



# Lysophosphatidic acid and its receptors: pharmacology and therapeutic potential in atherosclerosis and vascular disease



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## ABSTRACT

Lysophosphatidic acid (LPA) is a collective name for a set of bioactive lipid species. Via six widely distributed G protein-coupled receptors (GPCRs), LPA elicits a plethora of biological responses, contributing to inflammation, thrombosis and atherosclerosis. There have recently been considerable advances in GPCR signaling especially recognition of the extended role for GPCR transactivation of tyrosine and serine/threonine kinase growth factor receptors. This review covers LPA signaling pathways in the light of new information. The use of transgenic and gene knockout animals, gene manipulated cells, pharmacological LPA receptor agonists and antagonists have provided many insights into the biological significance of LPA and individual LPA receptors in the progression of atherosclerosis and vascular diseases. This review provides a comprehensive presentation of LPA with the highlight of the distinct role of its receptors in cell and animal models that relate to atherosclerosis and vascular diseases, and therefore provides new opportunities to reduce the burden of cardiovascular diseases. The recent drug development strategies that target LPA signaling pathways are also included in this review.

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## Contents

1. Introduction . . . . .	2
2. LPA and its production and degradation . . . . .	3
3. The in vitro studies of LPA and LPARs in the vasculature . . . . .	4
4. The in vivo studies of LPA and LPARs in the vasculature . . . . .	5
5. The signaling pathways of LPA . . . . .	6
6. Therapeutic implications of targeting LPA and its signaling pathways . . . . .	8
7. Conclusions . . . . .	9
Acknowledgments . . . . .	9
References . . . . .	9

**Abbreviations:** AC, adenylyl cyclase; ATX, autotaxin; cAMP, cyclic adenosine monophosphate; CVDs, cardiovascular diseases; DAG, diacylglycerol; DCs, dendritic cells; ECs, endothelial cells; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; GPCR, G protein-coupled receptor; HUVECs, human umbilical vein endothelial cells; IL-8, interleukin 8; LDL, low density lipoprotein; LPA, lysophosphatidic acid; LPAR, LPA receptor; LPP3, lipid phosphate phosphatase 3; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemoattractant protein-1; MCs, mast cells; MMPs, matrix metalloproteinases; NADPH, nicotinamide adenine dinucleotide phosphate; NF- $\kappa$ B, nuclear factor kappa-light-chain-enhancer of activated B cells; ox-LDL, oxidized-LDL; PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor; PLC, phospholipase; PPAR $\gamma$ , peroxisome proliferator-activated receptor  $\gamma$ ; PTKR, protein tyrosine kinase receptor; ROCK, Rho associated protein kinase; ROS, reactive oxygen species; S/TKR, serine/threonine kinase receptor; SMCs, smooth muscle cells; TGFBR1, TGF- $\beta$  type 1 receptor; TGF- $\beta$ , transforming growth factor  $\beta$ ; TLR4, toll-like receptor 4; TNF- $\alpha$ , tumor necrosis factor  $\alpha$ ; VSMCs, vascular smooth muscle cells.

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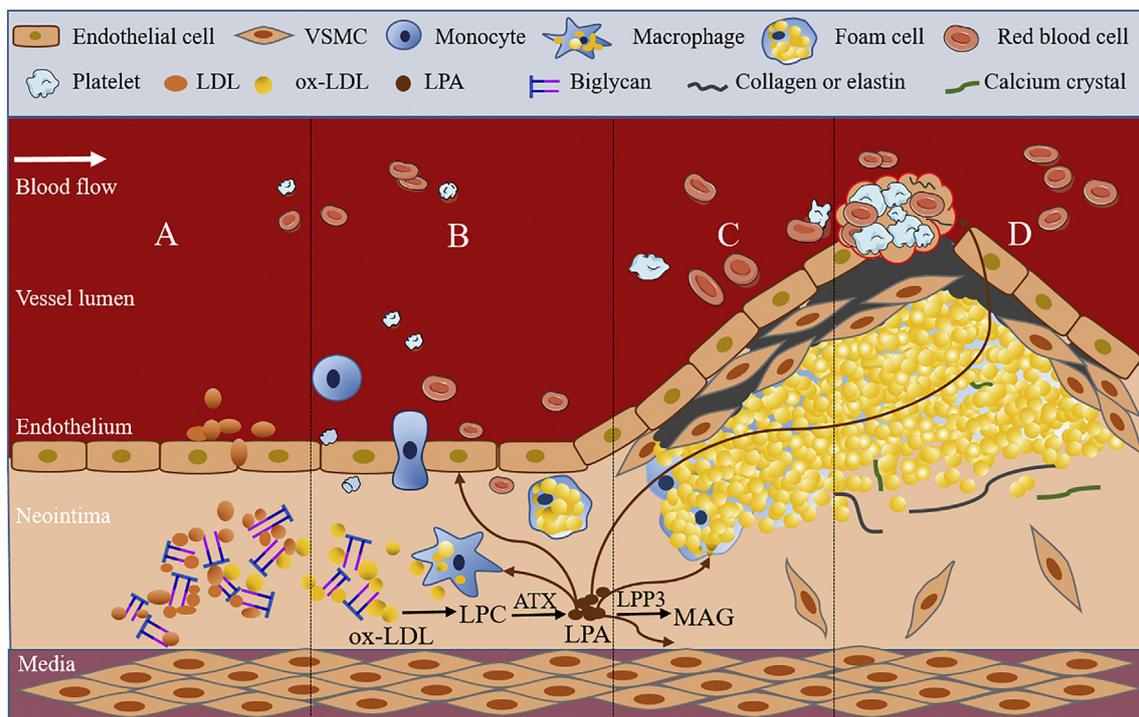
## 1. Introduction

Cardiovascular diseases (CVDs) are a group of diseases resulting from disorders of the heart and blood vessels. These diseases include coronary artery disease, cerebrovascular disease, peripheral artery disease, rheumatic heart disease, congenital heart disease, heart failure and many others (Benjamin et al., 2017). Despite the availability of a plethora of drugs to treat multiple risk factors, CVDs are still the leading causes of morbidity and mortality worldwide (Benjamin et al., 2017; McAloon et al., 2016). There is thus a need for the ongoing development of multiple new therapeutic strategies against CVDs (Roth et al., 2017).

The principal underlying pathology of most CVDs is atherosclerosis, which is a complex vascular pathological condition characterized by lipid deposition, endothelial dysfunction, vascular inflammation, matrix remodelling and fibroatheroma formation in the vessel wall (Fig. 1) (Gimbrone Jr & García-Cardeña, 2016; Libby, 2012; Ross, 1993; Weber & Noels, 2011; Williams & Tabas, 1995). In the vasculature, almost every type of cell is involved in the development of atherosclerosis (Linton et al., 2019; Tabas, García-Cardeña, & Owens, 2015). Among them, endothelial cells (ECs) form a dynamic interface (i.e., endothelium), which functions as a barrier between the circulating blood and the blood vessel wall. The dysfunction of this barrier by the lipid deposition is an early event in the development of atherosclerosis (Gimbrone Jr & García-Cardeña, 2016). With pathogenic signals, inflammatory cells are recruited to the sub-endothelium to propagate the atherosclerotic cascades (Libby, 2012). Vascular smooth muscle cells (VSMCs) proliferate and migrate into the intima, contributing to vascular intimal remodelling (Doran, Meller, & McNamara, 2008). Additionally, platelets are crucial for the formation of thrombosis after the rupture of the vulnerable atherosclerotic plaque (Davì & Patrono, 2007; Ruggeri, 2002). The activity of these vasculature cells and the

development of atherosclerosis are regulated by multiple signaling molecules that are present in the vascular system (Libby, Sukhova, Lee, & Galis, 1995; Sprague & Khalil, 2009). Lysophosphatidic acid (LPA) is a term for a series of endogenous signaling molecules (Moolenaar, 1995) which are generated during the metabolism of lipids. Different lines of in vitro and in vivo evidence suggest that LPA plays a crucial role in the development of atherosclerosis via multiple pathophysiological processes.

