Production of extracellular lysophosphatidic acid in the regulation of adipocyte functions and liver fibrosis

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Abstract

Lysophosphatidic acid (LPA), a glycerophospholipid, consists of a glycerol backbone connected to a phosphate head group and an acyl chain linked to sn-1 or sn-2 position. In the circulation, LPA is in sub-millimolar range and mainly derived from hydrolysis of lysophosphatidylcholine, a process mediated by lysophospholipase D activity in proteins such as autotaxin (ATX). Intracellular and extracellular LPAs act as bioactive lipid mediators with diverse functions in almost every mammalian cell type. The binding of LPA to its receptors LPA_{1-6} activates multiple cellular processes such as migration, proliferation and survival. The production of LPA and activation of LPA receptor signaling pathways in the events of physiology and pathophysiology have attracted the interest of researchers. Results from studies using transgenic and gene knockout animals with alterations of ATX and LPA receptors genes, have revealed the roles of LPA signaling pathways in metabolic active tissues and organs. The present review was aimed to summarize recent progresses in the studies of extracellular and intracellular LPA production pathways. This includes the functional, structural and biochemical properties of ATX and LPA receptors. The potential roles of LPA production and LPA receptor signaling pathways in obesity, insulin resistance and liver fibrosis are also discussed.

Key words: Autotaxin; Lysophosphatidic acid receptors; Obesity; Lysophosphatidic acid; Insulin resistance; Liver fibrosis

Core tip: Lysophosphatidic acid (LPA) is mainly derived from hydrolysis of lysophosphatidylcholine, a process mediated by lysophospholipase D activity in proteins such as autotaxin (ATX). The binding of LPA to its receptors LPA[1-6] activates multiple cellular signaling pathways and leads to changes. Studies using genetically modified animals have begun to reveal the roles of LPA pathways in metabolic active tissues and organs. The present review summarized recent progresses in the studies of extracellular and intracellular LPA production pathways; the functions, structural and biochemical properties of ATX and LPA receptors. Furthermore, the potential roles of LPA production and LPA receptor signaling pathways in obesity, insulin resistance and liver fibrosis are discussed.

INTRODUCTION

Lysophosphatidic acid (1- or 2-acyl-sn-glycerol 3-phosphate/radyl-glycerol-phosphate, LPA) is one type of water-soluble glycerophospholipid with molecular mass about 430-480 Da. All LPA molecules contain a glycerol backbone linked to a phosphate head group at sn-3 position and an acyl chain esterified to sn-1 or sn-2 position. Due to variation of the fatty acyl chain, LPA molecules are in different forms and derived from multiple sources, such as membrane lipids[1]. The LPA molecules produced extracellularly exert a variety of physiological responses after binding to their receptors as shown in Figure 1.

LPA was first identified as an active ingredient of Darmstoff by Vogt in 1957[2,3]. The term Darmstoff was used to describe a smooth-muscle-stimulating substance which was first observed with bath fluid of isolated intestine preparations[4]. The substance was acidic and soluble in many organic solvents, properties that distinguish Darmstoff from amines and polypeptides[5]. Results of acidic hydrolysis and paper chromatography showed that the smooth-muscle-stimulating activity of Darmstoff was due to a mixture of acidic phospholipids (PLs), one of which is an acetal phosphatidic acid[2,3]. In the 1960s, studies on smooth muscle and blood pressure suggested that LPA had biological activities[6,7]. Later on, various molecules of LPA species were isolated and identified from soy beans. It was shown that intravenous injection of LPA from crude soybean lecithin caused hypertension in rats and guinea pigs, but hypotension in cats and rabbits[8]. This raised intriguing questions regarding the activation mechanism of this lipid specie. Since then, the myriad

biological effects of LPA have drawn attention of biomedical scientists.

Subsequently, LPA in incubated serum at 36 ℃ for 18-24 h was shown to cause aggregation of feline and human platelets[9]. Whether LPA acts through its detergent-like physical property or its interaction with a specific receptor remained a critical question. Later, LPA was shown to stimulate cell proliferation in a pertussis toxin-sensitive manner[10]. This finding suggested that LPA acts through G protein-coupled receptor (GPCR). This information led to the cloning and identification of a GPCR, which is now known as LPA receptor 1 (LPA[1])[11]. It is known now that as a bioactive lipid mediator, LPA activates at least 6 specific GPCRs, named as LPA[1-6]. These GPCRs are coupled with several G_α proteins such as $G_{\alpha 12/13}$, $G_{\alpha q/11}$, $G_{\alpha i/o}$, and $G_{\alpha s}$. The binding of LPA to these receptors stimulates the activations of small GTPases, Ras, Rho, and Rac, and induces downstream actions[12].

The existence of extracellular LPA indicates its production outside a cell. Since autotaxin (ATX) was first identified from human plasma and found to be a lysophosphatidic acid-producing enzyme in 2002[13], the ATX-LPA receptor signaling pathway has been implicated in a variety of disease processes including the vascular and neural development, hair follicle development, tumor progression, lymphocyte trafficking, bone development, pulmonary fibrosis, fat mass regulation, cholestatic pruritus, neuropathic pain, embryo implantation, obesity and glucose homeostasis, spermatogenesis, fetal hydrocephalus, chronic inflammation, cellular proliferation, and smooth muscle contraction during development[14-18]. Both ATX and LPA have attracted the interest of researchers in an effort to understand their roles in pathophysiology and to develop new agents to treat above-mentioned pathological conditions.

EXTRACELLULAR AND INTRACELLULAR PRODUCTION AND DEGRADATION OF LPA

LPA and its common precursor lysophosphatidylcholine (LPC) can be found both extracellular and intracellular as signaling mediators and membrane components, respectively. Structurally, LPA is an acyl group esterified to the sn-1 or sn-2 position of the glycerol backbone. Due to the differences of acyl chain length, saturation and backbone position, various LPA chemical forms can be found in tissues and cells. Extracellular LPA is thought to mediate bioactive effects through LPA receptors[19]. Intracellular LPA is an important intermediate for the *de novo* biosynthesis of complex glycerolipids, including mono-, di-, and triglycerides, as well as PLs[20]. In addition, it has been thought that LPA can function as a ligand for transcription factor peroxisome proliferator-activated receptor γ (PPARγ)[21]. This indicates that LPA may play important roles in the regulation of gene expression.

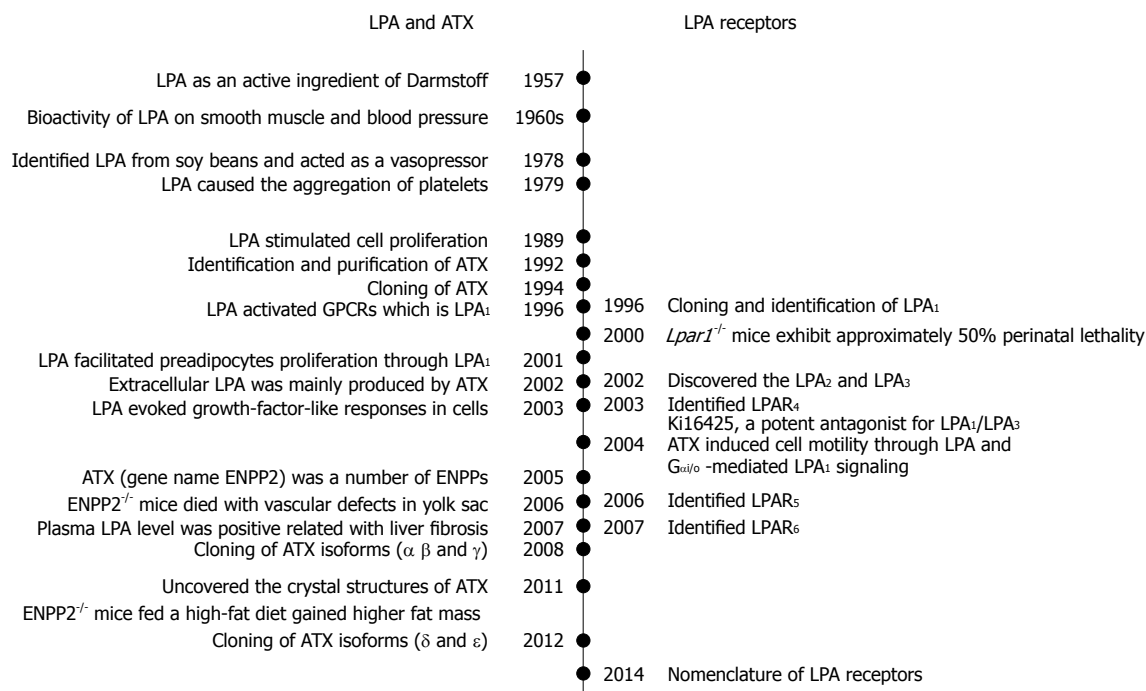|	LPA and ATX			LPA receptors
LPA as an active ingredient of Darmstoff	1957	●		
Bioactivity of LPA on smooth muscle and blood pressure	1960s	●		
Identified LPA from soy beans and acted as a vasopressor	1978	●		
LPA caused the aggregation of platelets	1979	●		
LPA stimulated cell proliferation	1989	●		
Identification and purification of ATX	1992	●		
Cloning of ATX	1994	●		
LPA activated GPCRs which is LPA$_1$	1996	●	1996	Cloning and identification of LPA$_1$
		●	2000	*Lpar1*$^{-/-}$ mice exhibit approximately 50% perinatal lethality
LPA facilitated preadipocytes proliferation through LPA$_1$	2001	●		
Extracellular LPA was mainly produced by ATX	2002	●	2002	Discovered the LPA$_2$ and LPA$_3$
LPA evoked growth-factor-like responses in cells	2003	●	2003	Identified LPAR$_4$ Ki16425, a potent antagonist for LPA$_1$/LPA$_3$
		●	2004	ATX induced cell motility through LPA and $G_{\alpha i/o}$ -mediated LPA$_1$ signaling
ATX (gene name ENPP2) was a number of ENPPs	2005	●		
ENPP2$^{-/-}$ mice died with vascular defects in yolk sac	2006	●	2006	Identified LPAR$_5$
Plasma LPA level was positive related with liver fibrosis	2007	●	2007	Identified LPAR$_6$
Cloning of ATX isoforms (α β and γ)	2008	●		
Uncovered the crystal structures of ATX	2011	●		
ENPP2$^{-/-}$ mice fed a high-fat diet gained higher fat mass				
Cloning of ATX isoforms (δ and ϵ)	2012	●		
		●	2014	Nomenclature of LPA receptors

