphorylation and downstream signaling.

LPA and S1P signaling were shown to interact with various other signaling pathways (e.g., PDGF). The physiological and pathological importance of these crosstalks are discussed elsewhere, in greater depth [77,96].

Concluding remarks

Lipid-endothelial interactions are directly linked to atherosclerosis, thrombosis and other cardiovascular diseases. The recognition that lipids can exert a wide variety of cellular outputs besides serving as mere structural polypeptides and cellular fuel has provided tremendous motivation for the study of the signaling cascades activated by lipids within ECs. The establishment of new genetic tools in mice, enabling EC specific gene deletion, together with the use of small animal models to examine endothelial-lipid interactions *in vivo*, has already facilitated great progress in the field. Nevertheless, many important questions still remain controversial. For instance, the specific receptors through which certain lipid classes (e.g. LDL) induce their biological effects within ECs have not yet been identified, hampering the attempts to study their downstream signaling pathways. Additionally, putative interactions between lipids and known angiogenic factors/receptors (for instance LDL and VEGFR1) are being revealed, which raise questions regarding alternative mechanisms through which lipids may modulate angiogenic responses especially under pathological conditions. An additional layer of complexity when studying the endothelial response to lipid stimuli is the fact that ECs are heterogeneous in regards to the expression of lipid receptors. This variation may derive from the specific functions of different host tissues with distinct metabolic needs. Finally, while ECs possess the machinery required to oxidize lipid derivatives (i.e. fatty acids), energy production by fatty acid oxidation seems to account for only very little of the ATP generated by ECs, which are in essence highly glycolytic. Future studies will most likely be directed at exploring both the metabolic and signaling aspects of lipid-endothelial interactions, as well as the link between these two processes that seem to be much more interlaced than previously thought.

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