Since the elucidation of the role of LPA in eliciting calcium responses in smooth muscle cells (SMCs) (Vogt, 1963), LPA has been intensively investigated regarding the pathogenesis of atherosclerosis (Schober & Siess, 2012; Siess & Tigyi, 2004; Sugden & Holness, 2011). LPA has a wide array of effects on vasculature cells (Smyth, Cheng, Miriyala, Panchatcharam, & Morris, 2008; Sugden & Holness, 2011), making it a potential pathogenic factor. Moreover, exogenous administration of LPA in rodents and other mammalian species results in acute vasoconstriction and increased blood pressure (Tigyi et al., 1995; Tokumura, Fukuzawa, & Tsukatani, 1978; Tokumura, Yotsumoto, Masuda, & Tanaka, 1995). Local infusion of LPA in carotid arteries induces progressive vascular neointima formation (Shen, Zou, Li, Zhang, & Guo, 2018; Yoshida et al., 2003; Zhang et al., 2004). More interestingly, LPA is largely produced by activated platelets as the concentration of LPA in serum is much higher than that in the plasma (Eichholtz, Jalink, Fahrenfort, & Moolenaar, 1993). This observation suggests LPA production can be accelerated under pathological conditions that involve platelet activation, such as inflammation and atherosclerosis. Indeed, the level of LPA is elevated in the serum of patients with acute heart attacks (Chen et al., 2003). The circulating LPA level is also increased in patients with acute coronary syndrome (Dohi et al., 2012) with blood at culprit coronary arteries possessing higher LPA levels, compared to the systemic arterial circulating blood (Dohi et al., 2013). In addition, LPA is found accumulated in atherosclerotic plaques (Rother et al., 2003;



**Fig. 1.** The major stages during the progression of atherosclerosis. A. The retention of low density lipoprotein (LDL) cholesterol in the neointima. Inside the neointima, LDL is trapped by proteoglycans, mainly biglycan. B. The trapped LDL gets oxidized forming immunogenic oxidized-LDL (ox-LDL), which then generates lipid factors, such as lysophosphatidic acid (LPA). These LDL and its derivative factors have multiple effects on the vasculature cells, specially causing the damage of endothelium, promoting the following inflammatory responses: (i) recruitment of monocytes into the neointima; (ii) conversion of monocytes into macrophages, which take up ox-LDL; and (iii) transformation of the lipid laden macrophages into foam cells. C. The cluster of foam cells, termed "fatty streak", leads to the devastating stage: (i) vascular smooth muscle cells (VSMCs) migrate to the sub endothelium layer to secrete collagen and elastin to form the fibrous cap; (ii) VSMCs also deposit calcium crystal in the neointima contributing to the formation of atherosclerotic plaque. D. If the atherosclerotic plaque is unstable, it can rupture thus leading to the formation of thrombosis. ATX, autotaxin; LPC, lysophosphatidylcholine; LPP3, lipid phosphate phosphatase 3; MAG, monoacylglycerol.

(Siess et al., 1999). These observations indicate that elevated LPA accelerates the progression of CVDs.

The biological responses to LPA is achieved through the activation of at least six known G protein-coupled LPA receptors (LPARs) (LPAR1-LPAR6) (Choi et al., 2010; Kihara, Maceyka, Spiegel, & Chun, 2014). These receptors are classified into endothelial differentiation gene (EDG) family (LPAR1-LPAR3) and non-EDG family (LPA4-LPAR6) receptors (Fukushima, Ishii, Tsujiuchi, Kagawa, & Katoh, 2015). In addition, peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) (McIntyre et al., 2003; Zhang et al., 2004), GPR87 (Tabata, Baba, Shiraishi, Ito, & Fujita, 2007), GPCR35 (Oka, Ota, Shima, Yamashita, & Sugiura, 2010) and P2Y10 (Murakami, Shiraishi, Tabata, & Fujita, 2008) have also been suggested to mediate LPA signaling (Velasco, O'sullivan, & Sheridan, 2017). The expression and function of each LPAR in the vasculature is cell and context dependent. For example, the mRNA of LPAR1-LPAR6 are differentially expressed in human atherosclerotic lesions as compared to normal arteries, indicating individual LPARs may play a distinct role in the initiation and progression of atherosclerosis (Aldi et al., 2018). Thus, it is important to assess the individual role of each type of LPAR in the context of vascular diseases, which might lead to uncovering new therapeutic targets against CVDs. This review will focus on the current knowledge of LPA, LPARs and their signaling pathways in vascular disease settings and attempt to unravel the presently unresolved issues and future promising areas for investigation.

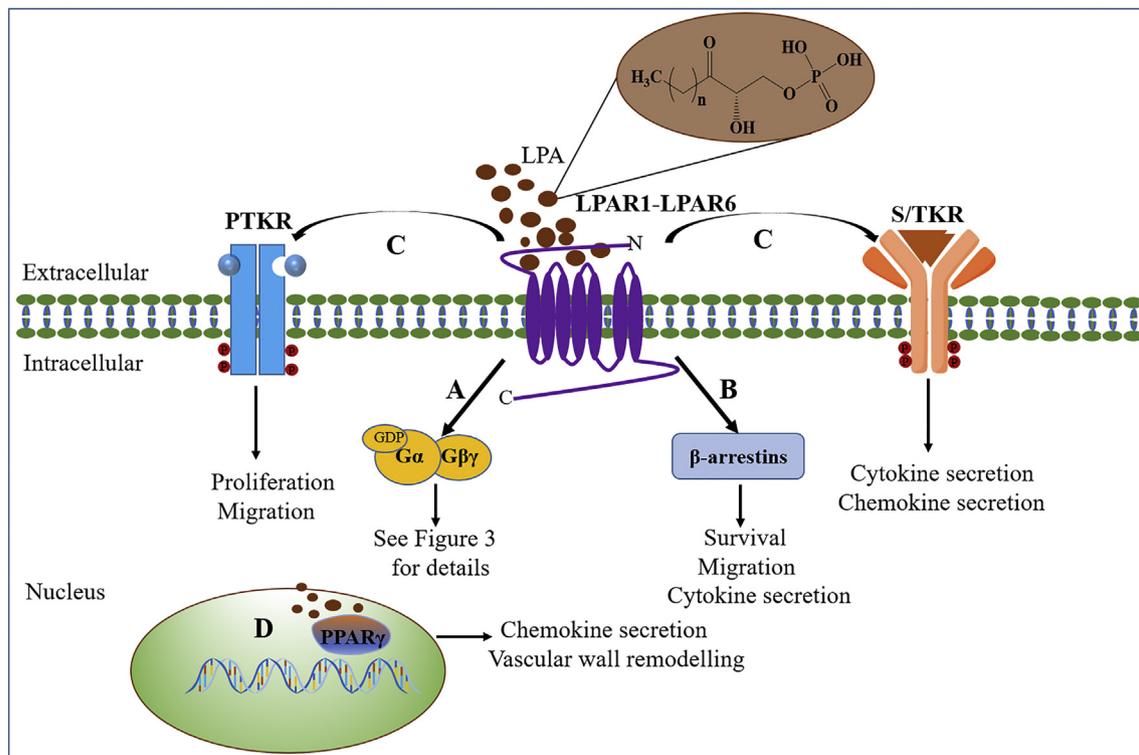
## 2. LPA and its production and degradation

LPA (molecular weight: 430–480 Da), belonging to the glycerol-based lysophospholipid family, comprises a glycerol backbone, a phosphate head group and a single tail of acyl chain of varied length and saturation (Yung, Stoddard, & Chun, 2014) (Fig. 2). The 16:0, 18:1, 18:2 and 20:4 LPA isoforms are the predominant forms in blood, while 18:1 LPA is the most commonly used laboratory form for signaling studies

(Choi et al., 2010). Generally, the unsaturated forms are more biologically potent than the saturated forms (Aoki, 2004; Hayashi et al., 2001; Zhang et al., 2004). Moreover, these LPA isoforms differ in affinity for distinct LPARs (Bandoh et al., 2000). For example, 2-acyl-LPA rather than 1-acyl-LPA is the preferred ligand of LPAR3 and LPAR6 (Bandoh et al., 1999; Yanagida et al., 2009). 18:1 LPA is the favored ligand of LPAR4 (Noguchi, Ishii, & Shimizu, 2003), while ether-linked 1-alkyl-LPA has a specific binding affinity to LPAR5 (Williams et al., 2009). LPA is widely distributed in tissues and biological fluids with varied concentrations. The LPA concentration in healthy individual plasma is around 0.7  $\mu$ M (Baker et al., 2002). However, its concentration in serum can be 10 times higher (Pamuklar et al., 2009) and can exceed 10  $\mu$ M under pathogenic conditions (Sugimoto, Takuwa, Yoshioka, & Takuwa, 2006; Yung et al., 2014).