Figure 1 Chronological events related the identifications of lysophosphatidic acid, ecto-nucleotide pyrophosphatase/phosphodiesterase/autotaxin and lysophosphatidic acid receptors. On the left side, it shows the events associated with the identifications of LPA molecules and ATX for its production. On the right side, it shows the cloning events of LPAR$_{1-6}$. LPA: Lysophosphatidic acid; ATX: Autotaxin; LPA$_{1-6}$: Lysophosphatidic acid receptor 1-6; ENPP2: Ectonucleotide pyrophosphatase/phosphodiesterase family member 2.

Pathways for LPA production

As shown in Figure 2, there are five major pathways for LPA production, (1) the lysophospholipids-ATX (LPLs-ATX) pathway, (2) the phosphatidic acid - phospholipase A$_1$ or A$_2$ (PA-PLA$_1$/PLA$_2$) pathway, (3) the *de novo* glycerophosphate acyltransferase (GPAT) synthesis pathway, (4) the monoacylglycerol kinase (MAGK) pathway, and (5) the oxidative modification of low-density lipoprotein (LDL) pathway. Despite recent advances in the identification of the enzymes responsible for LPA production, the regulation of these enzymes still remains obscure.

LPLs-ATX pathway

In the first pathway, LPLs generated from PLs by PLA$_1$ or PLA$_2$ are converted to LPA by a plasma enzyme ATX[22,13], which we will describe in later part of this article. A major source of extracellular LPA is LPC, other LPLs such as lysophosphatidylserine and lysophosphatidylethanolamine can also be enzymatically processed to produce LPA. This pathway accounts for the majority of circulating LPA.

PA-PLA$_1$/PLA$_2$ pathway

LPA is also produced intracellularly as an intermediate for the synthesis of other glycerolipids[20]. LPA can be produced enzymatically from intracellular organelles such as mitochondria and endoplasmic reticulum. Phosphatidic acid (PA) is first generated from PLs or diacylglycerol by phospholipase D enzymes (PLD$_1$ and PLD$_2$) and diacylglycerol kinase (DGK) activities,

respectively. Then, one acyl group is removed from the sn-1 position by PLA$_1$ or at the sn-2 position by PLA$_2$ enzymes to generate LPA. This pathway may be more important in specific tissues with expression of DGK such as the brain and skin[23].

De novo GPAT synthesis pathway

GPATs catalyze the first step in glycerolipid synthesis, *i.e.*, the conversion of glycerol-3-phosphate (G3P) to LPA by the transfer of fatty acids from acyl-CoA. Since GPAT exhibits the lowest specific activity of enzymes in the *de novo* triacylglycerol (TAG) and PLs synthesis pathways, it has been considered to be the rate limiting enzyme for them[24]. Many studies have been published on the regulation of TAG synthesis and its relevance to obesity and insulin resistance. GPAT activity in mitochondria was shown to be regulated by fatty acid-binding protein (FABP)[25,26]. It has been shown that mitochondrial GPAT activity was inhibited by LPA. FABP reversed the inhibition of LPA through the binding and extracting LPA from the mitochondrial outer membrane. The extracted LPA was converted to PA by microsomes, where acylglycerophosphate acyltransferases (AGPATs) are located[25,26]. These results suggested that FABP regulated the *de novo* synthesis of PA through the stimulation of mitochondrial GPAT and transport of LPA from mitochondria to microsomes.

MAGK pathway

Lipid phosphate phosphatases (LPPs) are also involved in the LPA turnover. LPPs can be found extracellularly
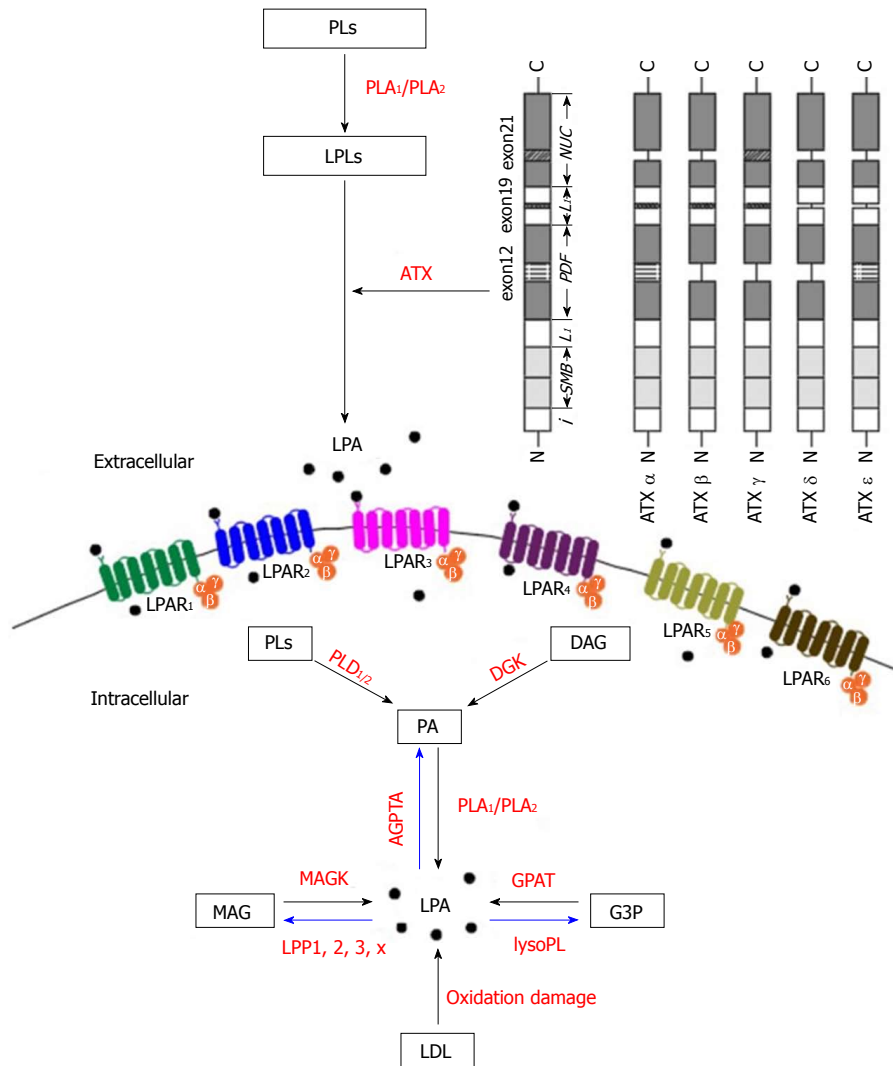
Figure 2 Biochemical pathways of lysophosphatidic acid synthesis and degradation. LPA can be produced extracellularly and intracellularly as signaling mediators and membrane components, respectively. There are five major pathways for LPA production, (1) the lysophospholipids-ATX (LPLs-ATX) pathway, (2) the phosphatidic acid - phospholipase A₁ or A₂ (PA-PLA₁/PLA₂) pathway, (3) the *de novo* glycerophosphate acyltransferase (GPAT) synthesis pathway, (4) the monoacylglycerol kinase (MAGK) pathway, and (5) the oxidative modification of low-density lipoprotein (LDL) pathway. In the upper right corner of the figure, there are catalytically active isoforms (ATXα, ATXβ, ATXγ, ATXδ and ATXε), which are expressed in different tissues. PLs: Phospholipids; PLA₁/PLA₂: Phospholipase A1/2; LPLs: Lysophospholipids; ATX: Autotaxin; ATXα-δ: Protein structure scheme of the domains of ATX; LPA: Lysophosphatidic acid; DAG: Diacylglycerol; DGK: Diacylglycerol kinase; PLD₁/₂: Phospholipase D1/2; PA: Phosphatidic acid; AGPAT: Acylglycerophosphate acyltransferase; MAG: Monoacylglycerol; MAGK: Monoacylglycerol kinase; LPP: Lipid phosphate phosphatase; G3P: Glycerol-3-phosphate; lyso PL: Lysophospholipase; GPAT: Glycerophosphate acyltransferase; LDL: Low-density lipoprotein; i: Intramembrane domain; SMB: N-terminal somatomedin B-like domains; L₁: L₁ linker region; PDF: Phosphodiesterase domain; L₂: L₂ linker region; NUC: C-terminal nuclease-like domain; LPA₁₋₆: Lysophosphatidic acid receptor 1-6.

