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Regulation of angiogenesis by phospholipid lysophosphatidic acid

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TABLE OF CONTENTS

1. ABSTRACT

Lysophosphatidic acid (LPA) as a bioactive phospholipid signaling mediator is emerging as an important regulator of endothelial cell functions and angiogenesis. Many studies have shown that LPA is an active player in regulating the processes of endothelial cell migration, proliferation, and differentiation, all essential in angiogenesis. Through modulating angiogenesis associated gene expression, LPA also promotes pathological angiogenesis. Intriguingly, the angiogenic signaling mechanisms mediated by LPA have been linked to specific G-protein coupled receptors and down stream MAPK including Erk1/2, p38 and JNK, protein kinase D (PKD-1), Rho kinase (ROCK), and the NF-kappa B signaling pathways. LPA regulates angiogenic responses via a complex signaling network, and LPA signaling is integrated and transduced to the nucleus to coordinate the transcription of different angiogenic genes. Investigation of these mechanisms will provide novel and valuable insights into the understanding of endothelial cell biology and angiogenic programs. This knowledge will facilitate designs for better therapies for the ischemic cardiovascular diseases and malignant tumors.

2. INTRODUCTION

Many phospholipids are involved in a very complex intracellular signaling network through binding target proteins, subsequently inducing a conformational change that is instrumental in passing on signals or in opening an ion channel (1). Lysophosphatidic acid (LPA) is a bioactive phospholipid present in almost all mammalian cells and tissues, and functions as a potent signaling molecule. The variety of LPA functions suggests that this signaling lipid is important in cardiovascular development and diseases, tumor progression, reproductive disorders, osteoarthritis, neuropathic pain and neuropsychiatric disorders, and fibrosis (2).

LPA is produced via different mechanisms (Figure 1). It can be produced extracellularly by the action of secretory phospholipase A2 (sPLA2) on microvesicles released from activated cells and lipoprotein oxidation. In plasma, LPA is biosynthesized by thrombin-activated platelets through stimulated release of phospholipases A1 (PLA1), and PLA2. However, the hydrolysis of phosphatidic acids (PAs) by PLA1 and PLA2 to produce LPA is thought to occur mainly inside the cell or in the cell

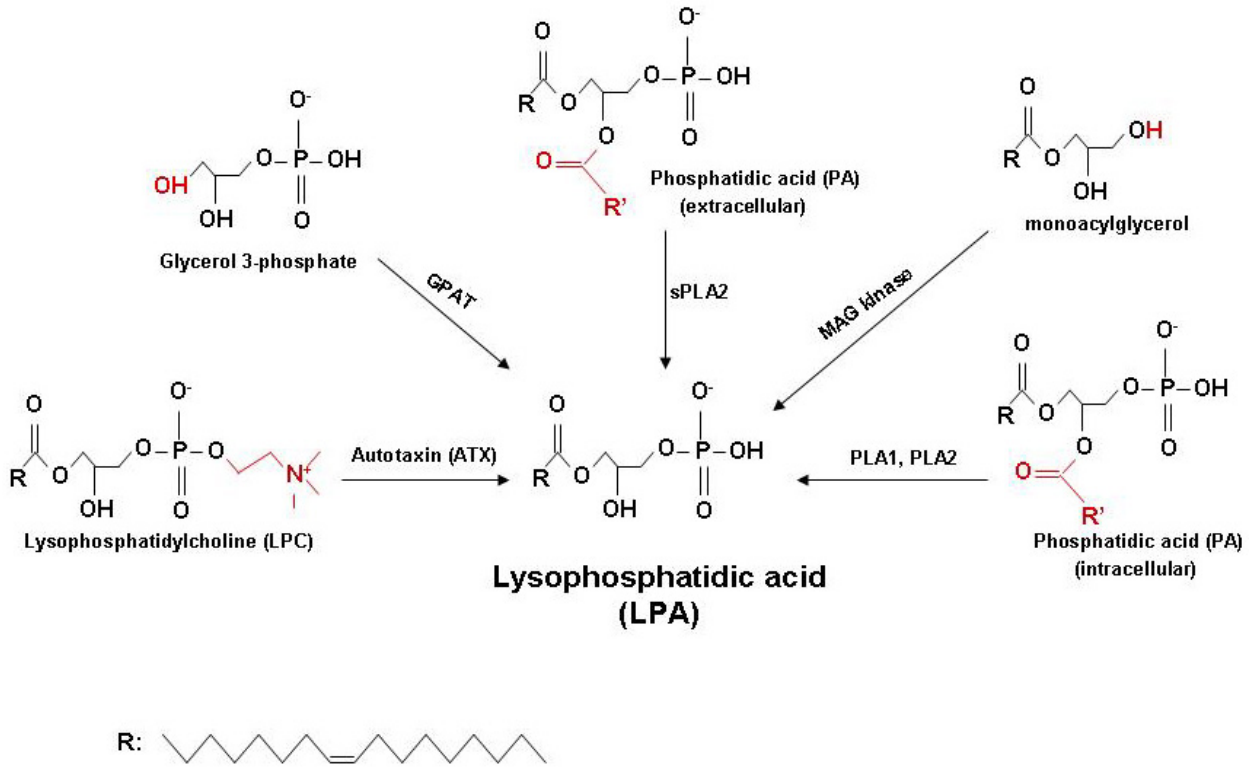

Figure 1. Conversion of LPA from different precursors by various enzymes in vivo. The chemical groups highlighted in red in LPA precursors denote the difference between the specific precursor and LPA.

membrane. A well-characterized lysophospholipase D (autotaxin or ATX) produces LPA by removing the choline group from lysophosphatidylcholine in the plasma membrane. In the cardiovascular system, ATX dysregulation contributes to cardiovascular diseases such as atherosclerosis and cardiac ischemia. Elegant reviews have discussed the roles of LPA on differential cell signaling in cardiovascular diseases and malignant tumors (2-4). In this review we will focus on LPA in the regulation of angiogenesis.