LPA can be generated from various phospholipid precursors by several enzymes. The crucial enzyme in LPA production is autotaxin (ATX), which has lysophospholipase D activity and converts lysophospholipids, such as lysophosphatidylcholine, lysophosphatidylserine, lysophosphatidylethanolamine and lysophosphatidylinositol into LPA and corresponding headgroups (Tokumura et al., 2002; Tokumura et al., 2002). The other enzymes contributing to LPA production are phospholipase A1 and phospholipase A2, which hydrolyse the cell membrane located phosphatidic acids into LPA (Aoki, 2004; Sonoda et al., 2002). LPA can also be produced intracellularly via the acylation of glycerol-3-phosphate (Aoki, 2004; Aoki et al., 2002). Moreover, monoacylglycerol (MAG), the dephosphorylation product of LPA, can be rephosphorylated by acylglycerol kinase to form LPA (Bektas et al., 2005).

The inactivation of LPA in the circulation is also mediated by various enzymes. Three types of lipid phosphate phosphatases (LPPs), specially, LPP3 (Busnelli, Manzini, Parolini, Escalante-Alcalde, & Chiesa, 2018), play a vital role in LPA degradation which removes the phosphate head group to generate a receptor inactive lipid product, MAG



**Fig. 2.** Current known LPA signal transduction pathways in the vasculature. Currently, lysophosphatidic acid (LPA) can elicit its signals via at least four manners, each of which controls different downstream responses in the vasculature. A. LPA signals via classical G protein mediated signaling pathway. B. LPA signals via  $\beta$ -arrestin constituted signaling system. C. LPA signals via LPAR transactivated protein tyrosine kinase receptors (PTKR) or serine/threonine kinase receptors (S/TKRs). D. LPA signals via nucleus receptor PPAR $\gamma$ .

(Mueller, Ye, Morris, & Smyth, 2015). LPA acyltransferase is another enzyme which can convert LPA back into its precursors. In addition, LPA can be converted into glycerol-3-phosphate by phospholipases (Pagès, Simon, Valet, & Saulnier-Blache, 2001). The main pathway for LPA generation and degradation in the atherosclerotic plaque is shown in Fig. 1.

### 3. The in vitro studies of LPA and LPARs in the vasculature

A growing body of in vitro studies has demonstrated the impact of LPA and its receptors in the vasculature, such as enhanced cell proliferation and migration, elevated adhesion molecule expression, increased chemokines and cytokines secretion (Sugden & Holness, 2011). The complex interplay of these LPA-induced effects initiates and accelerates atherosclerosis. LPA has an effect on almost every type of vasculature cell, including platelets, monocytes, macrophages, dendritic cells, T-lymphocytes, vascular ECs, VSMCs and fibroblasts (Sugden & Holness, 2011). However, the exact receptor that initiates these effects in different vasculature cells is still unclear. This review seeks to reveal the current knowledge in this area.

#### 3.1. The effects of LPA and LPARs in platelets

In the vasculature, platelets are one of the major sources of LPA production (Aoki, Inoue, & Okudaira, 2008). LPA in turn regulates platelet shape (Siess et al., 1999), aggregation (Rother et al., 2003), platelet-monocyte aggregation (Haserück et al., 2004) and fibronectin matrix assembly (Olorundare, Peyruchaud, Albrecht, & Mosher, 2001). These effects can contribute to thrombosis, another critical pathogenic condition of vascular diseases. Among the lipids present in oxidized low density lipoprotein (ox-LDL) and atherosclerotic plaques, LPA is the major functional lipid molecule that is responsible for human platelet morphological changes (Siess et al. (1999). LPA-induced human platelet shape changes and aggregation are inhibited by the selective LPAR1 and LPAR3 antagonist, DGPP (8:0), indicating the regulatory role of LPAR1/3 (Rother et al., 2003). LPAR5 is the most abundantly expressed receptor in human platelets (Amisten, Braun, Bengtsson, & Erlinge, 2008; Khandoga, Pandey, Welsch, Brandl, & Siess, 2010). Two LPAR5 antagonists, H2L5987411 and H2L5765834, inhibit LPA-stimulated platelet shape changes, suggesting a prominent role of LPAR5 in LPA-mediated human platelet activation (Williams et al., 2009). Similarly, LPAR5 is the functional LPAR in platelets when examining two types of human MK cell lines (Meg-01, Dami) (Khandoga et al., 2010). Molecular silencing of LPAR5 inhibits platelet shape alterations induced by LPA in MK cells. LPAR2 suppression and LPAR3 activation promote megakaryopoiesis in K562 human erythroleukemia cells (Ho et al., 2015), suggesting roles of other LPARs in platelet regulation. In contrast, activation of LPAR3 inhibits platelet differentiation in human hematopoietic stem cells (CD34<sup>+</sup> HSCs) (Lin et al., 2018). These findings demonstrate the involvement of the different LPARs in the regulation of platelet activity.

#### 3.2. The effects of LPA and LPARs in endothelial cells

ECs line the innermost layer of blood vessel walls and play a vital role in maintaining vascular hemostasis (Gimbrone Jr & García-Cardena, 2016). LPA has a wide array of effects in ECs and is therefore critical for the hemostasis of the vasculature. In ECs, LPA stimulates adhesion molecule expression (Shimada & Rajagopalan, 2010), cytokine and chemokine secretion (Lin, Chen, Chen, & Lee, 2006), stress-fiber and gap formation (Siess et al., 1999), endothelial-leukocyte interactions (Rizza et al., 1999), and regulates the permeability of the endothelium (Alexander, Patton, Christman, Cuiper, & Haselton, 1998; Amerongen, Vermeer, & Van Hinsbergh, 2000).

The elevated adhesion molecules, cytokines and chemokines on the surface of ECs can trigger and favour monocyte and lymphocyte transmigration across the endothelium (Ramji & Davies, 2015; Sprague &

Khalil, 2009). In human umbilical vein endothelial cells (HUVECs), LPA via LPAR1 and LPAR3 and multiple downstream effectors, including Gα<sub>i</sub> protein (Lee et al., 2004; Palmetshofer, Robson, & Nehls, 1999) and RhoA-ROCK2-NF-κB (Shimada & Rajagopalan, 2010) leads to diverse adhesion molecule expression, such as E-selectin, vascular cell adhesion molecule-1 and intercellular adhesion molecule-1. In addition to the stimulation of adhesion molecule expression, LPA also induces cytokine and chemokine secretion by ECs. LPA-enhanced interleukin 8 (IL-8) and monocyte chemoattractant protein-1 (MCP-1) expression is mediated through a Gα<sub>i</sub>-RhoA-ROCK-NF-κB dependent pathway (Lin et al., 2006). The use of Ki16425 (a LPAR1/3 inhibitor) and GW9662 (PPARγ inhibitor) both reduce the LPA-mediated secretion of IL-8 and MCP-1 (Gustin, Van Steenbrugge, & Raes, 2008). Silencing of LPAR1 and LPAR3 in HUVECs suppresses IL-8 and MCP-1 associated gene expression (Lin et al., 2007). LPAR1 and LPAR3 are also involved in vascular endothelial growth factor-C expression, in vitro and in vivo EC tube formation (Lin et al., 2008), endothelial-dependent chemokine (C-X-C motif) ligand 1 (CXCL1) expression and monocyte adhesion (Zhou et al., 2011). LPA-stimulated endothelial hypoxia-inducible factor, (HIF)-1α, is the upstream effector of CXCL1 (Akhtar et al., 2015). Hence, LPA-LPAR1/3-HIF-1α-CXCL1 is an axis involved in the acceleration of atherosclerosis. Recently, it has been proposed that LPA via LPAR1/3-PKD-1 (protein kinase D-1)-FoxO1 (forkhead box protein O1) signaling axis downregulates CD36 expression and the propagation of the angiogenic signaling (Ren et al., 2016), which indicates that the diverse functions of LPA via ECs contributes to the development of atherosclerosis.