or intracellularly in endoplasmic reticulum or Golgi, where they dephosphorylate LPA, which leads to the formation of monoacylglycerol (MAG)[27]. MAG may then be phosphorylated by MAGK and thus participate in another round of LPA signaling[20]. Thus, the production of LPA is regulated by the availability of precursors as well as the expression of catalytic enzymes.

Oxidative modification of LDL
LPA was found as an active molecule on oxidized and modified LDL, in where it may contribute to platelet activation, endothelial cell stress-fiber and gap formation[28,29]. LPAs on these lipoproteins activate platelets through G-protein coupled LPA receptors and

a Rho/Rho kinase signaling pathway, which leads to platelet shape change and subsequent aggregation. The biologically active LPA-like products generated by this non-enzymatic oxidation co-migrated with an authentic LPA standard in thin layer chromatography. LPA was found to be accumulated in atherosclerotic plaques, which might act to activate platelets. The level of LPA is very high in the human carotid atherosclerotic lesion, suggesting the roles in thrombogenesis and rupture[28,29]. An alternative explanation to the generation of LPA from oxidized LDL is that ATX might be activated. The acyl/alkyl composition, the precursor of LPA and the mechanism responsible for LPA generation in the oxidized LDL remain to be addressed in the future.

Pathways for the degradation of LPA

There are three major pathways that degrade LPA as shown in Figure 2. The first is the removal of phosphate to form MAG by LPPs[30]. LPA has a half-life of 3 min when it is added to cells expressing LPP[31]. Four isoforms of LPP have been cloned and characterized in mammals, LPP1/PAP-2α/PAP-2α1[32], LPP1α/PAP-2α2[33], LPP2/PAP-2c/PAP-2α[34] and LPP3/PAP-2β/PAP-2β[32]. The second LPA degradation pathway involves the action of AGPAT enzymes, also known as lysophosphatidic acid acyltransferase. These microsomal enzymes catalyze the transfer of an acyl group from acyl-CoA to LPA to form PA. Proteins with AGPAT activity include a family of transmembrane enzymes[35], and membrane associated proteins involved in membrane fission such as endophilin[36] and C-terminal-binding protein/brefeldin A-ADP ribosylated substrate[37]. The third pathway for LPA degradation involves the hydrolysis of the acyl group from the G3P head group by the action of lysophospholipases. The majority of characterized lysophospholipases act on LPC[38].

ECTO-NUCLEOTIDE PYROPHOSPHATASE/ PHOSPHODIESTERASES

The ecto-nucleotide pyrophosphatase/phosphodiesterase (ENPP) family contains seven members with structurally similar catalytic domains that hydrolyze phosphodiester bonds in various substrates, including nucleoside triphosphates, LPLs, and choline phosphate esters[39,40]. ATX, or ENPP2, is the best-characterized member of ENPP family. ENPPs are defined by their ability to hydrolyze phosphodiester bonds of various nucleotides *in vitro*[41-43]. ATX/ENPP2 was originally identified as a tumor cell-motility-stimulating factor from the conditional medium of A2058 human melanoma cells[44]. Since the addition of pertussis toxin reduced cellular motility, ATX's effects were thought to involve $G_{i/o}$-mediated signaling[39,44]. ATX can be secreted as a 100 kDa glycoprotein. It is produced by multiple tissues including adipose tissue[45,46]. It is believed that the circulated ATX/ENPP2 is degraded by the liver[47]. Extracellular LPA was found to be present in sub-micromolar ranges. The responsible enzyme was identified to be ATX[13,22]. ATX-mediated autocrine signaling induces cell motility through LPA production and $G_{i/o}$-mediated LPA receptor signaling[48].

From both a structural and evolutionary point of view, ENPP family members have been categorized into two subgroups, ENPP1-3 and ENPP4-7[39]. ENPP1-3 all have two N-terminal somatomedin B-like (SMB) domains, a central phosphodiesterase (PDE) domain and a C-terminal nuclease (NUC)-like domain as shown in Figure 2. ENPP4-7 only have similarity in the PDE domain. The crystal structures of mouse[49] and rat[50] ATX show loops on both sides of the catalytic domain, which may help to determine the binding specificity. The SMB and PDE domains are connected by the first loop (L_1 linker region), whereas the PDE and NUC domains are connected by the second loop (L_2 linker region)[49].

The secreted ATX is a constitutively active glycoprotein with a N-terminal signal peptide sequence containing a furin cleavage site[46]. The other ENPPs are transmembrane or anchored proteins. In addition to its pyrophosphatase/phosphodiesterase activities, ATX has lysophospholipase D (lysoPLD) activity. The N-terminal signal peptide of the ATX precursor is removed first, and then, the remaining part is cleaved by proprotein convertases before the active ATX with lysoPLD activity is released into the extracellular environment, which converts LPC into LPA and choline[47]. The structure of PDE domain has a lipid binding pocket and a nearby tunnel allowing entry of substrates and release of products[51]. The NUC domain has been thought to maintain the rigidity of the PDE domain, and the two N-terminal SMB domains mediate binding of ATX to integrin[52]. This binding brings ATX to the cell membrane, which allows the production of LPA in a location close to its receptors[51,53,54].

The structures of ATX in complex with diverse LPAs show distinct conformations after different acyl chains occupy the binding pocket[49]. LPAs with saturated chains bind in the hydrophobic pocket in a more elongated fashion, whereas LPAs with unsaturated chains have a bent conformation due to the presence of carbon-carbon double bond(s). For LPA (22:6), the acyl chain shows a U-shaped conformation in the binding pocket[49]. ATX prefers LPC species with shorter and unsaturated acyl chain as substrates, and the rank order is 14:0 > 16:0 > 18:3 > 18:1 > 18:0. All these show that ATX is able to hydrolyze LPCs with different lengths and saturations of acyl chains to produce the corresponding LPAs.

The cDNA of ATX/ENPP2 was cloned in 1994[55]. After that, its homology with phosphodiesterases was revealed, and the cloning and tissue distribution of the three human and mouse isoforms (α, β and γ) were determined in 2008[50]. Two more isoforms (δ and ε) were identified in 2012[56]. The ATX gene is located on mouse chromosome 15 and on human chromosome 8. The human and mouse ATX gene structures are conserved[50]. The mouse ATX gene spans more than 80 kb and contains at least 27 exons. The three splicing sites in exons 12, 19 and 21 can theoretically result in eight isoforms, in which five were detected. These isoforms are catalytically active (ATX α-δ) and expressed in different tissues. They are ATXα (ATXm), ATXβ (ATXt), ATXγ (PD-Iα)[50], ATXδ and ATXε[56]. ATXβ and ATXδ, which are the most and second most abundant isoforms, respectively, share similar biochemical characteristics (Figure 2). Houben *et al*[53] characterized that a 52-residue polybasic insertion corresponding to exon 12 in ATXα isoform confers specific binding to heparan sulfate proteoglycans thereby targeting LPA production to the plasma membrane. This is another potential mechanism for localizing ATXα to cell membranes and for LPA production in close proximity to LPA receptors. Exon 12 encodes a 52-amino acid insertion of the mouse ATXα and ATXε

isoforms (amino acids 324-375), whereas exon 21 encodes an additional 25-amino acid of the murine ATXγ isoform (amino acids 593-617). Novel isoforms ATXδ and ATXε have a 4-amino acid deletion on exon 19. This complex way of exon arrangement has been maintained through evolution. Human ATX exhibit 93% sequence identity with rodent ATX while all important residues are highly conserved[49].