Angiogenesis in the adult occurs by sprouting from existing blood vessels, a process that requires EC proliferation and migration and remodeling of extracellular matrix. Abnormal angiogenesis and endothelial cell functions are hallmarks of cancer, ischemic and inflammatory diseases (5-7). Evidence for the role of LPA signaling in angiogenesis is emerging and provocative. LPA itself is proangiogenic, and knocking out some of the LPA receptors will also have an impact on angiogenic processes (8-14).

3. REGULATION OF ANGIOGENESIS ASSOCIATED GENE EXPRESSION

Angiogenesis is associated with the chemokines and growth factors produced from macrophages, neutrophils and other inflammatory cells including MCP-1, IL-8, TNF and VEGF. The NF-κB pathway regulates angiogenesis which may be related to the expression of the gene products (15-17). Actually, LPA can regulate angiogenesis through altering angiogenic gene expression in a cell type-specific manner (Table 1). Using granulosa-lutein cells obtained from women undergoing *in vitro* fertilization, Chen et al. demonstrated that LPA increases IL-8 and IL-6 expression through LPA receptors and the NF-κB dependent pathway, leading to increased permeability of HUVEC monolayer (18). These angiogenic properties are also primed by LPA through induction of the expression of granulocyte-monocyte colony stimulating factor (GM-CSF), Gro-alpha, MCP-1, and IL-6 in breast cancer cells (17). In human endometrial stromal cells, LPA enhances IL-8 expression, but not VEGF or IL-6, through the LPA_1 receptor via the NF-κB pathway. In human first-trimester trophoblast cells, LPA also regulates angiogenesis and the innate immune system in the early pregnancy by inducing chemokine production (19).

LPA shares several biological functions with macrophage migration inhibitory factor (MIF), including promotion of tumor cell growth and associated angiogenesis. MIF plays an important role in the immune system as well as in tumorigenesis and angiogenesis (20). LPA actually increases MIF and VEGF expression in a murine colon cancer cell line. The proangiogenic effect of LPA occurs via MIF interaction with VEGF that is accomplished by modulating Ras-MAPK and Ras-Akt/PI3K signaling (21). Meanwhile, the tumors derived from MIF knockdown colon cells show reduced size (21), implicating MIF-mediated angiogenesis in promoting

Table 1. LPA and gene expression relevant to angiogenesis

Cell type	Gene	Up/down	Phenotype	Ref.
Granulosa lutein cells	IL-8	up	Increased permeability of HUVEC monolayer	7
	IL-6	up		
Breast cancer cells	GM-CSF	up	Angiogenesis	5
	Gro-alpha	up		
	MCP-1	up		
	IL-6	up		
Human endometrial microvascular ECs	IL-8	up	Increased migration, permeability,	6
	Gro-alpha	up	Capillary tube formation and proliferation	
	MCP-1	up		
Murine colon cancer cells	MIF	up	Angiogenic response	63
	VEGF	up		
Human ECs	IL-8	up	?	54
	MCP-1	up		
Smooth muscle progenitor cells	CXCL12	up	SPC mobilization andneointima formation	61
Microvacular cells	CD36	down	Angiogenesis	45, 57
Primary HUVECs	CTGF	up	Angiogenesis	33

tumor growth. Recently, LPA was demonstrated to activate Rho kinase (ROCK) signaling and MAPK p38, JNK and NF-κB pathways in ECs. This leads to increased IL-8 and MCP-1 mRNA and protein expression but NF-κB signaling is not involved in inducing MCP-1 expression (22).

Studying the role of LPA in the expression of inflammatory cytokines in endothelial cells will help in understanding the role of EC interaction with other cell types including macrophages and leukocytes in the development of angiogenesis. LPA regulates inflammatory responses in human ECs via LPA_1 and LPA_3 receptors by enhancing angiogenic gene expression (23). In HUVECs, LPA upregulates intercellular adhesion molecule-1 (ICAM-1/CD54) expression, which enhances the interaction between leukocytes and the endothelium through a G_i-, NF-κB-, and possibly Rac-dependent mechanism (24). The results suggest that LPA may promote wound healing and inflammation. LPA also mediates ICAM-1 and VCAM-1 expression through activating the Rho kinase-NF-κB pathway via LPA_1, implicating the role of LPA in endothelial barrier integrity and trans-endothelial migration of leukocytes during inflammation (25). Our group showed that LPA promotes angiogenesis by switching off CD36 signaling, an important antiangiogenic pathway in microvasculature (26, 27). This biological effect is mediated by protein kinase PKD-1 via LPA receptors LPA_1 and LPA_3 (26). The human microvascular endothelial cells (HMVECs) appear to express higher level of LPA_3 than LPA_1 (26), which was proposed to be selectively activated by unsaturated LPA species and showed a strong preference for sn-2 versus the sn-1 acyl-LPA regioisomer (28). These studies suggest a role for LPA in inflammatory angiogenesis.

LPA has been recognized as a potent eNOS activator and used to study NO-related responses in endothelial cells and in the nuclear organelles isolated from these cells (29, 30). The nucleus may indeed function as a potential organelle for LPA intracrine signaling in the regulation of pro-inflammatory and angiogenic gene expression (29, 31) probably via nuclear LPA_1-mediated signaling and coordinating with nuclear transcription factor peroxisome proliferator-activated receptor (PPARγ) (32). Therefore, it could be interesting to understand how extracullar LPA communicates with nuclear LPA signaling to regulate EC gene transcription. Understanding this intracellular LPA-mediated nuclear signaling in endothelial cells and angiogenic gene transcription will provide valuable insights into the transcriptional regulation of angiogenesis.

4. REGULATION OF ENDOTHELIAL CELL PROLIFERATION AND MIGRATION

The endothelial cells that are essential to angiogenesis line the inner surface of the blood vessels. The normal physiological functions of endothelial cells are essential in regulating angiogenesis. LPA has been shown to impact EC functions including proliferation, migration, and modulation of EC inflammatory responses in the vascular system.