Increased permeability of the endothelium is a critical event across all stages of the atherosclerotic process (Komarova, Kruse, Mehta, & Malik, 2017). The effect of LPA on permeability of the endothelium is controversial. In HUVECs, LPA via LPAR6-Gα<sub>13</sub>-RhoA-ROCK pathway induces actin stress fibre formation and increases cell permeability (Amerongen et al., 2000; Yukiura, Kano, Kise, Inoue, & Aoki, 2015a, 2015b). LPA also decreases the permeability of the endothelium (Alexander et al., 1998). More recently, the LPAR1 antagonist (AM966) acting through LPAR1-Gα<sub>12/13</sub>-RhoA pathway leads to human lung microvascular EC barrier disruption, thus indicating a role for LPAR1 in regulation of the permeability of endothelium (Cai, Wei, Li, Suber, & Zhao, 2017). These observations provide evidence for the requirement of LPAR1, LPAR3, LPAR6 and PPARγ for the function of ECs.

#### 3.3. The effects of LPA and LPARs in inflammatory cells

Leukocyte recruitment and subsequent induction of an inflammatory response are hallmarks of the progression of atherosclerosis (Ross, 1993, Libby, 2006, Little et al. 2011). LPA promotes the progression of atherosclerosis via its various effects on inflammatory cells, for example, promoting inflammatory cell migration (Takeda et al., 2016), cytokine secretion (Kozian et al., 2016) and macrophage transformation (Chen et al., 2017).

LPA via its receptors regulates the migration of inflammatory cells. Inefficient movement of LPAR2-deficient lymphocytes within lymph nodes indicates the active role of LPAR2 in lymphocyte transmigration across the high endothelial venules basal lamina (Bai et al., 2013). Moreover, movement of T-cells within the lymph nodes is induced by LPA via a LPAR2-ROCK-myosin II dependent pathway (Takeda et al., 2016). In addition to LPAR2, LPAR4 also plays a crucial role in the transmigration of lymphocytes across high endothelial venules into lymph nodes. Specifically, lymphocytes accumulate heavily within the high endothelial venules EC layer in LPAR4 null mice, compared to LPAR6 null mice and wild-type mice (Hata et al., 2015).

LPA also regulates macrophage-related processes, such as monocyte-macrophage transformation (Ray et al. 2017), ox-LDL uptake (Chang, Hsu, Lin, Chiang, & Lee, 2008), macrophage-foam cell transformation (Chen et al., 2017) and matrix metalloproteinase (MMP) secretion (Gu et al., 2017). LPA-induced ox-LDL uptake in macrophages is

mediated via the LPAR3-G $\alpha_i$ -SR-A (the class A scavenger receptor) pathway (Chang et al., 2008). LPA promotes foam cell formation in Raw 264.7 macrophages by downregulating LPAR1/3-Akt dependent class B scavenger receptor type I (SRBI) expression, a receptor promoting the efflux of free cholesterol (Chen et al., 2017). The application of the LPAR1/3 inhibitor (Ki16425) and Akt inhibitor (MK-2206) both prevent foam cell formation and enhance SRBI expression (Chen et al., 2017). Furthermore, MMPs, particularly MMP9 (Sluijter et al., 2006; Vandooren, Van Steen, & Opdenakker, 2013), are principle mediators of extracellular matrix remodeling and degradation, which contribute to atherosclerotic plaque instability (Ketelhuth & Bäck, 2011; Werb, 1997). In THP-1-derived macrophages, the attenuated MMP9 expression in LPAR2 knockdown cells and NF- $\kappa$ B inhibited (using PDTC) cells suggests a modulating role of LPA in the expression of MMP9 via the LPAR2-NF- $\kappa$ B pathway (Gu et al., 2017). Similarly, in human THP-1 cells, the downregulation of LPA-induced MMP9 expression by toll-like receptor 4 (TLR4) knockdown indicates the regulation role of LPA via TLR4 other than the classical LPARs (Zhou, Yang, Li, Liu, & Lei, 2018).

Activated mast cells (MCs) release pro-inflammatory mediators, increase vascular permeability and facilitate migration of circulating inflammatory cells to the site of MC activation (Bot, Shi, & Kovanen, 2015; Theoharides et al., 2012). LPA accelerates this process by regulating the activity of MCs (Bot et al., 2013). LPA stimulates MC viability through LPAR1/3-G $\alpha_i$  dependent and PPAR $\gamma$ -dependent pathways (Bagga et al., 2004). In primary human cord blood MCs, LPA via LPAR5 induces calcium fluxes and secretion of macrophage inflammatory protein-1 $\beta$ , a potent chemokine associated with immune cell activation (Lundequist & Boyce, 2011). Evidence has also been provided in the human mast (HMC-1) and the mouse microglia (BV-2) cell lines where inhibition of LPAR5 prevents LPA-mediated secretion of MCP-1 in both cell lines (Kozian et al., 2016). These results suggest LPAR5 is a functional LPAR on MCs.

Other than the effects on T cells, macrophages and mast cells, LPA also regulates the function of neutrophils and dendritic cells (DCs). For instance, LPA induces the calcium influx in human neutrophils (Itagaki, Kannan, & Hauser, 2005). In DCs, LPA enhances human DC maturation and promotes immature cells into the intima to facilitate the development of atherosclerosis (Chen et al., 2006). LPAR3 is the receptor that regulates the migration of immature murine DCs to LPA, as the motility was reduced by LPAR3 antagonist VPC32179 or LPAR3 silencing (Chan et al., 2007). Furthermore, LPA inhibits LPS stimulated DC activation via a LPAR2-dependent manner with the evidence that LPAR2-deficient DCs are hyperactive both in vitro and in vivo (Emo et al., 2012).

#### 3.4. The effects of LPA and LPARs in vascular smooth muscle cells

VSMCs have a pivotal role in the initiation and progression of atherosclerosis (Chamley-Campbell, Campbell, & Ross, 1979; Doran et al., 2008). During atherosclerosis, VSMCs proliferate and migrate to the intima, contributing to the thickening of the neointima and fibroatheroma formation (Doran et al., 2008). LPA can induce dedifferentiation (Hayashi et al., 2001), proliferation (Tokumura et al., 1994), migration (Kim, Keys, & Eckhart, 2006) and contraction (Wang et al., 2009) of VSMCs and hence is a major contributor to the development of the neointima in which atherosclerosis subsequently develops.

LPA via the mitogen-activated protein kinases (MAPKs), ERK and p38, induces rat aortic VSMC dedifferentiation resulting in cell proliferation and migration (Hayashi et al., 2001). Phenotypic modulation is a critical feature of the behaviour of VSMCs, contributing to the development of atherosclerosis in multiple ways (Campbell & Campbell, 1985). LPA stimulated phenotypic modulation of rat aortic VSMCs is regulated by PPAR $\gamma$  (Zhang et al., 2004). LPA-induced rat aortic VSMC phenotypic modulation is mediated by LPAR3, as LPA-induced cell phenotypic modulation is inhibited by LPAR1/3 antagonist but this inhibitory effect is lost when coadministered with a highly selective LPAR3 agonist (Zhou, Niu, & Zhang, 2010). The role of PPAR $\gamma$  and LPAR3 in VSMCs

phenotypic dedifferentiation in vitro is thus correlated. LPA-induced VSMC migration and proliferation is mediated by G $\alpha_q$  protein as VSMCs with transient infection of adenovirus encoding G $\alpha_q$ , an inhibitor of G $\alpha_q$ , display decreased LPA-induced VSMC migration and proliferation (Kim et al., 2006). Aortic SMCs isolated from LPAR1 knockout mice and LPAR1/2 double knockout mice have been used to demonstrate that LPAR1 and LPAR2 are not required for LPA stimulated VSMCs dedifferentiation but are essential in VSMCs proliferation and migration (Panchatcharam et al., 2008). Recent data have demonstrated that LPA-induced migration of murine VSMCs is regulated through LPAR1-CCN1-integrin-focal adhesion kinase (FAK) axis (Wu et al., 2014).