ATX has a broad profile of tissue expression, with relatively high levels in the blood, brain, kidney, and lymphoid organs[57-59]. Secretion of ATX leads to high concentration in cerebrospinal fluid and in the endothelial venules of lymphoid tissues[60-62]. The cellular sources of plasma ATX are incompletely understood. Nevertheless, adipocytes may be a source[63,64]. ATX is also stored in platelets and released during their activation[65,66]. Circulating ATX is rapidly taken up by the scavenger receptors of liver sinusoidal endothelial cells, and then degraded by the liver[46]. Thus, like insulin, ATX is largely removed from the circulation through first passage by the liver. For the ATX isoforms, high expression levels of ATXβ and ATXγ mRNA were detected in peripheral tissues and the brain, whereas ATXα was shown the lowest expression level in both the central nervous system and peripheral tissues among the three isoforms in human. In mice, ATXβ is widely expressed in the brain and peripheral tissues, and ATXγ and ATXα showed little variation in their distribution[50]. Human brain and retina showed relatively higher expression level of ATXα than that of ATXβ and ATXγ, whereas the expression levels of ATXδ and ATXε in the small intestine and spleen are higher than that in other tissues[56].

LPA has been quantified in a variety of species, tissues, and fluids, including neural tissue, cerebrospinal fluid, fertilized hen white, seminal fluid, tears, plasma, serum, urine, saliva, and aqueous humor[67-69]. The formation of LPA species depends on the precursor PLs, which can vary by acyl chain length and degree of saturation. The term LPA most often refers to 18:1 oleoyl-LPA (1-acyl-2-hydroxy-sn-glycero-3-phosphate), as it is the most commonly one. Other chemical forms of LPA can be observed in various biological systems that have concentrations ranging from low nanomolar to micromolar levels[67,70]. LPA concentrations in human and rat blood can range from 0.1 μmol/L in plasma and up to 10 μmol/L in serum, which is well over the apparent nanomolar kDa of LPA$_{1-6}$[71-74]. The LPA molecules containing 18:2, 20:4, 16:1, 16:0, and 18:1 acyl chains are particularly abundant in plasma[75-77]. Current methods to detect LPA include indirect enzymatic assays[73], TLC-GC, LC-MS, and LC-MS/MS[78-80].

LPA RECEPTORS-MEDIATED LPA SIGNALING

LPA acts as a potent mitogen, which was previously known as "ventricular zone gene-1 (vzg-1)" due its high level in the embryonic neuroproliferative layer of the cerebral cortex[11,12]. The cloning and functional identification of LPA$_1$ led to determination of other receptor genes based upon sequence homology[81-83]. This is particularly true for the "endothelial differentiation gene" (EDG) members[84] that include LPA and sphingosine 1-phosphate receptors. Then, two other LPA receptors, LPA$_2$ and LPA$_3$ (also known as EDG4, and EDG7), were subsequently discovered based on shared homology with LPA$_1$(EDG2)[85]. Later on, LPA$_4$ (P2RY9, GPR23)[86], LPA$_5$ (GPR92)[87] and LPA$_6$ (P2RY5, GPR87)[88] were identified. They share 35% amino acid homology to the purinergic (P2Y) family of GPCRs, as compared to less than 20% homology to LPA$_1$, suggesting that LPA$_{4-6}$ are more closely related to the P2Y receptors[52]. Here, LPA$_1$-LPA$_6$ are for proteins, and their gene symbols are *LPAR1-LPAR6* for human and *Lpar1-Lpar6* for non-human[89].

All LPA receptors signal through at least one of the four heterotrimeric G$_α$ proteins (G$_{α12/13}$, G$_{αq/11}$, G$_{αi/o}$, and G$_{αs}$)[12,90], resulting in downstream signals that produce diverse physiological and pathophysiological effects (Figure 3). G$_{α12/13}$-mediated LPA signaling regulates cytoskeletal remodeling, cell migration and invasion through activation of Rho pathway proteins[91]. Rho signals to c-jun N-terminal kinase (JNK) and p38 through Rho-associated kinase (ROCK) and protein kinase N. The LPA-coupled G$_{αq/11}$ protein primarily regulates Ca^{2+} homeostasis through phospholipase C (PLC), which generates the second messengers IP$_3$ and diacylglycerol (DAG)[92-94]. G$_{βγ}$ and G$_{αi/o}$ subunits mediate the activation of phosphatidylinositol 3-kinase (PI3K) which results in the stimulation of the Akt pathway and increase of protein translation after the activation of the mammalian target of rapamycin (mTOR) signaling pathway. Activation of PI3K by G$_{βγ}$ subunits also stimulates the activity of Rac, leading to cell migration and JNK regulation of pro-inflammatory gene expression, and Ras activity, leading to the stimulation of Raf- mitogen-activated protein kinase (MEK)-extracellular signal-regulated kinase (ERK) pathway to promote the expression of genes involved in proliferation and invasion. G$_{αi/o}$, besides PI3K, also stimulates the Ras-Raf-MEK-ERK pathway promoting cell survival and other functions[95,96]. G$_{αs}$ can activate adenylyl cyclase and increase cAMP concentration upon LPA stimulation[97]. However, the same enzyme is also inhibited by G$_{αi/o}$, showing the complexity of signaling pathways after the activation of LPA receptors[98].

All six LPA receptors can be stimulated by 1-acyl-LPAs, which show different potencies. LPA$_3$ and LPA$_6$ prefer unsaturated 2-acyl-LPA, while LPA$_5$ likes ether-linked 1-alkyl-LPA species[99,100]. In addition, lysophosphatidylserine, lysophosphatidylinositol, and lysophosphatidyletethanolamine, have been thought to activate these receptors as well[101]. Different LPA molecules may have preference to different subtypes of LPA receptors[102]. Table 1 summarizes PLA receptors expression profiles and their known physiological functions in humans and mice.
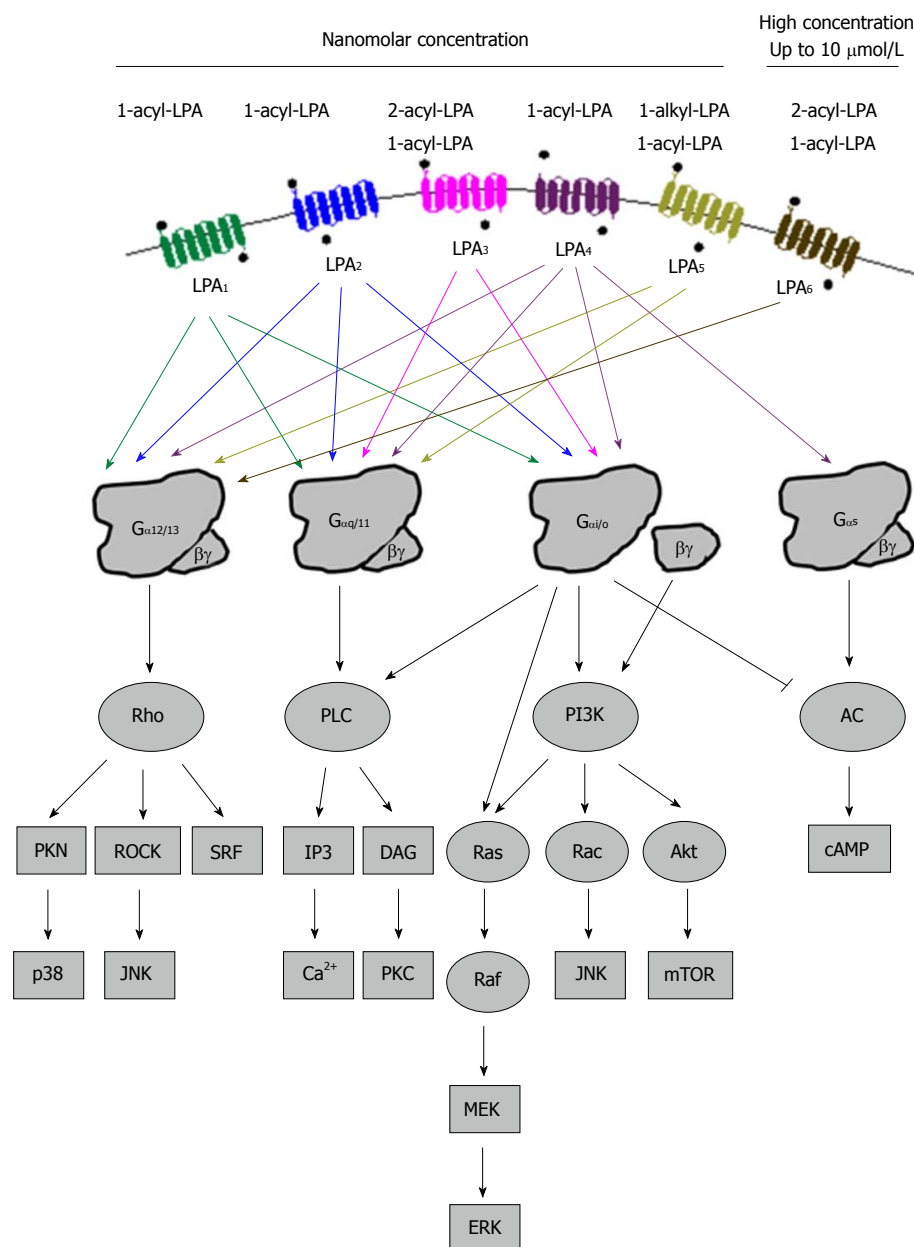
Figure 3 Summary of lysophosphatidic acid activated intracellular signaling pathways *via* the six cognate lysophosphatidic acid receptors. PLC: Phospholipase C; PI3K: Phosphatidylinositol 3-kinase; AC: Denylyl cyclase; PNK: Polynucleotide 5'-hydroxyl-kinase; ROCK: Rho-associated kinase; JNK: c-jun N-terminal kinase; SRF: Serum response factor; IP3: Inositol 1,4,5-triphosphate; DAG: Diacylglyerol; PKC: Protein kinase C; MEK: Mitogen-activated protein kinase; ERK: Extracellular signal-regulated kinase; Akt: Protein kinase B; Mtor: Mammalian target of rapamycin.