The ECs possess high plasticity, allowing them to sense and respond to angiogenic signals. In this process, *de novo* proliferation is an important step. Fibroblast growth factor (FGF) and LPA have convergent signaling that stimulates EC proliferation through their distinct receptors. LPA triggers proliferation of bovine aortic endothelial cells (BAECs) in concentrations lower than those required to stimulate proliferation of human foreskin fibroblasts (33), indicating that the ECs are more sensitive to LPA. This may result from differences in receptor expression that can be affected by cell density. It has been reported that receptor LPA_3 maximally expresses at low cell density and minimally expresses in dense cell population, which is positively associated with cell proliferation (34). However, receptor LPA_1 plays a negative role in regulating cell proliferation, and its expression profile is opposite to that of LPA_3. In human umbilical vein ECs (HUVECs), LPA has been shown to induce stress fiber formation (9) but not proliferation (35,

36). Yet, it enhances HUVEC proliferation in an EGFR transactivation-dependent manner (37). Intriguingly, in human endometrial microvascular ECs, LPA stimulates proliferation and capillary tube formation by inducing IL-8 from trophoblasts (19). These results suggest that LPA regulates angiogenesis via modulating EC growth in an endothelial cell type and stimulant dependent manner.

EC migration is another essential character for angiogenesis. LPA acts directly on the ECs to regulate cell migration. However, it appears that EC migration in response to LPA is restricted to specific conditions. LPA only stimulates migration of certain ECs on certain extracellular matrix proteins. In fetal bovine heart ECs, LPA stimulates cell migration on a fibronectin matrix by remodeling the cytoskeleton. This may require a balance between Gi and Rho signaling over a broad dose range, whereas VEGF or FGF-2 can induce HUVEC migration over a narrow dose range, producing a bell-shaped curve (38, 39). In bovine pulmonary artery ECs (BPAECs), LPA regulates migration by recruiting hydrogen peroxide-inducible clone 5, a paxillin family member, to the focal adhesions and to the pseudopodia. This is accomplished via activating MAPK/Erk1/2 pathway (40). In the absence of a gradient, LPA still increases migration, showing its chemokinetic feature with a small chemotactic component, similar to FGF-2 (38, 39). This is different from VEGF-induced migration, which is primarily chemotactic. On the contrary, HUVECs, bovine adrenal microvascular ECs, bovine lung microvascular ECs, and BPAECs do not respond significantly to LPA in terms of migration (38, 39). Furthermore, LPA does not induce a directional migration (chemotaxis) in HUVECs (35, 36), or in primary human microvascular endothelial cells (HMVECs) (26).

Mechanistically, MAPK/Erk1/2 signaling does not appear to be involved in LPA-stimulated migration in certain EC types. Instead, the Gi-mediated pathway, which diverges upstream from Mek-1, and a balance between Rho and Gi activation is critical for this process (38). Our own results indicate that LPA regulates EC migration indirectly as it attenuates TSP-1 inhibition of migration stimulated by FGF-2 (33). This is attributed to suppressing CD36 transcription in HMVECs, in which PKD-1 signaling is involved (26). VEGF can induce ATX expression, and consequently LPA and LPA_1 receptor production, via VEGFR-2 in HUVEC (41). ATX and VEGF cooperation may regulate EC migratory responses via Akt2 (41). In addition, through stimulating IL-8 protein expression in trophoblasts, LPA indirectly promotes migration in human endometrial microvascular Ecs (19). Finally, LPA also appears to upregulate VEGFR-2 expression in the microvascular ECs (our unpublished data) that may lead to tip cell positioning (42), an important step for proangiogenic migration and sprouting. Taken together, LPA differentially regulates EC migration in a cell type and matrix-specific manner. Investigation into LPA on endothelial tip cell behavior and relevant signaling pathways involved may add more insights into the biological significance of LPA-induced EC motility in branching morphogenesis.

5. EMERGING EVIDENCE OF LPA IN ANGIOGENESIS

More and more studies focus on the role of LPA signaling in angiogenesis. Using the chicken chorio-allantoic membrane assay, Rivera-Lopez et al. reported that LPA is proangiogenic *in vivo* in 2008 (43). LPA is also involved in EC capillary tube formation during inflammatory angiogenesis. As a bioactive lipid mediator, it is present in biological fluids during endothelial damage or injury (37) that may be associated with proangiogenesis in wound healing. The long transmembrane isoform of carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1-L) promotes branching morphogenesis after monolayer injury, which is related to LPA-induced RhoA activation (44). LPA produced by mild oxidized LDL accumulates in the lipid-rich core of atherosclerotic plaques. This may be associated with angiogenesis in atherosclerotic lesions (9, 10).

The phenotypes of LPA receptor knockout mice provide further insight into the role of LPA in angiogenesis. A small proportion of $Lpar1^{-/-}$ and $Lpar1^{-/-}/Lpar2^{-/-}$ mice were born with frontal cranial hematomas (11, 14). In addition, LPA_3 receptor deletion resulted in anomalous spacing and timing of blastocyst implantation with reduced uterine expression of cyclooxygenase-2 (12), an important player in angiogenesis (13). Most recently, LPA receptor 4 has been associated with vascular and lymphatic development (8). A subset of LPA_4-deficient embryos showed dilated blood and lymphatic vessels with impaired recruitment of mural cells.

In the tumor microenvironment, LPA stimulates not only growth and invasion of cancer cells but also angiogenesis. The latter mainly results from regulation of the expression and function of proangiogenic and antiangiogenic factors. Tumor angiogenesis is predominantly driven by VEGF, a proangiogenic growth factor expressed by many solid cancers (45, 46). LPA induces VEGF expression in a variety of cell types in the tumor milieu. The cancer-derived LPA stimulates VEGF secretion from human adipose tissue-derived mesenchymal stem cells (hASCs). This may be via multiple signaling pathways involving Rho kinase, ERK, PLC, and phosphoinositide-3-kinase (47). LPA also stimulates proangiogenic VEGF production in peritoneal mesothelial cells (48). In ovarian cancer cells, the expression of multiple VEGF variants is stimulated by LPA through c-Myc and Sp-1 transcription factors independent of HIF-1α (49). In the multiple myeloma cell line, U266, the Burkitt's lymphoma cell line, BJAB, and the chronic lymphocytic leukemia-like cell line I-83, LPA induces VEGF transcription via the activation of c-Jun N-terminal Kinase (JNK) and NF-κB (50), an important determinant of tumor aggressiveness. In summary, the studies support the essential role of LPA-mediated VEGFs and their receptor production in proangiogenic responses in malignant tumor. The paracrine effect of LPA-LPA_1-mediated VEGF

expression also promotes tumor angiogenesis within the tumor milieu. This may be associated with *in vivo* differentiation of the human adipose tissue-derived mesenchymal stem cells (hASCs) to carcinoma-associated fibroblasts that produce VEGF (51). However, the effect can be abrogated by treatment of hASCs with LPA_1 and LPA_3 receptor antagonist, or LPA_1 knockdown in the hASCs.