Cytokines are mediators for most bio-responses of VSMCs (Sprague & Khalil, 2009). LPA stimulates the release of IL-6 and MCP-1 in human aortic VSMCs (Hao, Tan, Wu, Xu, & Cui, 2010). Notably, these cytokines are also highly expressed on atherosclerotic lesions and can exacerbate atherosclerosis (Gu et al., 1998; Schieffer et al., 2000). LPA-induced IL-6 release is mediated by LPAR1-PKC (protein kinase C)-p38 $\alpha$  pathway (Hao et al., 2010), whereas LPA-induced MCP-1 secretion is via Rac-1-mediated NADPH oxidase-dependent ROS generation (Kaneyuki et al., 2007). Many cytokines, such as IL-6 and TNF- $\alpha$  (tumor necrosis factor  $\alpha$ ) can modulate VSMC contraction and vascular tone (Sprague & Khalil, 2009). The role of LPA on vascular tone is complex; LPA can induce vasodilation via endothelial dependent LPAR1-PLC (phospholipase C)-NOS (nitric oxide synthase) (Ruisanchez et al., 2014) pathway and also cause vasoconstriction in VSMCs by cross talk between LPA/LPAR1 and TXA2/TP pathways (Dancs et al., 2017). Taken together, the data indicates that LPAR1, LPAR2 and LPAR3 are the prominent functional receptors on VSMCs.

The retention of low density lipoprotein (LDL) in the sub-endothelium by proteoglycans, mainly biglycan, is the initiating event and a central pathogenic process in atherogenesis (Williams & Tabas, 1995; Williams & Tabas, 1998; Nakashima et al., 2007; Little, Ballinger, Burch, & Osman, 2008). Hyperelongation of glycosaminoglycan (GAG) chains on biglycan leads to increased binding of lipoproteins and enhanced entrapment in the blood vessel wall (Little et al., 2008). A number of growth factors, such as thrombin (Ivey & Little, 2008), platelet-derived growth factor (PDGF) (Getachew et al., 2010), endothelin (Little, Burch, Getachew, Al-aryahi, & Osman, 2010) and transforming growth factor- $\beta$  (TGF- $\beta$ ) (Burch et al., 2010; Burch et al., 2010), act on VSMCs to induce the production and secretion of biglycan with hyperelongated GAG chains. Blocking the effect of growth factors that cause hyperelongation of GAG chains on biglycan reduces the lipid binding in vitro and decreases lipid deposition in the vessel wall in mouse models of atherosclerosis (Ballinger et al., 2010; Getachew et al., 2010; Kijani, Vázquez, Levin, Borén, & Fogelstrand, 2017). However, little is presently known in relation to the effect of LPA and LPARs on GAG chain hyperelongation which can be a potential area of future investigation.

#### 4. The in vivo studies of LPA and LPARs in the vasculature

Genetic manipulation in animal models has enabled detailed studies on the role of LPA and its receptors in vascular diseases. ATX (encoded by Enpp2 gene) is the primary enzyme involved in LPA production with the evidence that the heterozygous ATX deficient mice result in 50 percent LPA reduction (Tanaka et al., 2006; Van Meeteren et al., 2006), while the ATX overexpressing mice have elevated circulating LPA levels compared to the wild-type (Pamuklar et al., 2009). Moreover, ATX gene manipulated in vivo models have been established to investigate the essential impact of LPA in vascular diseases. ATX-deficient mice experience embryonic lethality (around E9.5-10.5) due to aberrant vessel formation in the yolk sac and embryos (Tanaka et al., 2006; Van Meeteren et al., 2006). Similarly, ATX-deficient zebrafish embryos display severe vascular defects, which are also observed in LPAR1/4 double knockdown zebrafish embryos (Yukiura et al., 2011). However, mice

overexpressing ATX demonstrate prolonged bleeding time and attenuated thrombosis (Pamuklar et al., 2009). Overexpression of ATX in mice embryonic period also causes severe vascular abnormalities and results in embryonic death (~E9.5) (Yukiura et al., 2015a, 2015b).

LPP3 (encoded by PLPP3 or PPAP2B) is the major enzyme involved in LPA degradation and thus has a specific role in vascular pathophysiology (Busnelli et al., 2018; Wu et al., 2015). LPP3 deficient mouse embryos show embryonic lethality (~E9.5) due to abnormal vasculogenesis (Escalante-Alcalde et al., 2003). Recently, liver-specific LPP3 deletion was shown to aggravate the development of atherosclerosis through the modulation of the plasma lipids in apoE<sup>-/-</sup> mice (Busnelli et al., 2017). Furthermore, adult mice lacking LPP3 in endothelial and hematopoietic cells exhibit enhanced inflammatory responses and impaired angiogenesis (Panchatcharam et al., 2014). It is LPP3 deficiency in endothelial not hematopoietic cells that leads to increased vascular permeability (Panchatcharam et al., 2014). Administration of the ATX inhibitor (PF8380) and pan-LPA receptor antagonist (BrP-LPA) into LPP3 null mice both attenuates inflammation-induced endothelial permeability (Panchatcharam et al., 2014). Thus, the role of ATX-LPA-LPP3 axis for development of vascular system has been recognised.

Direct manipulation of LPARs in animal models has provided evidence supporting the critical role of different LPARs in vascular disease. The vascular leak produced by lung injury is markedly attenuated in LPAR1 knockout mice (Tager et al., 2008). In LDL receptor null mice, administration of the LPAR1/3 antagonist Ki16425 reduces the development of atherosclerotic plaques by reducing MCP-1 and plasma LDL cholesterol levels (Kritikou et al., 2016). Moreover, LPAR4 was recently implicated as a key regulator of LPA signaling in the vascular system. LPAR4 null mice display high embryonic lethality (~30%) due to defective blood and lymphatic vessel formation characterized by edema and hemorrhage (Sumida et al., 2010). In addition, LPAR4 mediates the LPA stimulated fine vascular networks formation in mice models through G $\alpha_{12/13}$  mediated RhoA/ROCK signaling, suggesting the regulatory role of LPAR4 in angiogenesis (Eino et al., 2018; Takara et al., 2017). More recently, LPAR4 via the promotion of inflammatory cell migration enhances the development of atherosclerosis which is attenuated in LPAR4 deficient mice (Yang et al., 2019). In another mouse model, LPAR4-G $\alpha_{12/13}$ -Rho/ROCK signaling is mainly responsible for LPA-initiated hypertensive response, whereas LPAR6 is critical receptor for vasoactivity and vascular development (Kano et al., 2019). These lines of evidence suggest that LPAR1, LPAR4, LPAR6 are the main mediators in vascular system homeostasis with the precise underlying molecular mechanisms remaining to be elucidated.