LPA$_1$

LPA$_1$ is the first LPA receptor identified based on studies of LPA in the brain[11]. LPA$_1$ couples to three G$_\alpha$ proteins - G$_{\alpha 12/13}$, G$_{\alpha q/11}$, and G$_{\alpha i/o}$, which can result in the activation of downstream pathways including Akt, Rho, Ras, and PLC (Figure 3). These pathways mediate many cellular responses initiated by LPA$_1$ such as neurodevelopment regulation, cell proliferation, differentiation, apoptosis and survival, cell-cell contact through a variety of mechanisms[68,84,103-106]. *Lpar1$^{-/-}$* mice exhibit about 50% perinatal lethality, which was attributed to the defective development of olfaction. The survived ones had reduction of body size, craniofacial dysmorphism, and loss of Schwann cells[107]. Dysregulation at glutamatergic

synapses was observed in *Lpar1$^{-/-}$* mice[108]. When the original *Lpar1$^{-/-}$* mouse line was expanded, a spontaneous variant named "Málaga LPA1" arose. They showed more severe brain defects than the original *Lpar1$^{-/-}$* line mice did[109]. The loss of LPA$_1$ in animals seems to modulate the development of several diseases including cancer, obesity, neuropathic pain, fibrosis and male infertility[110].

LPA$_2$

The amino acid sequence of LPA$_2$ is about 50% identical to that of LPA$_1$, and it associates with G$_{\alpha i/o}$, G$_{\alpha q/11}$, and G$_{\alpha 12/13}$, the same as LPA$_1$[106] (Figure 3). These G proteins use Ras, PI3K/Rac, PLC/DGA and Rho to mediate their

Table 1 Expression pattern of lysophosphatidic acid receptors and their known physiological functions in humans and mice

Name	Information	Previous orphan names	Major expression tissue (high to low level)	Knockout effects in mouse	Biological functions	Ref.
LPA1	Human chromosome locus 9q31.3; 41.1 kDa[1]; 364 aa[2]; Identity[3] 97.3% Mouse chromosome locus 4, 32.2 cM; 41.1 kDa; 364 aa	*vzg-1*, *edg-2*, 39.4 kDa; 344 aa *mrec1.3*, *lpA1*	Brain, placenta, urinary bladder, uterus, testis, lung, small intestine, heart, stomach, kidney, spleen, thymus, and skeletal muscle. Brain, heart, lungs, stomach, intestine, placenta, kidneys, spleen, uterus, testes.	Perinatal lethality, retarded growth, defective olfaction, reduced body size, craniofacial dysmorphism with blunted snouts, and increased apoptosis in sciatic nerve Schwann cells.	Neurodevelopment regulation, cell proliferation, differentiation, apoptosis and survival, cell-cell contact through serum-response element activation, cell migration and cytoskeletal organization, Ca^{2+} homeostasis, cAMP-regulated cellular processes and adenylyl cyclase inhibition	Yung *et al*[68], 2014; Archbold *et al*[138], 2014; Choi *et al*[139], 2008; Anliker *et al*[103], 2013; Sakai *et al*[104], 2013; Wittpoth *et al*[98], 1999; An *et al*[82], 1998; Contos *et al*[107], 2000; Contos *et al*[106], 2000; Fukushima *et al*[84], 2001.
LPA2	Human chromosome 19p13.11; 39.1 kDa; 351 aa; Identity 83.5% Mouse chromosome 8, 33.91 cM; 38.7 kDa; 348 aa	*edg-4*, *lpA2*	Leukocytes, testis, prostate, spleen, thymus and pancreas. Kidney, testis, uterus, lung, stomach, spleen, thymus, postnatal brain, and heart.	Normal	Cell migration, viable and healthy, nervous system development and immune system regulation.	Yung *et al*[68], 2014; An *et al*[82], 1998; Contos *et al*[106], 2000b; Archbold *et al*[138], 2014; Ohuchi *et al*[111], 2008; Choi *et al*[110], 2010; Valentine *et al*[140], 2008; Xu *et al*[113], 2004; Lai *et al*[112], 2005; Contos *et al*[115], 2002; Choi *et al*[139], 2008.
LPA3	Human chromosomal locus 1p22.3; 40.1 kDa; 353 aa; Identity 91.2% Mouse chromosome locus 3, 71.03 cM; 40.3 kDa; 354 aa	*edg-7*, *lpA3*	Heart, testis, prostate, pancreas, lung, ovary, and brain. Lung, kidney, uterus, testis, small intestine, brain, heart, stomach, placenta, spleen, and thymus.	Delayed embryo implantation, embryo crowding, and reduced litter size for female null mutants.	Male and female reproductive physiology, inflammation, cell Ca^{2+} homeostasis and cAMP regulation, vertebrate left-right patterning during embryogenesis.	Yung *et al*[68], 2014; Bandoh *et al*[83], 1999; Im *et al*[116], 2000; Contos *et al*[107], 2000a; Zhao *et al*[117], 2015; Ye *et al*[118], 2010; Hama *et al*[119], 2010; Lai *et al*[120], 2012
LPA4	Human chromosome Xq21.1; 41.9 kDa; 370 aa; Identity 98.4% Mouse chromosome X region D; 41.9 kDa; 370 aa	P2Y9/GPR23	Ovaries, thymus, pancreas, brain, heart, small intestine, testis, prostate, colon, and spleen. Heart, ovary, skin, thymus, and bone Marrow.	Inhibition of its differentiation into osteoblasts in human mesenchymal stem cell line; For mouse: increased trabecular bone volume, number, and thickness; pericardial effusions, severe edema and hemorrhage, abnormally dilated blood and lymphatic vessels and lymph sacs, and impaired pericyte recruitment.	ROCK-dependent cell aggregation and N-cadherin-dependent cell adhesion, cAMP accumulation, differentiation of immortalized hippocampal progenitor cells, negatively cell motility regulation and osteogenesis.	Yung *et al*[68], 2014; Ohuchi *et al*[111], 2008; Choi *et al*[110], 2010; Liu *et al*[123], 2010; Mansell *et al*[124], 2010; Liu *et al*[125], 2009; Sumida *et al*[126], 2010; Yanagida *et al*[71]; Lee *et al*[97], 2007; Rhee *et al*[121], 2006; Lee *et al*[122], 2008
LPA5	Human chromosome 12p13.31; 41.3 kDa; 372 aa; Identity 79.0% Mouse chromosome 6, 59.21 cM; 41.4 kDa; 372 aa	GPR92	Spleen, heart, small intestine, placenta, colon, and liver. Small intestine, lung, heart, stomach, colon, spleen, thymus, skin, liver, platelets, mast cells, gastrointestinal lymphocytes, and dorsal root ganglia.	Reduced lung metastasis by melanoma cells.	Neurite retraction, stress fiber formation, receptor internalization, water absorption, Ca^{2+} mobilization and cAMP accumulation, LPA-induced release of chemokine ligand 4 in mast cells.	Yung *et al*[68], 2014; Lee *et al*[87], 2006; Lee *et al*[141], 2015; Amisten *et al*[142], 2008; Lundequist *et al*[129], 2011; Araki *et al*[130], 2014; Lin *et al*[128], 2010; Yanagida *et al*[143], 2013

| LPA[6] | Human chromosome 13q14.2; 39.4 kDa; 344 aa; Identity 93.0% | P2Y5 | Hair, skin. | Hypotrichosis | Hair development, increased intracellular Ca^{2+}, reduced forskolin-stimulated cAMP accumulation, and ERK1/2 activation | Yanagida *et al*[144], 2011; Yanagida *et al*[143], 2013; Raza *et al*[135], 2014; Dong *et al*[145], 2014; Lee *et al*[141], 2015; Lee *et al*[133], 2009 |
| | Mouse chromosome 14, region D3; 39.4 kDa; 344 aa | | Hair, immune cells. | | | |

