

DEVELOPMENT AT A GLANCE

Lysophosphatidic acid signalling in development

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ABSTRACT

Lysophosphatidic acid (LPA) is a bioactive phospholipid that is present in all tissues examined to date. LPA signals extracellularly via cognate G protein-coupled receptors to mediate cellular processes such as survival, proliferation, differentiation, migration, adhesion and morphology. These LPA-influenced processes impact many aspects of organismal development. In particular, LPA signalling has been shown to affect fertility and reproduction, formation of the nervous system, and development of the vasculature. Here and in the accompanying poster, we review the developmentally related features of LPA signalling.

KEY WORDS: Lysophospholipid, GPCR, Progenitor, Proliferation, Differentiation, Survival, Migration, Chemotaxis, Trafficking, Adhesion

Introduction

Lysophospholipids are found both as minor membrane components and as extracellular signalling mediators in numerous tissues and fluids. A major lysophospholipid form is lysophosphatidic acid

(LPA), which acts through G protein-coupled receptors (Yung et al., 2014). LPA signalling influences the survival, proliferation, differentiation, migration, adhesion and morphology of a range of cell types during development. These include neural progenitor cells (NPCs), astrocytes and oligodendrocytes in the nervous system (Anliker et al., 2013; Fukushima et al., 2002), endothelial cells during vascular formation and maintenance (Chen et al., 2013; Yukiura et al., 2011), cells of the reproductive system (Ye and Chun, 2010), osteoblasts and osteoclasts during bone development (Lapierre et al., 2010; Liu et al., 2010), proliferating pre-adipocytes (Valet et al., 1998), and cells of the immune system (Chan et al., 2007; Goetzl et al., 2000; Zhang et al., 2012). Here, we review how LPA is produced and metabolised during development, how it signals and how it influences the development of a number of tissues and organs. Other important lysophospholipids include sphingosine 1-phosphate, which have been reviewed elsewhere (Kihara et al., 2014; Mendelson et al., 2014).