Neointimal lesions characterized by the accumulation of cells within the intima are a prelude to the development of atherosclerotic plaques (Stary et al., 1994). LPA can induce neointimal lesion development when applied to uninjured arteries (Cheng et al., 2009). PPAR $\gamma$  and LPAR1 are required to elicit LPA-induced vascular wall remodelling and atherogenesis. LPA via stimulating VSMC proliferation and migration triggers arterial wall remodelling in the rat carotid artery model (Yoshida et al., 2003) and this effect is completely blocked by the PPAR $\gamma$  antagonist, GW9662, and mimicked by PPAR $\gamma$  agonists (Zhang et al., 2004). Specially, LPAR1, LPAR2 and LPAR1/2 double knockout mice behave like wild type mice, whereas PPAR $\gamma$  null mice fail to show PPAR $\gamma$  ligand-induced arterial wall remodelling (Cheng et al., 2009). Matricellular protein CCN1 is associated with LPA-LPAR1-induced SMC migration in vitro (Wu et al., 2014). An in vivo experiment demonstrates that CCN1 is highly expressed in neointimal lesions produced by LPA infusion of carotid artery in a mouse model (Hao et al., 2016). CCN1 siRNA knockdown and LPAR1 knockout inhibit LPA-induced neointimal formation (Hao et al., 2016), suggesting a key role of LPAR1-CCN1 axis in SMC migration and vascular remodelling. LPARs also enhance the development of neointimal lesions caused by other physical vascular injuries. LPAR1 and LPAR2 are upregulated following ligation vascular injury (Panchatcharam et al., 2008). LPAR1/2 double knockout partially protects the mice from the injury-induced

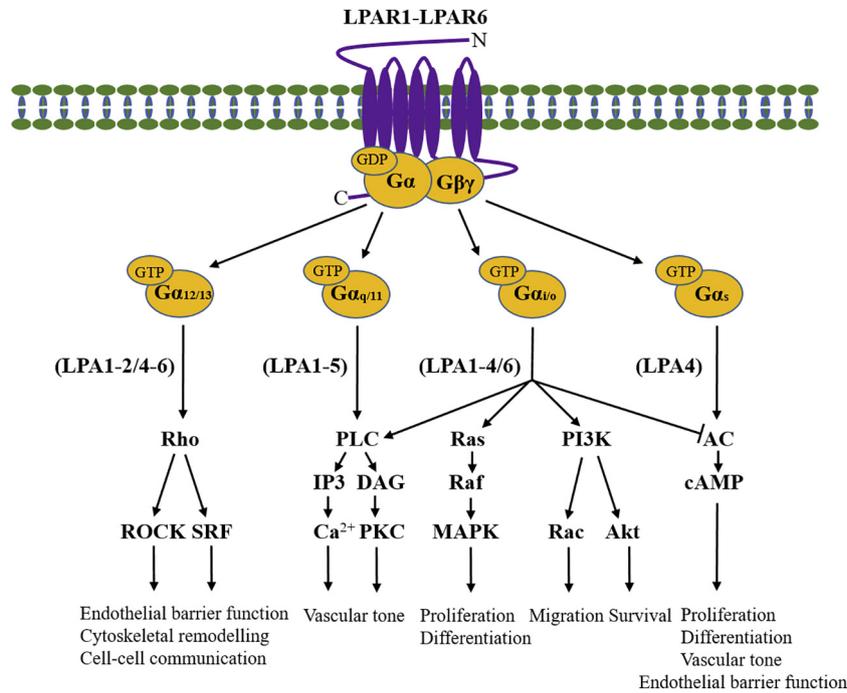
neointimal hyperplasia while LPAR1 null mice develop larger neointimal lesions after injury due to the upregulation of LPAR3 (Panchatcharam et al., 2008). In a wire-induced carotid injury mouse model, LPAR1 mRNA is downregulated while LPAR3 mRNA is upregulated at one week after injury (Subramanian et al., 2010). The use of the LPAR1/3 antagonist, Ki16425, inhibits neointimal formation due to the diminished neointimal accumulation of both SMCs and macrophages (Subramanian et al., 2010). Consistent with these results, in a carotid artery balloon injury rat model, intraperitoneal injection of 1 mg/kg LPA enhances vascular neointimal hyperplasia via the modulation of proliferation, autophagy, inflammation and oxidative stress of neointima (Shen et al., 2018). These observations suggest that PPAR $\gamma$  and LPAR1/2/3 are the critical regulators of vascular remodelling and thus essential for the progression of atherosclerosis.

## 5. The signaling pathways of LPA

GPCR signaling involves three signal transduction mechanisms (Daub, Weiss, Wallasch, & Ullrich, 1996; Little et al., 2010; Little, Burch, et al., 2011; Little, Chait, and Bobik, 2011; Burch, Getachew, Osman, Febbraio, & Little, 2013; Kamato et al., 2016). The first is classical mechanism by which the signal is transmitted in a direct way: ligand  $\rightarrow$  receptor  $\rightarrow$  coupled G protein  $\rightarrow$  intracellular effectors (Pierce, Premont, & Lefkowitz, 2002; Simon, Strathmann, & Gautam, 1991; Sutherland, Robison, & Butcher, 1968). For example, thrombin via protein activated receptor (PAR-1) and G $\alpha_q$  leads to increased intracellular calcium (Neylon, Nickashin, Little, Tkachuk, & Bobik, 1992). The second is mediated by two types of  $\beta$ -arrestins which trigger signaling by recruiting scaffold signaling proteins to the receptor (Lefkowitz & Shenoy, 2005). The third mechanism is transactivation dependent signaling in which the agonist binds to its cognate GPCR complex to activate second cell surface receptors, either a protein tyrosine kinase receptor (PTKR) (Daub et al., 1996) or serine/threonine kinase receptor (S/TKR) (Burch et al., 2013; Kamato et al., 2016; Little et al., 2010), without involvement of new protein synthesis (Little, Burch, et al., 2011; Little, Chait, and Bobik, 2011). Apart from the transmembrane receptors, the nuclear receptor PPAR $\gamma$  also plays a critical role in LPA signal transduction (McIntyre et al., 2003). Below we review these mechanistic pathways in the context of LPA signaling (Fig. 2).

### 5.1. LPA signaling via classical GPCR signaling pathway

The six LPARs show distinct affinity to G $\alpha$  proteins: LPAR1 (G $\alpha_{12/13}$ , G $\alpha_{q/11}$ , G $\alpha_{i/o}$ ), LPAR2 (G $\alpha_{12/13}$ , G $\alpha_{q/11}$ , G $\alpha_{i/o}$ ), LPAR3 (G $\alpha_{q/11}$ , G $\alpha_{i/o}$ , G $\alpha_s$ ), LPAR4 (G $\alpha_{12/13}$ , G $\alpha_{q/11}$ , G $\alpha_{i/o}$ , G $\alpha_s$ ), LPAR5 (G $\alpha_{12/13}$ , G $\alpha_{q/11}$ ), LPAR6 (G $\alpha_{12/13}$ , G $\alpha_{i/o}$ ) (Fig. 3) (Stoddard & Chun, 2015; Yung et al., 2014). Among them, G $\alpha_q$  protein is the regulator of LPA-stimulated MMP-2 and MMP-9 secretion in LPAR1-expressing B103 cells (Kato et al., 2012). G $\alpha_q$  protein also acts as a regulator during the proliferation and migration of VSMCs (Kim et al., 2006). G $\alpha_i$  coupling to LPAR1 leads to VSMC contraction (Dancs et al., 2017), while coupling to LPAR3 promotes ox-LDL uptake in macrophages (Chang et al., 2008). LPA via the LPAR1/2-G $\alpha_{12/13}$ -RhoA-ERM pathway induces ovarian cancer cell migration (Park et al., 2018). Upon LPA stimulation, LPAR4 and LPAR5 via the coupling of G $\alpha_{12/13}$  leads to Rho-mediated neurite retraction and stress fiber formation while the binding of G $\alpha_{q/11}$  resulting in increased intracellular Ca<sup>2+</sup> through PLC and the second messengers, inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) (Lee, Rivera, Gardell, Dubin, & Chun, 2006). In addition, LPA via LPAR6 induces G $\alpha_{12/13}$ -ROCK-dependent morphological changes in HUVECs (Yanagida et al., 2009). Recent research suggests LPAR6-G $\alpha_{12/13}$ -Rho pathway is involved in blood-brain barrier permeability and brain edema (Masago et al., 2018). The classical signaling pathway is one of the principle pathways by which LPA exhibits its intracellular effects.



**Fig. 3.** The G protein mediated LPA signaling in the vasculature. The classical signaling of LPA is associated with heterotrimeric G-proteins. The LPARs via their preferred G proteins elicit diverse downstream signaling cascades that include Rho family small GTPase, phospholipase C (PLC), mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K), and adenylyl cyclase (AC). These different signaling cascades regulate pleiotropic effects in the vasculature, including the endothelial barrier function and vascular tone, etc. ROCK, Rho-associated kinase; SRF, serum response factor; IP3, inositol 1,4,5-trisphosphate; DAG, diacylglycerol; PKC, protein kinase C; cAMP, cyclic adenosine monophosphate.