[1]Molecular mass were obtained from UniProt[146]; [2]aa means amino acids; [3]Identities between human and mouse lysophospholipid receptors were calculated in UniProt[146]. *vzg-1*: Ventricular zone gene-1; *edg*: Endothelial differentiation gene.

down-stream signals, which may regulate cell survival and migration[107]. LPA[2] regulates cell survival and cell migration in the development of nervous system and functions of immune system[68,90,106,110,111]. The focal adhesion molecule thyroid receptor-interacting protein 6[112,113] and several PDZ-domain and zinc finger proteins[114] interact with LPA[2]. The PDZ-binding domain of LPA[2] regulates Na$^+$/H$^+$ exchanger regulatory factor 2 activity, and activates PLC-3 and Akt/ERK signaling pathways. These pathways stimulate cell migration, enhance survival, and alter gene expression, accounting for the functions attributed to LPA[2]. *Lpar2*$^{-/-}$ mice are viable and healthy, while those null for both *Lpar1* and *Lpar2* show features essentially consistent with those of *Lpar1*$^{-/-}$[115]. These data suggest functional redundancy of LPA[2] with LPA[1].

LPA[3]

LPAR3/Lpar3 was cloned based upon homology to already identified LPA receptor genes using degenerated primers in a PCR-based cloning strategy[83,116]. LPA[3] couples with G$_{\alpha q/11}$ and G$_{\alpha i/o}$ to mediate adenylyl cyclase inhibition, PLC activation and Ca^{2+} mobilization, and Ras activation[105] (Figure 3). LPA[3] prefers 2-acyl-LPAs containing unsaturated fatty acids[83]. It mediates the activation of a series of physiological processes such as male and female reproductive physiology, inflammation, cell Ca^{2+} homeostasis and cAMP regulation[107,117-119]. LPA[3] appears to determine vertebrate left-right patterning during embryogenesis as downregulation of *Lpar3* or inhibition of LPA[3] activity disrupted patterning process in zebrafish[120]. *Lpar3*$^{-/-}$ mice are viable with no reported neural deficits, even though LPA[3] is found in the frontal cortex, hippocampus, and amygdala[83,116]. On the other hand, female *Lpar3*$^{-/-}$ mice have a delayed embryo implantation, and reduced litter size[117].

LPA[4]

The first so-called non-EDG LPA receptor was identified in 2003, and named as LPA[4]. It shares homology (approximately 20%) with LPA[1-3], and it is more closer to the P2Y receptor family[86]. LPA[4] was identified by screening orphan receptors using calcium mobilization as a readout for ligand-induced signals. LPA4 couples with G$_{\alpha 12/13}$, G$_{\alpha q/11}$, G$_{\alpha i/o}$ and G$_{\alpha s}$[97], and activates Rho/ROCK to induce neurite retraction and stress fiber formation[71,97] (Figure 3). It induces ROCK-dependent cell aggregation

and N-cadherin-dependent cell adhesion[71]. LPA[4] is only LPA receptor that activates G$_{\alpha s}$ to induce cAMP level[97]. The activation of LPA[4] was thought to regulate the differentiation of immortalized hippocampal progenitor cells[121]. In addition, the activation of LPA[4] could inhibit LPA-induced cell migration, but LPA exposure increased lamellipodia formation and transwell movement of LPA[4] null cells, indicating an increased sensitivity[122]. It shows the ability of LPA[4] to negatively regulate cell motility and indicates that differential effects may be achieved by simultaneously expressing multiple LPA receptors. *LPAR4*-deficient human mesenchymal stem cells lost ability to differentiate into osteoblasts[123]. While adult *Lpar4*$^{-/-}$ mice appear grossly normal[122], they exhibit increased trabecular bone volume, number, and thickness[124,125]. LPA[4] pathway seems to inhibit osteogenesis. *Lpar4*$^{-/-}$ mice had reduction of prenatal survival rate during embryo development, which is accompanied by changes such as pericardial effusions, severe edema and hemorrhage[126].

LPA[5]

LPA[5,] the fifth LPA receptor, was identified in 2006[87,127]. It shares about 35% homology with *LPAR4*, and 22% homology with *LPAR1-3*[87]. LPA[5] couples with G$_{\alpha 12/13}$ and G$_{\alpha q/11}$, which mediate neurite retraction, stress fiber formation, and receptor internalization in LPA[5]-expressing cell lines[87] (Figure 3). It also activates G$_{\alpha q/11}$ to increase intracellular calcium mobilization, and cAMP accumulation *via* a non-G$_{\alpha s}$ mechanism, suggesting the involvement of other G-proteins[87,127]. LPA[5] signaling may also affect intestinal water absorption[128]. This is achieved through the LPA-induced recruitment of Na$^+$/H$^+$ exchanger 3 to the microvilli mediated by the interaction between LPA[5] and Na$^+$/H$^+$ exchanger regulatory factor 2. Additionally, LPA[5] is the main LPA receptor responsible for LPA-induced release of chemokine ligand 4 in mast cells[129]. Interestingly, LPA[5] in B16 melanoma cells, prefers alkyl-LPA (18:1) to acyl-LPA (18:1)[99]. *Lpar5*$^{-/-}$ null mice exhibit reduced lung metastasis by melanoma cells compared with wild type ones[130].

LPA[6]

The most recently identified LPA receptor is LPA[6]. It was first isolated from a chicken T cell library and named receptor 6H1 in 1993[131], and then, renamed to P2Y5 because of sequence homology with P2Y receptors in

1996[132]. LPA6 couples with G$_\alpha$-protein G$_{\alpha12/13}$ (Figure 3). Its activation by LPA causes cAMP accumulation, changes in cell morphology, and guanosine 5'-3-O-(thio) triphosphate binding[71]. When LPA6 was expressed together with a G$_\alpha$ protein, LPA stimulation increased intracellular Ca^{2+} level, and decreased forskolin-induced cAMP level and ERK activation in intestinal cells[133]. LPA6 has been thought to be involved in familial hair loss[134,135]. Mutations of lipase member H and LPA6 in patients with hypotrichosis are respectively associated with a decrease in LPA production and abnormal LPA6 activation in cells[134,136,137]. These findings demonstrate the roles of LPA6 and LPA signaling may be therapeutic targets for the treatment or prevention of human hair loss[138-146].

LPA RECEPTOR SIGNALING IN OBESITY AND INSULIN RESISTANCE

Recently, obesity has become major public health concern, particularly in the United States. According to 2015 Center of Disease Control and Prevention estimates, more than one-third of adults (34.9% or 78.6 million) and 17% of youth in the United States were obese in 2011-2014[147]. Obesity is associated with the development of chronic metabolic diseases including diabetes, heart disease, stroke, and some types of cancer. The long-term effects of being overweight correlate with premature death, cardiovascular disease, metabolic morbidities, and asthma, among other problems[148]. Both environmental factors and genetic factors contribute to the obesity development. Many factors modulate the propensity to accumulate fat in cells, including an increased ratio of adipocyte precursor cells to differentiated adipocytes[149].

LPA receptor signaling regulates adipogenesis

Obesity is associated with adipocyte hypertrophy and hyperplasia. Hypertrophy results in excessive TAG accumulation in adipocytes. Hyperplasia results in recruitment of new adipocytes *via* proliferation and differentiation. LPA was found to induce proliferation of 3T3F442A preadipocytes, indicating the role of LPA signaling in fat storage[150]. LPA stimulation increases the growth of 3T3F442A cells *via* LPA1, which activates the Ras-Raf-MEK-ERK pathway, and of the focal adhesion kinase[20,151].

It has been reported that *Lpar1*$^{-/-}$ mice exhibited greater adiposity than the control mice without alteration of feeding behavior, despite of lowered body weight[107]. Interestingly, *Lpar1*$^{-/-}$ mice were resistant to diet-induced obesity that may result at least in part from alterations in leptin production[64]. Mature adipocytes express more ATX than preadipocytes. When secreted from adipose tissue, ATX may promote preadipocyte proliferation. Its expression was up-regulated during adipocyte differentiation, and in *db/db* mice[44,45].