Altering antiangiogenic signaling can be another way by which LPA regulates angiogenesis. In mouse dermal fibroblasts, LPA downregulates thrombospondin-1 (TSP-1) (52), the first discovered important endogenous antiangiogenic factor in tumor angiogenesis (6, 53). This is regarded as an important step in the generation of the pro-angiogenic tumor stroma. Interestingly, in bovine aortic endothelial cells (BAECs), exogenous TSP-1 inhibits the baseline mitogenic activity in response to LPA. Recombinant mouse TSP-2 also inhibits LPA-stimulated BAEC proliferation in a dose range similar to that of TSP-1 (33). LPA can abrogate the antiangiogenic effect of TSP-1 by suppressing the transcription of CD36, the receptor of TSP-1 and -2 in HMVECs (26). This indicates that LPA may initiate angiogenesis by counteracting the inhibitory effect of TSPs through a feedback mechanism in the microvasculature.

Studies in ATX, a motility stimulating protein and key enzyme in the production of LPA, also support the role of LPA in tumor angiogenesis. ATX was originally identified as an autocrine factor present in melanoma cell culture medium that stimulated tumor cell motility (54), and demonstrated to promote tumor angiogenesis (41). Recently, a link between ATX expression, LPA, and VEGF signaling has been reported in ovarian cancer cell lines via a positive feedback mechanism. Exogenous VEGF stimulates ATX production, resulting in enhanced extracellular LPA production while the elevated LPA modulates VEGF responsiveness by inducing VEGFR-2 expression through receptor LPA_4 (55). However, PLCγ signaling increases the level of a secreted form of ATX, producing LPA and promoting regression of blood vessels (56). This may be explained by the fact that LPA-mediated EC motility leads to disorganization/regression of tube formation. Moreover, mice that under- or overexpressed members of this intrinsic destabilization pathway show either delayed or accelerated regression of blood vessels (56). Therefore, ECs could be instructed to engage a PLCγ-dependent intrinsic destabilization pathway leading to the production of soluble ATX and LPA and inhibiting proliferative diabetic retinopathy and solid tumors growth. Lung cancer and glioblastoma multiforme are highly angiogenic but resistant to antiangiogenic therapy. Linkous et al. employed syngenic glioblastoma and Lewis lung carcinomas cell lines injected subcutaneously in cPLA2α-deficient mice to investigate the effects of cPLA2 expression on tumor growth and vascularity. Their results demonstrated the key regulatory roles of cPLA2 and LPA in tumor angiogenesis, suggesting cPLA2 as a novel molecular target for antiangiogenic therapy in addition to VEGF signaling (57). Interestingly, cytosolic phospholipase A2α (cPLA2α) is an enzyme that specifically recognizes the sn-2 acyl bond of phospholipids and catalytically hydrolyzes membrane glycerophospholipids to produce LPA (58). In addition, BrP-LPA, a dual activity LPA receptor antagonist and autotaxin inhibitor, reduces tumor angiogenesis and promotes tumor regression in an engineered three dimensional lung cancer xenograft model (59). Taken together, these results support LPA as a potential regulator of angiogenesis via different signaling pathways (Figure 2).

6. SUMMARY AND PERSPECTIVE

The biomedical importance of signaling triggered by LPA has become apparent and increasingly significant in the field of cardiovascular biology and oncology. Emerging evidence demonstrates that LPA regulates angiogenic gene expression via different signaling pathways such as intracellular signaling PKD-1, MAPK/Erk1/2, PI3K/Akt, NFκB, or Rho pathway, subsequently impacting EC behavior and the angiogenic process (Figure 2). But the roles of LPA in angiogenesis and signaling via its cognate receptors are likely to be species-, tissue-, and cell-specific. In vascular endothelial cells, the field is only beginning to determine the specific biochemical events and mechanisms that are initiated by different LPA receptors. It should be interesting to study the biological outcomes of activation of the individual receptors and integration of intracellular signaling in endothelial cells. An understanding of endothelial-specific cellular and molecular mechanisms of LPA will also allow the development of novel strategies for therapeutic intervention involving angiogenesis. Studies focusing on the molecular signaling signatures of LPA via different receptors and in different endothelial cell types will aid in deciphering the cues involved and in the optimization of therapeutic approaches.

Phospholipids including LPA may be involved in major components of a complex intracellular signaling network in the ECs. Recently, Bot et al. showed that LPA homeostasis is altered during atherosclerotic progression, favoring intracellular LPA accumulation in carotid artery plaques (10). Receptor LPA_1 localizes at the nucleus and LPA is able to bind to PPARγ for the regulation of gene expression (29, 32). It would be of interest to study how LPA functions as an intracellular messenger to couple intracellular signals to nuclear components including transcription factors, co-repressors, and co-activators to modulate specific angiogenic gene transcription. Moreover, LPA may also regulate gene transcription via modification of chromatin structure through epigenetic mechanisms.