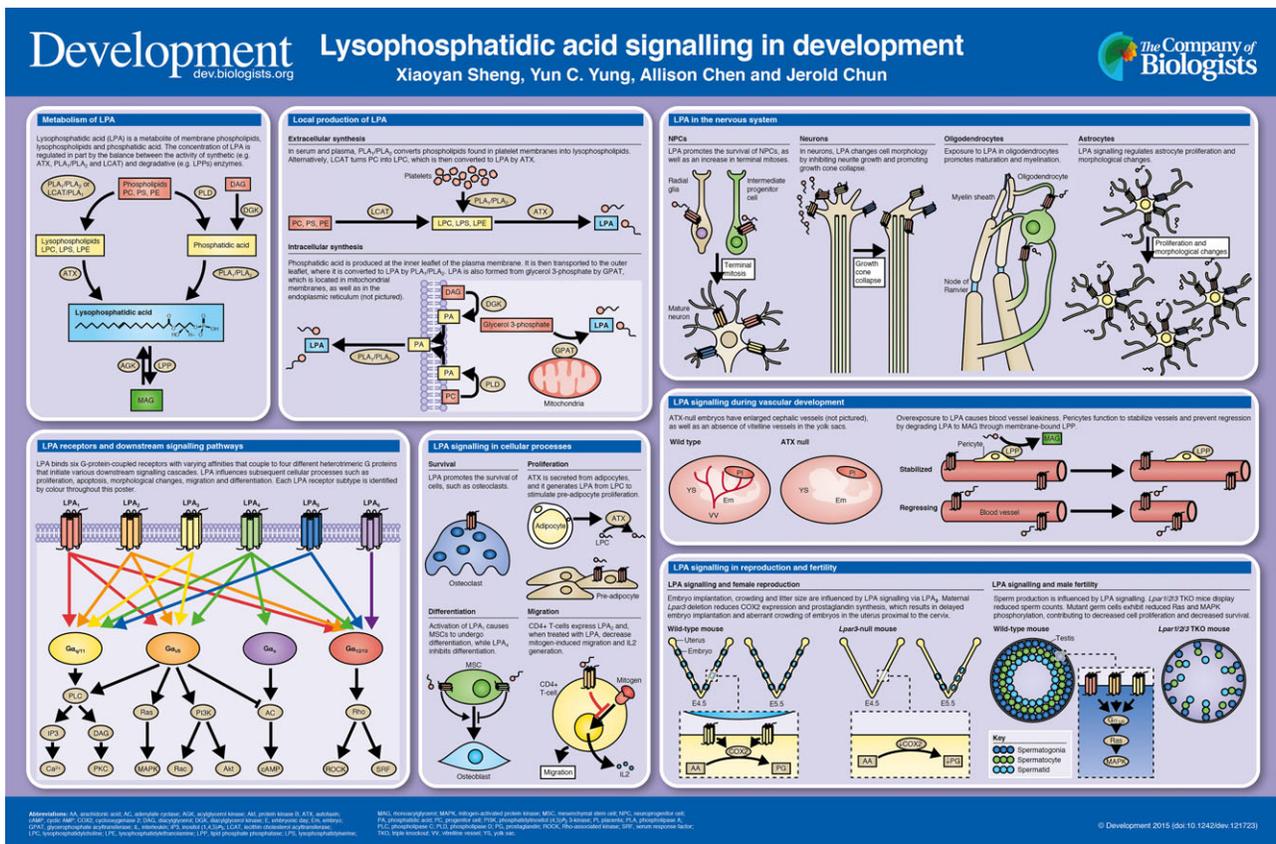
LPA production and metabolism

LPA – along with its most common precursor lysophosphatidylcholine (LPC) – is present both intracellularly and extracellularly in tissues and organ systems in various chemical forms that differ with regards to acyl chain length, saturation and backbone position. LPA is also found in

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many biological fluids, including plasma, serum, saliva, tears, aqueous humour, follicular fluid and cerebrospinal fluid at biologically meaningful concentrations (Aoki, 2004; Yung et al., 2014), and is usually bound to carrier proteins such as albumin. The total or specific forms of LPA present in a sample can be identified and quantified via colorimetric assays, mass spectrometry and other methods (Jesionowska et al., 2014).

A major source of extracellular LPA is LPC, which is converted by the enzyme autotaxin (ATX, gene name *Enpp2*) to liberate LPA and choline (Perrakis and Moolenaar, 2014). Other lysophospholipids such as lysophosphatidylserine (LPS) and lysophosphatidylethanolamine (LPE) can also be enzymatically processed to produce LPA. In addition, LPA can also be formed via the hydrolysis of phosphatidic acid (PA) by membrane-bound phospholipase A1 α or β (Aoki, 2004; Pagès et al., 2001).

While extracellular LPA acts as a signalling molecule through plasma membrane LPA receptors, intracellular LPA is also present and can also be an intermediate for the synthesis of other glycerolipids (Pagès et al., 2001). Intracellular LPA can be produced enzymatically from intracellular organelles such as mitochondria and the endoplasmic reticulum. For example, in mitochondria, membrane-bound glycerophosphate acyltransferase (GPAT) can convert PA to LPA (Pagès et al., 2001). Lipid phosphate phosphatase (LPP) enzymes, which exist extracellularly or intracellularly on the luminal surface of endoplasmic reticulum or Golgi membranes, can dephosphorylate and degrade LPA into monoacylglycerol (MAG) (Brindley and Pilquil, 2009). MAG may then be rephosphorylated by monoacylglycerol kinase (MGK) and thus participate in another round of LPA signalling (Pagès et al., 2001). Thus, the production of LPA is regulated by the availability of precursor metabolites as well as the expression of catalytic enzymes. Whether intracellularly produced LPA can cross the plasma membrane into the extracellular compartment is currently unclear.