The serum levels of LPC, the precursors of LPA,

increases gradually in rabbits fed a high-cholesterol diet for 12 wk. The levels of individual LPAs formed after the incubation of serum for 24 h elevated with the increase of the length of time that rabbits were fed a high cholesterol diet[152]. These studies indicate that feeding of a high-fat diet can cause an increase in the circulating level of LPA. Preadipocytes mainly express LPA1[153], and the mRNA level of *Lpar1* expression in preadipocytes is higher than that in mature 3T3-L1 adipocytes[154]. However, in human adipose tissue, obesity does not influence on *LPAR1* expression[155]. This discrepancy of LPA1 expression levels between human and mouse adipose tissues suggest that obesity promotes LPA synthesis rather than activation in adipose tissue.

The LPA-induced proliferation of preadipocytes[20,153,154] has been thought to be mediated through LPA1 and the activation of the Ras-Raf-MEK-ERK pathway[154,156,157]. LPA inhibits differentiation of white and brown preadipocyte cell lines, which include porcine preadipocyte cell line; mouse preadipocyte cell line, 3T3-L1 and 3T3F442A; and human Simpson-Golabi-Behmel Syndrome preadipocyte cells[153,154,158,159]. This inhibition is mediated by LPA1 *via* the Rho-ROCK pathway[160,161]. All these result in a down-regulation of PPARγ, and impaired responses of PPARγ-targeted genes to its ligands, which leads to reduced TAG accumulation, and expression levels of adipogenic genes[153,154].

The activation of Rho-ROCK pathway delayed the activation of the Wnt-signaling pathway, which has been partially attributed to the inhibited PPARγ expression and adipogenesis. When mice with the adipocyte-specific knockout of ATX gene (FATX-KO) were fed a high-fat diet, they had more fat mass and larger adipocyte size, but not adipocyte number, than the control mice did in the absence of any change of food intake. The deletion of ATX in mice appeared to lead sensitivity to diet-induced obesity, which might be due to elevated expression levels of PPARγ and its down-stream adipogenic genes in subcutaneous white adipose tissue. Interestingly, those knockout mice had improved glucose tolerance and less systemic insulin resistance than the control mice fed the same diet[63,161]. LPA stimulation seems to have anti-adipogenic effect in white adipocytes[153] and in brown preadipocytes[159]. Aforementioned experiments seem to indicate that ATX-LPA receptor signaling pathway may inhibit the development of adipose tissue (Figure 4).

On the other hand, others reported that ATX promotes preadipocytes proliferation and differentiation into adipocytes, thereby promoting adipocyte hyperplasia and obesity. It was showed that deletion of ATX results in smaller body weight gain, smaller fat pad weights and adipocyte numbers, less insulin resistance and glucose tolerance in heterozygous *Enpp2*$^{+/-}$ mice and adipocyte-specific FATX-KO mice fed a high-fat diet than their littermates controls[162]. Moreover, the FATX-KO improved brown adipose tissue function,
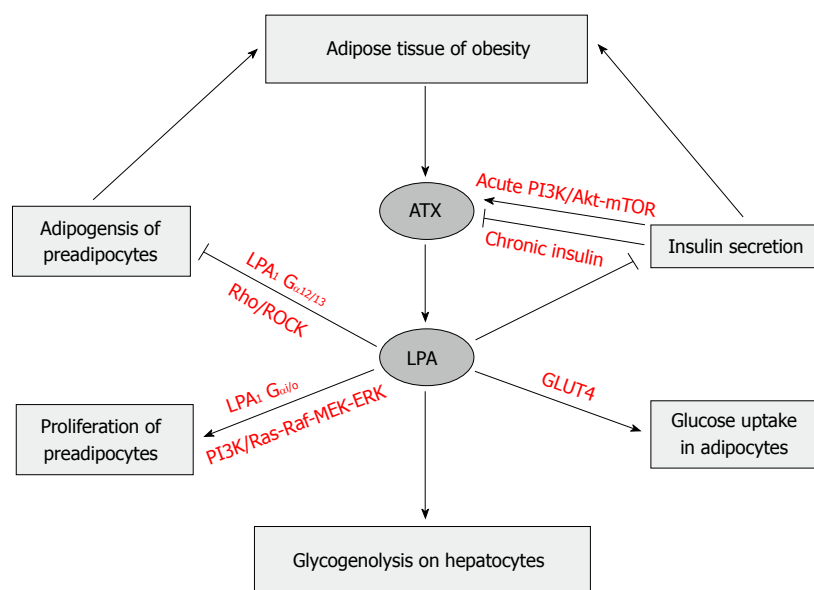
Figure 4 Autotaxin-lysophosphatidic acid signaling axis regulates adipose tissue development and glucose homeostasis in obesity. In adipose tissue, especially in mature adipocytes, the elevated expression of AXT leads to production of LPA and then induced proliferation of preadipocytes *via* LPA_1 through Ras-Raf-MEK-ERK pathway. On the other hand, LPA inhibits differentiation of white and brown preadipocytes, which is mediated by LPA_1 *via* the Rho-ROCK pathway. Short-term insulin treatment increases ATX secretion in adipocytes *via* PI3K/Akt-mTOR pathway, whereas long-term insulin treatment reduces ATX activity. LPA produced by ATX in obesity has a tonic inhibitory effect on glucose homeostasis through inhibition of insulin secretion in isolated pancreas islets, increase of glucose transport in myocyte and adipocytes *via* GLUT4 translocation in a PI3K dependent manner, and elevation of glycogenolysis in hepatocytes. ROCK: Rho-associated kinase; PI3K: Phosphatidylinositol 3-kinase; MEK: Mitogen-activated protein kinase; ERK: Extracellular signal-regulated kinase; Akt: Protein kinase B; mTOR: Mammalian target of rapamycin; GLUT4: Glucose transporter type 4.

increased energy expenditure, and improved systemic metabolism. Transgenic mice expressing the human ATX/ENPP2 gene under the control of $\alpha1$ antitrypsin gene promoter became sensitive to diet-induced obesity due to reduced expression of brown adipose tissue-related genes in peripheral white adipose tissue and accumulated significantly more fat without any change of locomotor activities, thermogenic profiles, and systemic metabolism[159]. In mice, ATX is highly expressed in visceral white adipose tissue and brown adipose tissue and is downregulated in adipose tissue hypertrophy[162]. In human, ATX expression is higher in subcutaneous than in visceral fat, and the latter fat pad in obese subjects has higher ATX expression level than that in non-obese subjects, which is correlated with leptin expression[155]. The circulating ATX levels correlated negatively with body mass index, and mRNA levels of ATX were reduced in subcutaneous fat from obese subjects[162]. Moreover, ATX expression in adipose tissues may be negatively regulated by LPA through a feedback regulatory mechanism, which may involves inflammatory cytokines such as tumor necrosis factor α (TNF-α) and interleukin-1β[163].

ATX-LPA receptor signaling axis exerts a negative effect on glucose homeostasis in obesity

Earlier studies found that increases in adipocyte size correlated with insulin resistance, and increased risk of type 2 diabetes[164,165]. The expression of ATX is increased in the adipose tissue of obese and insulin-resistant subjects and mice[44,155,166]. It has been shown that LPA also regulates glucose metabolism[167,168].

LPA was found to enhance glucose uptake in a dose-dependent manner in both GLUT4myc L6 myotubes and 3T3-L1 adipocytes, a process that was attributed to the increase of GLUT4 translocation in a PI3K dependent manner. Moreover, the effect of LPA on glucose uptake was completely inhibited by pretreating cells with $LPA_{1/3}$ receptor antagonist Ki16425 and Gi inhibitor pertussis toxin[169]. LPA significantly lowered blood glucose levels in normal mice and streptozotocin-induced diabetic mice, suggesting the promotion of glucose usage, but not stimulation of insulin secretion[169].

The elevation of ATX expression in adipocytes of *db/db* mice occurred simultaneously with the development of hyperglycemia, and only 3 wk after the emergence of hyperinsulinemia in them[166]. ATX expression was up-regulated by treatment with TNFα, and down-regulated by rosiglitazone in 3T3F442A adipocytes[166]. The upregulation of ATX expression in adipocytes of *db/db* mice seems to be associated with the emergence of hyperglycemia rather than fat accumulation or hyperinsulinemia[166].

The plasma levels of LPC, as the precursor of LPA, are reduced in obese and type 2 diabetic mice, suggesting that it may regulate blood glucose level. This reduction may contribute to the impairment of glucose homeostasis[170]. Interestingly, adipocyte specific ATX knockout mice fed with a high-fat diet showed greater adiposity and better tolerance to glucose challenge than control mice[63], suggesting a negative effect of LPA on glucose homeostasis. Similarly, LPA production appears to impair glucose disposal probably through a reduction

of plasma insulin as pharmacological inhibition of LPAR$_{1/3}$ activation improves glucose homeostasis in obese and prediabetic mice[171]. Another possibility is that the progression of diabetes affects ATX expression in adipose tissue[162]. It has been shown that treatments with high concentrations of glucose and insulin led to ATX secretion in adipocytes. Short-term insulin treatment increased ATX activity, whereas long-term insulin treatment reduced the levels of ATX mRNA and protein, and its activity[172].