Sufficient evidence demonstrates the role of LPA signaling in EC functions and interactions with leukocytes, expression of angiogenic gene transcription and in regulation of angiogenic processes. This highlights the importance of LPA in vascular biology. LPA production and the LPA receptor subtypes expressed by different cell types in the vascular system are important potential drug targets for manipulating pathological angiogenesis and thrombosis (60). However, the mechanistic role of LPA in angiogenesis has only started to be appreciated, with many

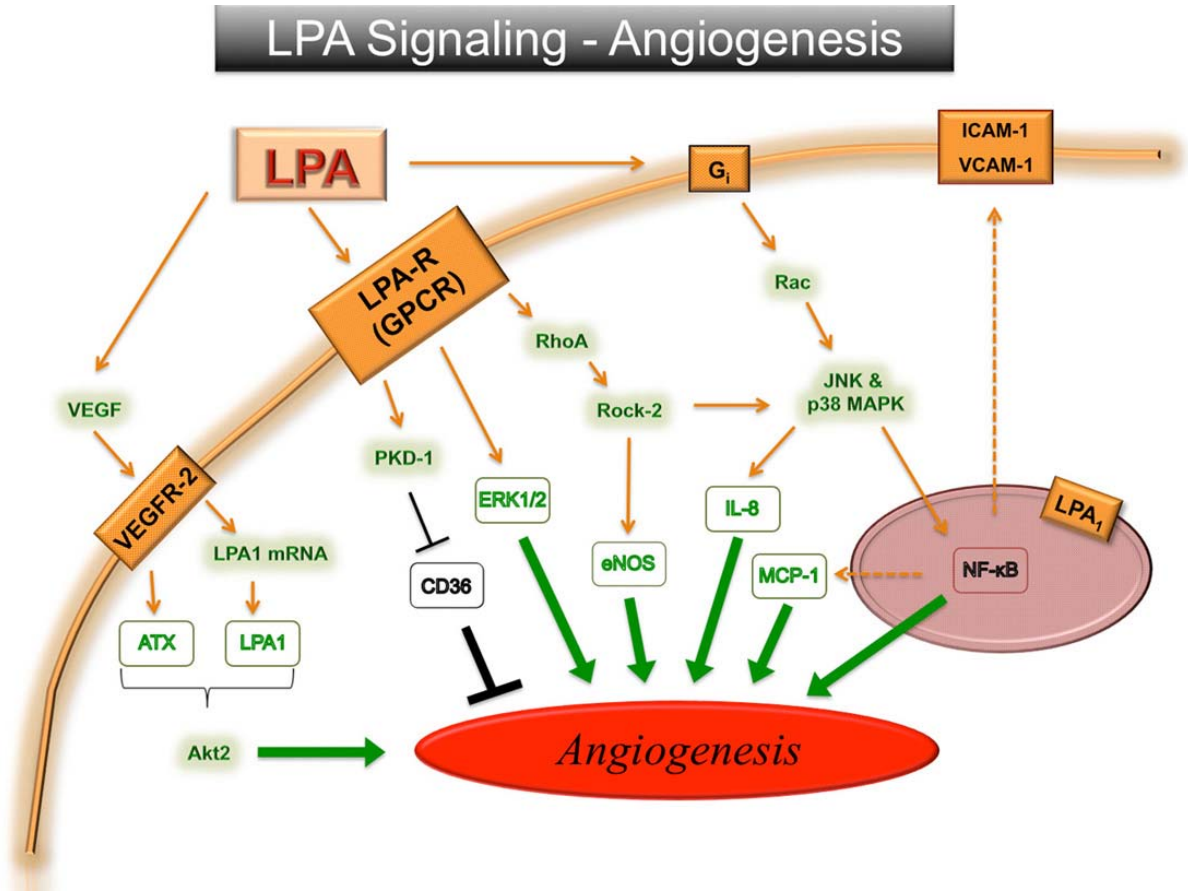

Figure 2. LPA mediates angiogenic signaling in endothelial cells and regulates angiogenesis through the cytoplasmic and nuclear signaling molecules shown in the figure.

questions remaining to be addressed. For instance, how does LPA-mediated signaling coordinate with transcriptional machinery to regulate angiogenic gene transcription in endothelial cells? In terms of targeting the vasculature, the questions are:

- What is the role of LPA in tumor endothelial cells and endothelial tip cells?
- What is the role of individual LPA receptor or receptor combinations in transduction of LPA signaling and angiogenesis?
- How do the localization and expression levels of LPA receptors affect LPA signaling? Is this signaling also regulated by receptor modification?
- What role does nuclear LPA_1 receptor play in nuclear signaling and gene transcription?
- What is the potential benefit of LPA signaling in cardiac and limb ischemia and is this beneficial to the formation of functional vasculature?

Future studies are needed to address these interesting and challenging questions that could lead to better understanding of the roles of LPA in the cardiovascular system and tumor angiogenesis. Regulation of LPA signaling may reveal potential therapeutic targets in cardiovascular diseases and malignant tumors, as well as the cardiovascular complications of diabetes.

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8. REFERENCES

1. C Schultz: Challenges in studying phospholipid signaling. *Nat Chem Biol* 6(7), 473-475 (2010)

2. ME Lin, DR Herr, J Chun: Lysophosphatidic acid (LPA) receptors: signaling properties and disease relevance. *Prostaglandins Other Lipid Mediat* 91(3-4), 130-138 (2010)

3. SS Smyth, HY Cheng, S Miriyala, M Panchatcharam, AJ Morris: Roles of lysophosphatidic acid in cardiovascular physiology and disease. *Biochim Biophys Acta* 1781(9), 563-570 (2008)

4. GB Mills, WH Moolenaar: The emerging role of lysophosphatidic acid in cancer. *Nat Rev Cancer* 3(8), 582-591 (2003)

5. HF Dvorak: Angiogenesis: update 2005. *J Thromb Haemost* 3(8), 1835-1842 (2005)

6. B Ren, KO Yee, J Lawler, R Khosravi-Far: Regulation of tumor angiogenesis by thrombospondin-1. *Biochim Biophys Acta* 1765(2), 178-188 (2006)

7. B Ren, Y Deng, A Mukhopadhyay, AA Lanahan, ZW Zhuang, KL Moodie, MJ Mulligan-Kehoe, TV Byzova, RT Peterson, M Simons: ERK1/2-Akt1 crosstalk regulates arteriogenesis in mice and zebrafish. *J Clin Invest* 120(4), 1217-1228 (2010)