LPA receptors and downstream signalling pathways

Six LPA receptor (LPA_R) genes have been identified and characterized to date: *LPAR1-LPAR6* in humans and *Lpar1-Lpar6* in mice and non-human species (which encode the receptors LPA₁-LPA₆) (Kihara et al., 2014; Yung et al., 2014). These seven-transmembrane GPCRs bind different forms of LPA with varying affinities and activate specific heterotrimeric G protein pathways defined, in part, by G $\alpha_{12/13}$, G $\alpha_{q/11}$, G $\alpha_{i/o}$ and G α_s . The downstream signalling cascades involve well-known mediators such as Ras, Rho, Rac, Akt, MAPK, PKC and adenylate cyclase. The resultant activation of these signalling cascades then influences major cellular processes such as proliferation, apoptosis, morphological changes, migration and differentiation.

The normal development of diverse tissues can depend on LPA_R expression, which occurs in varying spatiotemporal patterns (Choi et al., 2010). For example, the developing mouse cerebral cortex mainly expresses *Lpar1* in the ventricular zone and correlates with the initiation, progression and decline of neurogenesis (Hecht et al., 1996). In addition, LPA_R function can be regulated through numerous mechanisms, including receptor desensitization, internalization and phosphorylation (Hernandez-Mendez et al., 2014).

Identifying roles for LPA signalling during development

Major insights into the early developmental roles for LPA came from the successful cloning of the first LPA receptor, LPA₁ (Hecht et al., 1996). *Lpar1*-null mice display 50% perinatal lethality with impaired suckling behaviour that is correlated with olfactory defects (Contos et al., 2000). In addition, these mice appear

growth restricted and are smaller in size compared with heterozygous or wild-type littermates (Hecht et al., 1996). This is consistent with the known embryonic expression patterns of LPA₁ in the brain, dorsal olfactory bulb, limb buds, craniofacial region, somites and genital tubercle (Hecht et al., 1996; Ohuchi et al., 2008). Significantly, *Lpar4*-null mice also display ~30% embryonic lethality, as well as haemorrhage and oedema in various organ systems. These defects result from abnormal mural cell recruitment and vessel stabilization in vascular and lymphatic vessels (Sumida et al., 2010). In addition, *Enpp2*-heterozygous mice display numerous developmental defects; they exhibit at least a 50% reduction in LPA plasma levels, as well as early growth retardation, mid-gestational embryonic lethality due to defects in blood vessel formation, neural tube defects, pericardial effusion, reduced number of somites and defective axial turning (Tanaka et al., 2006; van Meeteren et al., 2006; Yung et al., 2014). Thus, the widespread decrease and/or loss of LPA signalling caused by ATX removal produces severe defects that are partially recapitulated by the deletion of individual LPA_Rs. *Lpar1/Lpar2/Lpar3* triple-null mice also show similar embryo lethality (Ye et al., 2008). It is likely that other combinatorial deletions of LPA_Rs will produce defects that approach the *Enpp2*-null phenotype. Together, these initial studies indicated important roles for LPA signalling during embryo development and paved the way for further and more detailed analyses of LPA function in various developmental contexts.

LPA signalling and reproduction

LPA influences both male and female reproductive function. In males, *LPAR1-LPAR4* and *Lpar1-Lpar3* are detected in the human and mouse testis, respectively. Spermatogenesis from germ cells is decreased in *Lpar1/Lpar2/Lpar3* triple-null mice, as well as in single *Lpar1*, *Lpar2* or *Lpar3*-null mice. This results in a testosterone-independent reduction in mating activity and sperm count, as well as increased prevalence of age-related azoospermia (Ye et al., 2008).