In humans, ATX expression in adipose tissue significantly increased in diabetes patients in contrast with obese-only subjects[158,166]. Its expression in subcutaneous fat is higher than that in visceral fat. Nevertheless, ATX in visceral, but not subcutaneous, fat of obese subjects is higher than that in non-obese patients[155]. Interestingly, the circulating ATX levels in the blood were reduced in obese subjects[162]. The females have higher blood ATX level than males[173].

The variations of ATX expression were correlated with some clinical parameters. In obese patients, visceral fat ATX was positively correlated with diastolic arterial blood pressure, plasma leptin level, and expression levels of inducible nitric oxide synthase and apelin receptor[155]. In older and obese humans, plasma ATX correlated with fasting glucose, fasting insulin, and glucose level 2 h after an oral glucose tolerance test, and body mass index[173]. LPA produced by ATX in obesity has a tonic inhibitory effect on glucose homeostasis through inhibition of insulin secretion in isolated pancreas islets, increase of glucose transport in myocyte and adipocytes, and elevation of glycogenolysis in hepatocytes[164]. LPA was reported to activate glycogenolysis in hepatocytes *in vitro*[174], suggesting that LPA's effects on glucose homeostasis may be mediated by the liver. All these indicate that LPA production *via* ATX and its receptors activation may impact glucose homeostasis (Figure 4).

POSSIBLE ROLE OF LPA SIGNALING IN LIVER FIBROSIS

The liver plays a critical role in the control of glucose and lipid homeostasis. The disturbance of this homeostasis may lead to development of metabolic diseases such as type 2 diabetes[175] and nonalcoholic fatty liver disease (NAFLD)[176]. Liver fibrosis is a process that leads to the alteration of the hepatic architecture marked by the accumulation of proteins such as collagen in extracellular matrix. This is generally associated with the development of liver diseases such as NAFLD and hepatitis. If left untreated, the further development of these diseases and liver fibrosis will lead to cirrhosis, and liver failure, which needs liver transplantation for the treatment. Factors causing damages of hepatocytes result in activation of hepatic stellate cells (HSCs) and production of pro-inflammatory and pro-fibrotic factors, which will stimulate formation of accumulation of proteins in extracellular matrix[177].

The injuries caused by nutritional and environmental factors alter liver structures and functions, which may lead to the liver fibrosis[178]. The hepatic matrix is remodeled by the inflammatory responses after liver injury. Upon the stimulation, the generation of the liver matrix such as collagen, elastin, hyaluronan, proteoglycans and fibronectin is elevated, which is followed by remodeling processes. All these are associated with the activation of HSCs, and the change of local architecture and the reduction of liver functions. Excessive production and accumulation of extracellular matrix in the liver results in fibrosis, which can lead to liver cirrhosis[178]. In addition to HSCs, other cells responsible for the fibrosis include fibrocytes from hematopoietic stem cells, portal fibroblasts, bone marrow derived mesenchymal cells, epithelial-mesenchymal transition and endothelial to mesenchymal transition[178].

The excessive accumulation of lipids and alterations of their metabolism have been used to explain the etiology of type 2 diabetes, which is associated with profound changes of hepatic gene expression[175,179]. This alteration of hepatic lipid metabolism may cause the development of fibrosis. For example, the elevation of lipid peroxidation in zone 3 hepatocytes has been suggested with the development of fibrosis[180]. On the other hand, changes of fatty acid compositions in plasma phospholipids have been observed in subjects with fibrosis[181,182]. All these show that the alterations of plasma phospholipids in patients with metabolic diseases may play a role in the development of fibrosis.

It has been shown that serum ATX activity and LPA level increase with the development of liver fibrosis in patients with chronic hepatitis C[74,183-185], and with cholestasis and pruritus[186,187]. The association of elevated plasma ATX level with chronic liver disease (CLD) in patients suggests a shorter overall survival in a 10-year follow-up study[185]. Moreover, the increased expression level of hepatic ATX mRNA was found in the majority of publically available CLD and hepatocellular carcinoma (HCC) microarray data sets, suggesting an association of ATX with liver pathophysiology[185]. ATX and LPA levels increased in the plasma of patients with hepatitis C virus (HCV) infection, and positively associate with liver fibrosis stages[183,184,188,189]. HCV infection may stabilize the activity of hypoxia inducible factor in a PI3K dependent manner, which may increase ATX expression, and in turn induce liver fibrosis[190].

It has been shown that serum ATX level correlated with fibrosis grade, and is useful as its marker in liver fibrosis[191]. The higher expression level of *LPA2* mRNA has been associated with the poorer differentiation of HCC cells, and a higher *LPA6* mRNA level is associated with microvascular invasion of HCC. The high expression levels of *LPA2* and *LPA6* mRNA in HCC predict a high potential for malignancy. The elevated levels of *LPA6* and *LPA6* mRNA in conjunction with plasma ATX predict higher rate of recurrence after surgical removal of the tumors[192]. In addition, the plasma level of ATX

has been considered as a potential pathogenic factor and/or biomarker for nonalcoholic fatty liver disease in nondiabetic and obese women[193]. Moreover, the plasma ATX levels correlated with prognosis of cirrhosis (Child-Pugh score), showing the link of ATX and the severity of cirrhosis in patients with CLD[194].

In rats, plasma LPA level and serum ATX activity were increased in liver injury and were correlated with severity of the damage; the former in relation to the extent of fibrosis, and the latter in relation to the extent of hepatocyte damage[186,195]. In mice, different hepatotoxic stimuli linked with the development of different forms of CLD were shown to stimulate hepatocyte ATX expression, leading to increased LPA production, HSCs activation, and signals for fibrosis development[185].

LPA was first shown to stimulate rat HSCs proliferation through MAP kinase activation in 1998[196]. Then, LPA was shown to enhance HSCs contractility through modulation of cellular morphology and attachment to extracellular matrices *via* Rho-kinase[197,198]. LPA also inhibits the apoptosis of those cells through Rho/Rho kinase activation[199], suggesting its involvement in the pathogenesis of liver fibrosis. Moreover, LPA was shown to induce nuclear translocation of inducible nitric-oxide synthase in hepatocytes[200]. These findings demonstrate the possible involvement of LPA in the development of liver fibrosis.

CONCLUSION

LPA is a highly bioactive lipid mediator with a number of cellular sources and exerts its actions through a family of receptors coupling with GPCRs in various cell types. Here, we have discussed recent advances in pathways for extracellular and intracellular production of LPA, the functions as well as structural and biochemical properties of ATX and LPA receptors. For the past 20-30 years, the cloning and identification of proteins mediating LPA production and signal transduction pathways open a new field for us to understand relevance of these proteins in physiology and disease development. The association of LPA production and signal pathways with chronic metabolic diseases has been gradually realized. We have highlighted the roles of LPA signaling pathways in the obesity, insulin resistance and liver fibrosis.

The realization of the importance of LPA-mediated functions leads to more open questions begging for answers. (1) The regulations of enzymes involved in LPA synthesis and degradation pathways remain to be further investigated. Whether intracellularly produced LPA can cross the plasma membrane into the extracellular compartment is currently unclear. Additional enzymes or pathways for the production of LPA are still worth exploring. For example, phosphatidylglycerol was shown to be converted to LPA under the catalytic action of GPAT as reported[201]. (2) For ATX, an important player for the extracellular LPA production, how its activity is regulated, and how the newly produced LPA is released remain to be

addressed. Future analysis will undoubtedly shed some light on these. (3) There are many factors contributing to the pathophysiology of obesity and metabolic diseases. Therefore, the precise role of LPA signaling pathways in these diseases remains to be investigated further. In addition, mechanisms by which the LPA and its receptor signaling pathways in the differentiation of both white and brown adipocytes remain to be clarified. This may help for the control of lipid metabolism. (4) LPA seems to have a negative effect on glucose homeostasis in obesity. This was observed only in obese patients, but not in non-obese subjects. So future human studies should focus on more heathy subjects and compare with those parameters of obese patients. (5) LPA appears to inhibit insulin secretion. Whether this inhibitory effect is due to a direct action of LPA on pancreas islets or a possible regulation of liver glycogen mobilization and/or muscle glucose oxidation remains to be clarified. And (6) It was shown that the plasma LPA level and serum ATX activity both were increased in association with liver fibrosis. The underlying mechanism remains to be determined.

Taken together, the LPA signaling pathways contain multiple points that potentially involve in the development of obesity, liver fibrosis and related pathologies. The development of novel pharmacological modulators targeting intervention points may open new research fields and provide potential medicinal therapies to reduce human suffering. The prospects are bright for expanding insights and contributions in LPA biology.

ACKNOWLEDGMENTS

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