8. H Sumida, K Noguchi, Y Kihara, M Abe, K Yanagida, F Hamano, S Sato, K Tamaki, Y Morishita, MR Kano, C Iwata, K Miyazono, K Sakimura, T Shimizu, S Ishii: LPA4 regulates blood and lymphatic vessel formation during mouse embryogenesis. *Blood* 116(23), 5060-5070 (2010)

9. W Siess, KJ Zangl, M Essler, M Bauer, R Brandl, C Corrinth, R Bittman, G Tigyi, M Aepfelbacher: Lysophosphatidic acid mediates the rapid activation of platelets and endothelial cells by mildly oxidized low density lipoprotein and accumulates in human atherosclerotic lesions. *Proc Natl Acad Sci U S A* 96(12), 6931-6936 (1999)

10. M Bot, I Bot, R Lopez-Vales, CH van de Lest, JS Saulnier-Blache, JB Helms, S David, TJ van Berkel, EA Biessen: Atherosclerotic lesion progression changes lysophosphatidic acid homeostasis to favor its accumulation. *Am J Pathol* 176(6), 3073-3084 (2010)

11. JJ Contos, N Fukushima, JA Weiner, D Kaushal, J Chun: Requirement for the lpA1 lysophosphatidic acid receptor gene in normal suckling behavior. *Proc Natl Acad Sci U S A*97(24), 13384-13389 (2000)

12. X Ye, K Hama, JJ Contos, B Anliker, A Inoue, MK Skinner, H Suzuki, T Amano, G Kennedy, H Arai, J Aoki, J Chun: LPA3-mediated lysophosphatidic acid signalling in embryo implantation and spacing. *Nature* 435(7038), 104-108 (2005)

13. M Sahin, E Sahin, S Gumuslu: Cyclooxygenase-2 in cancer and angiogenesis. *Angiology* 60(2), 242-253 (2009)

14. JJ Contos, I Ishii, N Fukushima, MA Kingsbury, X Ye, S Kawamura, JH Brown, J Chun: Characterization of lpa(2) (Edg4) and lpa(1)/lpa(2) (Edg2/Edg4) lysophosphatidic acid receptor knockout mice: signaling deficits without obvious phenotypic abnormality attributable to lpa(2). *Mol Cell Biol* 22(19), 6921-6929 (2002)

15. I Garkavtsev, SV Kozin, O Chernova, L Xu, F Winkler, E Brown, GH Barnett, RK Jain: The candidate tumour suppressor protein ING4 regulates brain tumour growth and angiogenesis. *Nature* 428(6980), 328-332 (2004)

16. F Lerebours, S Vacher, C Andrieu, M Espie, M Marty, R Lidereau, I Bieche: NF-kappa B genes have a major role in Inflammatory Breast Cancer. *Bmc Cancer* 8, 41 (2008)

17. A Boucharaba, B Guillet, F Menaa, M Hneino, AJ van Wijnen, P Clezardin, O Peyruchaud: Bioactive lipids lysophosphatidic acid and sphingosine 1-phosphate mediate breast cancer cell biological functions through distinct mechanisms. *Oncol Res* 18(4), 173-184 (2009)

18. SU Chen, CH Chou, H Lee, CH Ho, CW Lin, YS Yang: Lysophosphatidic acid up-regulates expression of interleukin-8 and -6 in granulosa-lutein cells through its receptors and nuclear factor-kappaB dependent pathways: implications for angiogenesis of corpus luteum and ovarian hyperstimulation syndrome. *J Clin Endocrinol Meta.* 93(3), 935-943 (2008)

19. SU Chen, CH Chou, KH Chao, H Lee, CW Lin, HF Lu, YS Yang: Lysophosphatidic acid up-regulates expression of growth-regulated oncogene-alpha, interleukin-8, and monocyte chemoattractant protein-1 in human first-trimester trophoblasts: possible roles in angiogenesis and immune regulation. *Endocrinology* 151(1), 369-379 (2010)

20. V Veillat, C Carli, CN Metz, Y Al-Abed, PH Naccache, A Akoum: Macrophage migration inhibitory factor elicits an angiogenic phenotype in human ectopic endometrial cells and triggers the production of major angiogenic factors via CD44, CD74, and MAPK signaling pathways. *J Clin Endocrinol Metab* 95(12), E403-412 (2010)

21. B Sun, J Nishihira, M Suzuki, N Fukushima, T Ishibashi, M Kondo, Y Sato, S Todo: Induction of macrophage migration inhibitory factor by lysophosphatidic acid: relevance to tumor growth and angiogenesis. *Int J Mol Med* 12(4), 633-641 (2003)
[doi not found]

22. H Shimada, LE Rajagopalan: Rho-kinase mediates lysophosphatidic acid-induced IL-8 and MCP-1 production via p38 and JNK pathways in human endothelial cells. *FEBS Lett* 584(13), 2827-2832 (2010)

23. CI Lin, CN Chen, PW Lin, KJ Chang, FJ Hsieh, H Lee: Lysophosphatidic acid regulates inflammation-related genes in human endothelial cells through LPA1 and LPA3. *Biochem Biophys Res Commun* 363(4), 1001-1008 (2007)

24. H Lee, CI Lin, JJ Liao, YW Lee, HY Yang, CY Lee, HY Hsu, HL Wu: Lysophospholipids increase ICAM-1 expression in HUVEC through a Gi- and NF-kappaB-dependent mechanism. *Am J Physiol Cell Physiol* 287(6), C1657-1666 (2004)

25. H Shimada, LE Rajagopalan: Rho kinase-2 activation in human endothelial cells drives lysophosphatidic acid-

mediated expression of cell adhesion molecules via NF-kappaB p65. *J Biol Chem* 285(17), 12536-12542 (2010)

26. B Ren, J Hale, S Srikanthan, RL Silverstein: Lysophosphatidic acid suppresses endothelial cell CD36 expression and promotes angiogenesis via a PKD-1-dependent signaling pathway. *Blood* 117(22), 6036-6045 (2011)