In females, the expression of *LPAR1-LPAR3* is detected in granulosa-lutein cells (Chen et al., 2008), which are post-ovulation granulosa cells of the ovary that secrete progesterone. After fertilization, *Lpar3*-null mice display delayed embryo implantation, embryo crowding and reduced litter size (Ye et al., 2005). Such defects are also seen in mice that are genetically null for cyclooxygenase-2 (COX2), an enzyme that produces prostaglandins and that is downstream of LPA₃ signalling. Prostaglandin administration to *Lpar3*-null females can rescue delayed implantation and reduced litter size, while agonism of a thromboxane A₂ receptor partially rescues embryo crowding, confirming that LPA₃-mediated signalling is upstream of prostaglandin synthesis (Ye et al., 2012, 2005). Moreover, *Lpar3*-mediated alteration of metallo- and serine proteinases and collagen subtypes may be involved in the dynamic remodelling of the endometrial extracellular matrix that occurs in the peri-implantation uterus (Diao et al., 2011; Ye et al., 2011).

LPA in the nervous system

LPA signalling influences a number of developmental processes within the nervous system (Yung et al., 2015). LPA is found in varying abundance in the embryonic brain, neural tube, choroid plexus, meninges, blood vessels, spinal cord and cerebrospinal fluid at nanomolar to micromolar concentrations (Yung et al., 2014). LPA_Rs are differentially expressed in various neural cell types. For example, LPA₁ has effects on cerebral cortical growth and folding, growth cone and process retraction, survival, migration, adhesion, and proliferation. *Lpar1* expression is enriched in the ventricular zone (VZ) during embryonic cortical development, and is expressed

at lower levels in other cortical zones (Hecht et al., 1996; Yung et al., 2011). Accordingly, *Lpar1*-null mice display reduced VZ thickness, altered expression of neuronal markers and increased cortical cell death that perturbs the cortical layers in adults (Estivill-Torrus et al., 2008). The exposure of *ex vivo* cerebral cortices to LPA increases apical-basal thickness and produces gyri-like folds. This occurs through decreased apoptosis and increased NPC terminal mitosis in an *Lpar1/Lpar2*-dependent manner (Kingsbury et al., 2003).

LPA can also modulate neuronal morphology. For example, LPA inhibits neurite extension and promotes growth cone collapse by activating intracellular RhoA through LPAR-mediated signalling pathways (Yuan et al., 2003). LPA also induces changes in neuroblast morphology, controlling the transition from fusiform to round nuclear movements and the formation of F-actin retraction fibres through activation of the small GTPase Rho pathway (Fukushima et al., 2000). As an extracellular lipid signalling molecule, LPA can also affect process outgrowth (Campbell and Holt, 2001; Yuan et al., 2003) and the migration of early post-mitotic neurons during development (Fukushima et al., 2002).

Other nervous system-related cell types that are modulated by LPA signalling include oligodendrocytes, Schwann cells, microglia and astrocytes. Oligodendrocytes are the myelin-forming glial cells of the central nervous system (CNS). They predominantly express *Lpar1*; this expression is spatially and temporally correlated with oligodendrocyte maturation and myelination (Weiner et al., 1998). Moreover, in zebrafish, *enpp2* regulates the commitment of *olig2*-expressing progenitors into lineage committed oligodendrocyte progenitors, supporting the role for LPA in this process (Yuelling et al., 2012). In the mouse peripheral nervous system, Schwann cells depend upon LPA signalling for both survival (Contos et al., 2000; Weiner and Chun, 1999) and proper myelination (Anliker et al., 2013; Weiner et al., 2001), and they express *Lpar1*, *Lpar4* and *Lpar6* (Anliker et al., 2013). Microglia are the resident macrophages of the CNS and have developmental roles (Innocenti et al., 1983). Mouse microglia express *Lpar1* and possibly *Lpar3* (Moller et al., 2001). LPA signalling in microglia regulates proliferation, cell membrane hyperpolarization, chemokinesis, membrane ruffling and growth factor upregulation (Fujita et al., 2008; Moller et al., 2001; Schilling et al., 2002, 2004; Tham et al., 2003). Finally, LPA signalling in astrocytes, which are the most abundant glial type and express all LPARs, can regulate proliferation, actin stress fibre formation and morphological changes, and can indirectly promote neuronal differentiation (Manning and Sontheimer, 1997; Shano et al., 2008; Spohr et al., 2008; Suidan et al., 1997).