### 5.2. LPA signaling via $\beta$ -arrestin dependent pathway

While  $\beta$ -arrestins are known to desensitize the G protein-mediated GPCR signaling, they also elicit G protein-independent signaling cascades (Lefkowitz & Shenoy, 2005).  $\beta$ -arrestin 2 regulates LPAR1 endocytosis and LPA signal attenuation in mouse embryonic fibroblasts (Urs et al., 2005). G protein-independent  $\beta$ -arrestin 2 is the major mediator in LPA-induced ERK phosphorylation in murine fibroblast cells (Gesty-Palmer, El Shewy, Kohout, & Luttrell, 2005). In fibroblast cells from  $\beta$ -arrestin 2 knockout and  $\beta$ -arrestin 1/2 double knockout mice, the LPA-stimulated ERK activation is mediated by EGFR as the responses are inhibited by EGFR inhibitor, AG1478. In contrast, in  $\beta$ -arrestin 1 knockout cells and in wild-type cells, the LPA-induced ERK phosphorylation is insensitive to AG1478 (Gesty-Palmer et al., 2005). By recruiting CARMA3 to LPARs,  $\beta$ -arrestin 2 regulates LPA-mediated NF- $\kappa$ B activation and IL-6 expression in mouse embryonic fibroblasts (Sun & Lin, 2008). Moreover,  $\beta$ -arrestin 2 regulates LPA-induced cancer cell migration and invasion (Alemayehu et al., 2013; Li et al., 2009). In addition,  $\beta$ -arrestins downstream of RhoA/ROCK negatively control LPA-introduced Akt signals as the elevated pAkt levels are observed in  $\beta$ -arrestin 1/2 double knockout MEF cells (Lima-Fernandes et al., 2011). As discussed above,  $\beta$ -arrestins are playing a critical role in LPA signaling transduction.

### 5.3. LPA signaling via LPAR transactivation of kinase receptors

The initial paradigm changing observation of GPCR transactivation dependent signaling in Rat-1 fibroblasts utilized LPA as the GPCR agonist to investigate EGFR, a typical PTKR, transactivation (Daub et al., 1996). LPAR transactivation of the EGFR has also been demonstrated in human HaCaT keratinocytes and COS-7 cells; thereby extending the scope of this phenomenon (Daub, Wallasch, Lankenau, Herrlich, & Ullrich, 1997). LPA-induced transactivation of EGFR can be mediated via a ligand-independent mechanism (Daub et al., 1997) or a ligand-dependent mechanism which involves MMP mediated shedding of the

heparin-binding EGF-like growth factor (HB-EGF) (Prenzel et al., 1999). Many other downstream processes are dependent on LPAR transactivation of EGFR making it a key pathway for LPA signaling. For example, LPA-induced phosphoinositide 3-kinase (PI3K) activation depends on transactivation of EGFR (Laffargue et al., 1999). LPA via  $G\alpha_i$  mediated transactivation of EGFR stimulates the global protein synthesis in cultured rat aortic SMCs (Voisin et al., 2002). Similarly, LPA-induced coronary artery SMC migration is dependent on LPAR1- $G\alpha_i$  mediated transactivation of EGFR (Komachi et al., 2009). Furthermore, in HUVECs, LPA-upregulated vascular endothelial growth factor-C mRNA expression and subsequent tube formation of ECs in vitro and in vivo are regulated by LPAR1/3-MT1-MMP/MMP-2-COX-2 mediated transactivation of EGFR (Lin et al., 2008). Additionally, the LPAR2- $G\alpha_i$ -Src-EGFR-ERK axis is responsible for LPA-induced cell motility in ovarian cancer cells (Jeong et al., 2008). LPA-LPAR3- $G\alpha_{13}$ -RhoA-ROCK-EGFR signal axis contributes to human epithelial ovarian cancer cell migration (Cai & Xu, 2013).

LPA transactivation of EGFR occurs widely and is well characterized (Gschwind, Prenzel, & Ullrich, 2002; Inoue et al., 2011; Jeong et al., 2013; Shida et al., 2004; Tveteraas et al., 2016). However, LPA transactivation of PTKRs is not restricted to the EGFR. In CHO-K1 fibroblasts, LPA stimulates ERK signaling and cell proliferation via LPAR1 transactivation of insulin-like growth factor-I receptor (IGF-IR) (Olianas, Dedoni, & Onali, 2015). In L cells lacking EGFR, LPA-induced Shc and ERK activation are mediated by the platelet-derived growth factor receptor (PDGFR) and these responses are inhibited by a specific PDGFR antagonist, AG1296 (Herrlich et al., 1998). In Rat-1 cells expressing both EGFR and PDGFR, LPA-mediated EGFR transactivation pathway regulates the ERK activation (Herrlich et al., 1998), whereas in mesangial cells, LPA-initiated PDGFR transactivation pathway dominates (Goppelt-Strube, Fickel, & Reiser, 2000).

There is considerably more information on LPAR transactivation of PTKRs and much less is known about the recently described phenomena of GPCR mediated transactivation of S/TKRs (Burch, Ballinger, et al., 2010; Burch, Yang, et al., 2010; Kamato et al., 2015). TGF- $\beta$  type I receptor (TGFBRI) is a typical representative of S/TKR family. The binding of

integrin to the latent TGF- $\beta$  complex activates the TGF- $\beta$ , a canonical TGFBR1 agonist, which activates TGFBR1 and initiates downstream Smad-dependent and Smad-independent signaling (Derynck & Zhang, 2003). The mechanism of TGFBR1 transactivation has been investigated using different GPCR agonists (Burch et al., 2013; Scotton et al., 2009; Tatler et al., 2011). In human keratinocytes, LPA via TGFBR1 activates Smad3 without altering the release of TGF- $\beta$ , leading to cell growth arrest and chemotaxis (Sauer et al., 2004). In human VSMCs, thrombin mediates proteoglycan synthesis via TGFBR1 transactivation without the involvement of TGF- $\beta$  release (Burch, Ballinger, et al., 2010; Burch, Yang, et al., 2010) and *de novo* TGF- $\beta$  synthesis (Burch et al., 2013), suggesting the functionality of latent TGF- $\beta$  complex. However, there is data that LPA stimulates the release of TGF- $\beta$  which in turn leads to TGFBR1 activation and subsequent signaling cascades. LPA activates TGF- $\beta$  and initiates downstream Smad2 signaling through a LPAR2-G $\alpha_q$ -RhoA/ROCK-integrin  $\alpha$ V $\beta$ 6 dependent pathway in human lung epithelial cells (Xu et al., 2009), as well as in mouse proximal tubule cells (Geng et al., 2012). In human airway smooth muscle cells, LPA via  $\alpha$ V $\beta$ 5 dependent pathway induces TGF- $\beta$  activation and downstream plasminogen activator inhibitor-1 mRNA expression which is inhibited in the presence of TGF- $\beta$  antibody and  $\alpha$ V $\beta$ 5 neutralizing antibody (Tatler et al., 2011). In addition, LPA-mediated plasminogen activator inhibitor-1 mRNA expression is prevented by an actin assembly inhibitor, cytochalasin D, indicating the involvement of cytoskeletal changes and cell contraction in TGF- $\beta$  activation and therefore in TGFBR1 transactivation (Tatler et al., 2011). Taken together, these data provide insight for LPAR transactivation of PTKRs and S/TKRs in a cell dependent manner. The mechanisms involved in LPA transactivation dependent pathways are still elusive which will continue to be one of the promising areas of investigation.