27. RL Silverstein, M Febbraio: CD36, a scavenger receptor involved in immunity, metabolism, angiogenesis, and behavior. *Sci Signal* 2(72), re3 (2009)

28. Y Fujiwara, V Sardar, A Tokumura, D Baker, K Murakami-Murofushi, A Parrill, G Tigyi: Identification of residues responsible for ligand recognition and regioisomeric selectivity of lysophosphatidic acid receptors expressed in mammalian cells. *J Biol Chem* 280(41), 35038-35050 (2005)

29. F Gobeil, Jr., SG Bernier, A Vazquez-Tello, S Brault, MH Beauchamp, C Quiniou, AM Marrache, D Checchin, F Sennlaub, X Hou, M Nader, G Bkaily, A Ribeiro-da-Silva, EJ Goetzl, S Chemtob: Modulation of pro-inflammatory gene expression by nuclear lysophosphatidic acid receptor type-1. *J Biol Chem* 278(40), 38875-38883 (2003)

30. R Kou, J Igarashi, T Michel: Lysophosphatidic acid and receptor-mediated activation of endothelial nitric-oxide synthase. *Biochemistry* 41(15), 4982-4988 (2002)

31. B Ren, J Hale, D Ramakrishnan, RL Silverstein: HDAC 7 Couples LPA Signaling to Endothelial Cell CD36 Gene Regulation to Switch off TSP-1 Mediated Antiangiogenic Responses. *Circulation* 122, A12757 (2010)
[doi not found]

32. TM McIntyre, AV Pontsler, AR Silva, A St Hilaire, Y Xu, JC Hinshaw, GA Zimmerman, K Hama, J Aoki, H Arai, GD Prestwich: Identification of an intracellular receptor for lysophosphatidic acid (LPA): LPA is a transcellular PPARgamma agonist. *Proc Natl Acad Sci U S A* 100(1), 131-136 (2003)

33. TS Panetti, H Chen, TM Misenheimer, SB Getzler, DF Mosher: Endothelial cell mitogenesis induced by LPA: inhibition by thrombospondin-1 and thrombospondin-2. *J Lab Clin Med* 129(2), 208-216 (1997)

34. Y Xing, SH Ganji, JW Noh, VS Kamanna: Cell density-dependent expression of EDG family receptors and mesangial cell proliferation: role in lysophosphatidic acid-mediated cell growth. *Am J Physiol Renal Physiol* 287(6), F1250-1257 (2004)

35. F Wang, JR Van Brocklyn, JP Hobson, S Movafagh, Z Zukowska-Grojec, S Milstien, S Spiegel: Sphingosine 1-phosphate stimulates cell migration through a G(i)-coupled cell surface receptor. Potential involvement in angiogenesis. *J Biol Chem* 274(50), 35343-35350 (1999)

36. Y Yatomi, T Ohmori, G Rile, F Kazama, H Okamoto, T Sano, K Satoh, S Kume, G Tigyi, Y Igarashi, Y Ozaki: Sphingosine 1-phosphate as a major bioactive lysophospholipid that is released from platelets and interacts with endothelial cells. *Blood* 96(10), 3431-3438 (2000)
[doi not found]

37. HL Wu, CI Lin, YL Huang, PS Chen, CH Kuo, MS Chen, GC Wu, GY Shi, HY Yang, H Lee: Lysophosphatidic acid stimulates thrombomodulin lectin-like domain shedding in human endothelial cells. *Biochem Biophys Res Commun* 367(1), 162-168 (2008)

38. TS Panetti, J Nowlen, DF Mosher: Sphingosine-1-phosphate and lysophosphatidic acid stimulate endothelial cell migration. *Arterioscler Thromb Vasc Biol* 20(4), 1013-1019 (2000)

39. A Yoshida, B Anand-Apte, BR Zetter: Differential endothelial migration and proliferation to basic fibroblast growth factor and vascular endothelial growth factor. *Growth Factors* 13(1-2), 57-64 (1996)

40. C Avraamides, ME Bromberg, JP Gaughan, SM Thomas, AY Tsygankov, TS Panetti: Hic-5 promotes endothelial cell migration to lysophosphatidic acid. *Am J Physiol Heart Circ Physiol* 293(1), H193-203 (2007)

41. MM Ptaszynska, ML Pendrak, ML Stracke, DD Roberts: Autotaxin signaling via lysophosphatidic acid receptors contributes to vascular endothelial growth factor-induced endothelial cell migration. *Mol Cancer Res* 8(3), 309-321 (2010)

42. L Jakobsson, CA Franco, K Bentley, RT Collins, B Ponsioen, IM Aspalter, I Rosewell, M Busse, G Thurston, A Medvinsky, S Schulte-Merker, H Gerhardt: Endothelial cells dynamically compete for the tip cell position during angiogenic sprouting. *Nat Cell Biol* 12(10), 943-953 (2010)

43. CM Rivera-Lopez, AL Tucker, KR Lynch: Lysophosphatidic acid (LPA) and angiogenesis. *Angiogenesis* 11(3), 301-310 (2008)

44. MM Muller, BB Singer, E Klaile, B Obrink, L Lucka: Transmembrane CEACAM1 affects integrin-dependent signaling and regulates extracellular matrix protein-specific morphology and migration of endothelial cells. *Blood* 105(10), 3925-3934 (2005)

45. N Ferrara, KJ Hillan, HP Gerber, W Novotny: Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. *Nat Rev Drug Discov* 3(5), 391-400 (2004)

46. AK Olsson, A Dimberg, J Kreuger, L Claesson-Welsh: VEGF receptor signalling - in control of vascular function. *Nat Rev Mol Cell Biol* 7(5), 359-371 (2006)

47. ES Jeon, SC Heo, IH Lee, YJ Choi, JH Park, KU Choi, Y Park do, DS Suh, MS Yoon, JH Kim: Ovarian cancer-derived lysophosphatidic acid stimulates secretion of VEGF and stromal cell-derived factor-1 alpha from human

mesenchymal stem cells. *Exp Mol Med* 42(4), 280-293 (2010)