LPA signalling during vascular development

Embryonic vascular formation involves vasculogenesis (the growth of new vessels from angioblasts), angiogenesis (the sprouting of new blood vessels from pre-existing vessels via endothelial migration and extracellular matrix remodelling), and vessel maturation and stabilization (Eichmann and Thomas, 2013; Wacker and Gerhardt, 2011). The formation of specialized features, such as those in the blood-brain barrier, may also ensue. LPA signalling has effects on endothelial cell proliferation and migration (Teo et al., 2009). *Enpp2*-null mice display poor development of the yolk sac vasculature, including the presence of enlarged vessels as well as the absence of vitelline vessels. In these mice, early blood vessels form properly from angioblasts, but fail to develop into mature and stable vessels, as a result of decreased LPA levels (Tanaka et al., 2006; van Meeteren et al.,

2006). Consistent with this observation, the exposure of cultured allantois explants to exogenous LPA or ATX is not pro-angiogenic but rather maintains the stability of preformed vessels. *Lpar1*-null mice also display vascular defects such as cephalic frontal haematomas in a small fraction of embryos; the number of these haematomas is slightly higher in *Lpar1/Lpar2* double-null mice (Contos et al., 2000). The ATX-dependent production of LPA is also crucial for vascular formation in zebrafish; morpholino-based attenuation of ATX function results in aberrant vascular connection around the horizontal myoseptum. Although attenuation of individual LPA receptors does not produce such defects, the attenuation of both *Lpar1* and *Lpar4* function recapitulates the ATX-attenuated vascular defects (Yukiura et al., 2011). Thus, proper vessel stability relies on overlapping signalling from multiple LPA receptors. In support of this, *in vitro* studies demonstrate that overexposure to LPA can destabilize blood vessels and cause leakage, while pericytes, the contractile cells that ensheath the vessels, appear to stabilize them. In this context, membrane-bound LPPs on pericytes degrade LPA and prevent vascular regression (Motiejunaite et al., 2014).

Developmental roles for LPA in other cell types

LPA signalling has developmental influences on many other cell types. For example, during white pre-adipocyte differentiation, ATX expression is upregulated and the adipocytes release ATX, which then promotes LPA synthesis (Ferry et al., 2003). Furthermore, adipocyte-specific *Enpp2*-null mice fed a high-fat diet display decreased gains in body weight and adipose tissue when compared with wild-type mice on the same diet (Nishimura et al., 2014). This finding contrasts with results from a different group (Dusaulcy et al., 2011), although the discrepancy may be due to differences in mouse genetic background, in the timing of the initiation of high fat diet, or in peak ATX expression levels in pre-adipocytes. It has also been shown that LPA stimulates the proliferation of a pre-adipose cell line (Valet et al., 1998). In brown pre-adipocytes, LPA also induces proliferation by stimulating Erk1/2 via $G\alpha_i$ -dependent activation of PKC and Src. This pathway is insensitive to pertussis toxin (PTX) and involves PI3K (Holmström et al., 2010). Peroxisome proliferator-activated receptors (PPARs) play pivotal roles during adipogenesis. LPA does not act as a PPAR γ agonist in adipocytes but rather inhibits PPAR γ 2 expression and adipogenesis through LPA $_1$ (Simon et al., 2005).