#### 5.4. LPA signaling via nuclear receptor PPAR $\gamma$

PPAR $\gamma$  is a nuclear receptor at the crossroad of immune diseases, metabolic diseases and CVDs (de Dios et al., 2003; Lehrke & Lazar, 2005; Wahli & Michalik, 2012). In the vascular system, PPAR $\gamma$  can either prevent or promote the progression of CVDs. Mainly by reducing inflammatory marker expression, such as IL-1, IL-6 and TNF- $\alpha$ , PPAR $\gamma$  possesses an anti-atherosclerotic role (Hannan, Dilley, De Dios, & Little, 2003; Jiang, Ting, & Seed, 1998). In contrast, the effects of PPAR $\gamma$  on vascular system can also be negative as which has been proved by the cardiovascular side effects of different clinically used PPAR $\gamma$  ligands (Chandra, Miriyala, & Panchatcharam, 2017; Hamblin, Chang, Fan, Zhang, & Chen, 2009). LPA has been identified as a potential ligand of PPAR $\gamma$  (McIntyre et al., 2003). Via PPAR $\gamma$ , LPA stimulates vascular wall remodelling and atherogenesis (Cheng et al., 2009; Zhang et al., 2004). However, the precise role of LPA-PPAR $\gamma$  axis in the vasculature requires further investigation.

## 6. Therapeutic implications of targeting LPA and its signaling pathways

LPA and its receptors have been broadly implicated in a series of physiological and pathophysiological processes. Therapies targeting LPA biosynthesis, metabolism or signaling pathways could benefit the treatment of diverse diseases which are needing new treatment modalities. In the following sections we discuss the drug development strategies targeting this system with an aim to highlight their potential application in CVDs.

#### 6.1. Drug development strategies targeting and neutralizing LPA synthesis

ATX is responsible for the majority of extracellular and plasma LPA production (Tokumura, Kanaya, et al., 2002; Tokumura, Majima, et al., 2002), thus, it has received increasing interest as a target for medical intervention in recent years. In particular, many efforts have been made to

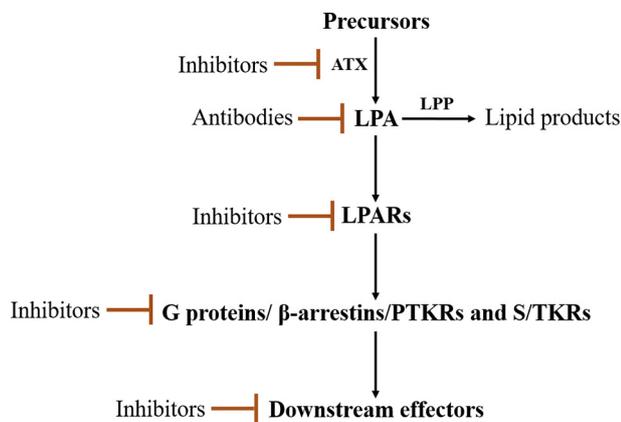
develop ATX inhibitors with the goal of reducing the level of bioactive LPA in the circulation (Nikolaou, Kokotou, Limnios, Psarra, & Kokotos, 2017). Among the available ATX inhibitors, GLPG1690 (Galapagos, Mechelen, Belgium) is the most advanced drug candidate and has completed phase 2 clinical trials (NCT02738801) for the management of idiopathic pulmonary fibrosis (Maher et al., 2018). During the phase 1 study, GLPG1690 reduced 18:2 LPA concentration in plasma (Van Der Aar et al., 2016). Up to this time, this drug candidate has progressed into phase 3 clinical trial which is now recruiting participants. The strategy of reducing or neutralising LPA using a monoclonal antibody (mAb) is also under evaluation. Specifically, the therapeutic potential of anti-LPA mAb has been shown in a traumatic brain injury mouse model, in which LPA-directed mAb B3 (Lpathomab) improved neurological outcomes (Crack et al., 2014). In its phase 1a clinical trial (NCT02341508), Lpathomab was well tolerated at all tested doses, and no serious adverse events or dose limiting toxicities were observed. Although these drug candidates are not designed for the treatment of CVDs, their potential in reducing circulating LPA raises the value of further studies in the context of CVDs.

#### 6.2. Drug development strategies targeting LPARs

LPARs, being GPCRs, are feasible therapeutic targets. Although progress has been made in this area, to date, there is still no drug approved by FDA or other drug administrative organisations. Nonetheless, there are candidate drugs undergoing preclinical and clinical trials. Among them, BMS-986020/AM152 (NCT01766817) and SAR-100842 (NCT01651143) which selectively target LPAR1 have entered clinical studies for the treatment of systemic sclerosis and related fibrotic diseases. SAR-100842 is a negative allosteric modulator, while BMS-986020 is acting as an orthosteric antagonist (Ellery et al., 2018). The recent double-blind randomized placebo-controlled phase 2a clinical trial (NCT01651143) on patients with early diffuse cutaneous systemic sclerosis shows SAR-100842 is safe, moderately effective and well tolerated in patients (Allanore et al., 2018).

#### 6.3. Drug development strategies targeting other LPA signal mediators

G proteins are one of the major downstream intermediates of LPA signaling, making them a potential target. Transient expression of G $\alpha_q$ I is capable of inhibiting LPA-induced migration and proliferation of VSMCs, which suggests the possibility of targeted gene therapy (Kim et al., 2006). ROCK is a pivotal signaling mediator which can trigger multiple downstream cascades and a central player in the vascular system and thus a critical therapeutic target (Satoh, Fukumoto, & Shimokawa, 2011). Fasudil, a ROCK inhibitor, targeting the ATP-dependent kinase domain of ROCK1 and ROCK2 has been approved for clinical use in Japan and China as a vasodilator to treat several vascular disorders (Shi & Wei, 2013). Furthermore, fasudil has undergone preclinical investigation in several neurodegenerative conditions (Chong, Ai, & Lee, 2017) and cancer treatments (Chin et al., 2015). Recently, the concept of "signaling bias" is gaining prominence, which raises the possibility of designing selective agonists or antagonists towards GPCRs and their G proteins,  $\beta$ -arrestins or the possible transactivation dependent receptors (Kenakin & Christopoulos, 2013). The ligands with specific effects on a particular receptor-linked effector system can emphasize beneficial signals and de-emphasize harmful signals, thereby benefiting the outcome of target therapy (Kenakin, 2017). G $\alpha_q$ -biased LPAR1 negative allosteric modulators have been characterized using high-throughput screening and label-free impedance assays (Shimizu & Nakayama, 2017).  $\beta$ -arrestin biased ligands have profound therapeutic value and are intensely investigated (Violin & Lefkowitz, 2007; Whalen, Rajagopal, & Lefkowitz, 2011), while nothing is currently known about targeting the LPAR mediated  $\beta$ -arrestin. In contrast, the biased ligand targeting GPCRs transactivation of specific receptors has gained less attention, which might be a promising area in the future.



**Fig. 4.** The potential therapeutic development strategies targeting LPA signaling pathway. Using autotaxin (ATX) inhibitors to reduce LPA generation; LPA monoclonal antibodies to reduce the level of LPA; LPA receptor antagonists to block the activation of LPA receptors (LPARs) thus block the initiation of signaling; Inhibitors toward G proteins,  $\beta$ -arrestins and protein tyrosine kinase receptors (PTKRs) or serine/threonine kinase receptors (S/TKRs) block the upstream signals; Inhibitors of downstream effectors are other alternative options for the LPA signaling blocking.

Although LPA has diverse pathogenic effects on initiation and progression of vascular diseases, the above mentioned potential therapeutic intervention targets can help to release the stress of LPA (Fig. 4).

## 7. Conclusions

The accumulating in vitro and in vivo evidence reviewed above demonstrates that LPA exerts an appreciable role in regulating atherosclerosis and vascular diseases. Elucidating the individual roles of each LPAR will benefit the strategic development of vascular diseases. Therefore, the distribution of known receptors in physical and pathological vasculature conditions and their corresponding precise molecular signaling pathways should be a continuous research focus. In order to decipher the complex role of LPA and LPARs, the specific LPAR or related signal protein deficient mutant models are required, which remains to be the biggest challenge today. Although a complicated area of cardiovascular pathology, the study of LPA regulation warrants a serious effort to determine the conditions in which a useful therapeutic agent might be developed.

## Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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