48. A Sako, J Kitayama, D Shida, R Suzuki, T Sakai, H Ohta, H Nagawa: Lysophosphatidic acid (LPA)-induced vascular endothelial growth factor (VEGF) by mesothelial cells and quantification of host-derived VEGF in malignant ascites. *J Surg Res* 130(1), 94-101 (2006)

49. Y Song, J Wu, RA Oyesanya, Z Lee, A Mukherjee, X Fang: Sp-1 and c-Myc mediate lysophosphatidic acid-induced expression of vascular endothelial growth factor in ovarian cancer cells via a hypoxia-inducible factor-1-independent mechanism. *Clin Cancer Res* 15(2),492-501 (2009)

50. X Hu, FJ Mendoza, J Sun, V Banerji, JB Johnston, SB Gibson: Lysophosphatidic acid (LPA) induces the expression of VEGF leading to protection against apoptosis in B-cell derived malignancies. *Cell Signal* 20(6):1198-1208 (2008)

51. ES Jeon, IH Lee, SC Heo, SH Shin, YJ Choi, JH Park, Y Park do, JH Kim: Mesenchymal stem cells stimulate angiogenesis in a murine xenograft model of A549 human adenocarcinoma through an LPA1 receptor-dependent mechanism. *Biochim Biophys Acta* 1801(11), 1205-1213 (2010)

52. W Kalas, P Klement, J Rak: Downregulation of the angiogenesis inhibitor thrombospondin 1 in fibroblasts exposed to platelets and their related phospholipids. *Biochem Biophys Res Commun* 334(2), 549-554 (2005)

53. B Ren, K Song, S Parangi, T Jin, M Ye, R Humphreys, M Duquette, X Zhang, N Benhaga, J Lawler, R Khosravi-Far: A double hit to kill tumor and endothelial cells by TRAIL and antiangiogenic 3TSR. *Cancer Res* 69(9), 3856-3865 (2009)

54. ML Stracke, HC Krutzsch, EJ Unsworth, A Arestad, V Cioce, E Schiffmann, LA Liotta: Identification, purification, and partial sequence analysis of autotaxin, a novel motility-stimulating protein. *J Biol Chem* 267(4), 2524-2529 (1992)
[doi not found]

55. MM Ptaszynska, ML Pendrak, RW Bandle, ML Stracke, DD Roberts: Positive feedback between vascular endothelial growth factor-A and autotaxin in ovarian cancer cells. *Mol Cancer Res* 6(3), 352-363 (2008)

56. E Im, R Motiejunaite, J Aranda, EY Park, L Federico, TI Kim, T Clair, ML Stracke, S Smyth, A Kazlauskas: Phospholipase Cgamma activation drives increased production of autotaxin in endothelial cells and lysophosphatidic acid-dependent regression. *Mol Cell Biol* 30(10), 2401-2410 (2010)

57. AG Linkous, EM Yazlovitskaya, DE Hallahan: Cytosolic phospholipase A2 and lysophospholipids in tumor angiogenesis. *J Natl Cancer Inst* 102(18), 1398-1412 (2010)

58. G Tosato, M Segarra, O Salvucci: Cytosolic phospholipase A2{alpha} and cancer: a role in tumor angiogenesis. *J Natl Cancer Inst* 102(18), 1377-1379 (2010)

59. X Xu, GD Prestwich: Inhibition of tumor growth and angiogenesis by a lysophosphatidic acid antagonist in an engineered three-dimensional lung cancer xenograft model. *Cancer* 116(7), 1739-1750 (2010)

60. G Tigyi: Aiming drug discovery at lysophosphatidic acid targets. *Br J Pharmacol* 161(2), 241-270 (2010)

61.S Ishii, K Noguchi, K Yanagida: Non-Edg family lysophosphatidic acid (LPA) receptors. *Prostaglandins Other Lipid Mediat* 89(3-4),57-65 (2009)

62. S Oka, R Ota, M Shima, A Yamashita, T Sugiura: GPR35 is a novel lysophosphatidic acid receptor. *Biochem Biophys Res Commun* 395(2), 232-237 (2010)

63. S Muehlich, N Schneider, F Hinkmann, CD Garlichs, M Goppelt-Struebe: Induction of connective tissue growth factor (CTGF) in human endothelial cells by lysophosphatidic acid, sphingosine-1-phosphate, and platelets. *Atherosclerosis* 175(2), 261-268 (2004)

64. F Gobeil, Jr., T Zhu, S Brault, A Geha, A Vazquez-Tello, A Fortier, D Barbaz, D Checchin, X Hou, M Nader, G Bkaily, JP Gratton, N Heveker, A Ribeiro-da-Silva, K Peri, H Bard, A Chorvatova, P D'Orleans-Juste, EJ Goetzl, S Chemtob: Nitric oxide signaling via nuclearized endothelial nitric-oxide synthase modulates expression of the immediate early genes iNOS and mPGES-1. *J Biol Chem* 281(23), 16058-16067 (2006)

Abbreviations: ATX: autotoxin, BAEs: bovine aortic endothelial cells, BPAECs: bovine pulmonary artery endothelial cells, CEACAM1: carcinembryonic antigen-related cell adhesion molecule 1, CTGF: connective tissue growth factor, ECs: endothelial cells, GPAT: glycerophosphate acyltransferase, GPCRs: G-protein coupled receptors, hASCs: human adipose tissue-deprived mesenchymal stem cells, HMVECs: human dermal microvascular endothelial cells, HRP: horseradish peroxidase, HUVECs: human umbilical vein endothelial cells, JNK: c-Jun N-terminal Kinase, LPA: lysophosphatidic acid, MAG: monoacylglycerol, MIF: macrophage migration factor, PAs: phosphatidic acids, PKD: protein kinase D, PLA: phospholipases A, ROCK: Rho kinase, S1P: sphingosine-1-phosphate, SPC: smooth muscle progenitor cell, TSP-1: thrombospondin-1

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