Immune cell development is also influenced by LPA signalling. For example, ATX and LPA induce naïve T-cell polarization, motility and entry into the lymph nodes by stimulating transendothelial migration (Zhang et al., 2012). It was also shown that freshly isolated human CD4 $^+$ T cells from peripheral blood mainly express LPAR2, and that the treatment of these cells with LPA decreases mitogen-induced IL2 generation and migration (Goetzl et al., 2000; Zhang et al., 2012; Zheng et al., 2000). In human immature dendritic cells, LPA induces PTX-sensitive calcium flux, actin polymerization and chemotaxis. However, in mature dendritic cells, LPA inhibits lipopolysaccharide (LPS)-mediated production of IL12 and TNF α , and increases IL10 in a PTX-insensitive manner (Panther et al., 2002). In an *in vitro* culture system mimicking human mast cell development, LPA synergizes with stem cell factor to stimulate mast cell proliferation. This LPA-induced proliferation can be attenuated by treatment with a LPA1/LPA3 antagonist, by PTX, and by an antagonist for PPAR γ . (Bagga et al., 2004). In human mast cells, LPAR5 appears to be the most prevalent receptor, the activation of which

induces macrophage inflammatory protein 1 β (MIP1 β) release (Lundequist and Boyce, 2011).

LPA signalling also modulates bone development. For example, LPA promotes the osteoblastic differentiation of human mesenchymal stem cells (hMSC-TERT cells), which express two LPAR genes, *LPAR1* and *LPAR4*. LPA₁ mediates osteogenesis by promoting osteoblast differentiation; this effect is opposed by LPA₄ signalling (Liu et al., 2010). *Lpar4*-null mice exhibit increased volume, number and thickness of bone trabeculae, consistent with a role for LPA₄ in inhibiting osteogenic differentiation. *Lpar1*-null mice also display significant bone defects, such as low bone mass and osteoporosis, which are likely produced by decreased osteoblastic differentiation from bone marrow mesenchymal progenitors (Gennero et al., 2011). LPA can also induce pleiotropic effects on osteoclast activity and function, acting via LPA₁, which can elevate intracellular Ca²⁺, activate nuclear factor of activated T cells (NFATc1) and promote their survival, and via an unidentified second G $\alpha_{12/13}$ -coupled LPAR, which can evoke and maintain retraction through reorganization of the actin cytoskeleton (Lapierre et al., 2010).

Recently, a role for LPA signalling in hair follicle development and growth has been uncovered. The LPAR p2y₅ (now known as LPA₆), for example, was shown to be crucial for normal human hair growth (Pasternack et al., 2008; Raza et al., 2014). In this context, lipase member H (LPH), also known as membrane-associated PA-selective PLA1 (mPA-PLA1), which uses phosphatidic acid as a substrate to produce LPA, contributes to human hair growth (Pasternack et al., 2009).

Perspectives

LPA signalling plays crucial roles in many developmental processes, impacting a number of organ systems and cell types. Further studies should continue to refine our understanding of LPAR expression and regulation. Importantly, it will be critical to fully characterize the spatiotemporal pattern of different LPA species, their metabolism and their interaction with LPAR subtypes.

It is notable that LPA arises from lipid precursors found in all cells of the body, thereby representing a vast reservoir of potential signalling molecules capable of acting in an autocrine or paracrine manner. LPA receptor signalling may overlap with and converge on pathways triggered by other signalling molecules that are important for development. It is thus probable that LPA signalling will broadly interface with these well-known mediators. Moreover, lipids play vital roles in the energetics of metabolism and thus developmental influences that alter metabolic states could well alter LPA signalling, both physiologically and pathophysiologically. Such influences may have special relevance to developmental disorders affecting the many organ systems that are influenced by normal LPA signalling, ranging from infertility and failure to thrive, to diseases manifesting in later life, such as those affecting the nervous system, as suggested by recent animal studies on foetal hydrocephalus (Yung et al., 2011), foetal brain hypoxia (Herr et al., 2011) and possibly neuropsychiatric disorders (Mirendil et al., 2015). Importantly, LPA receptors are part of a larger class of bona fide GPCR drug targets, raising the possibility of medicinally treating one or more of the myriad developmental disorders potentially impacted by LPA signalling.

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Competing interests

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