Review Article

Lysophospholipid receptor signaling in zebrafish development

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Abstract: Lysophospholipids are membrane-derived phospholipids with two well-known members, lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P). Both lipids have attracted attention for their roles in different physiological and pathological conditions via binding to G-protein-coupled receptors (GPCRs). LPA receptors are widely expressed with overlapping and distinct signaling and tissue distribution. They are closely related to the purinergic GPCRs and function via remodeling actin cytoskeleton or altering gene expression. LPA receptors are involved in neuronal, cardiovascular, immune and reproductive functions and also regulate bone and adipocyte development. S1P receptors are also critical in overlapping physiological processes despite those well-defined LPA and S1P-related functions in mammals, their roles in vivo especially during development are less well understood mainly due to the difficulty to study developmental processes in mammals. Zebrafish is an emergent vertebrate model particular suitable for in vivo and developmental research. Recently, more laboratories including ours have begun to explore lysophospholipid signaling in zebrafish and identified some previously known or unappreciated functions for lysophospholipids in embryogenesis, cardiovascular and neuronal development. Therefore, we will discuss those discoveries and compare them to our understanding of lysophospholipid signaling in mammalian studies.

Keywords: Lysophosphatidic acid (LPA); sphingosine-1-phosphate (S1P); embryogenesis, cardiovascular development; neuronal development

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Lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P) are membrane-derived lysophospholipids important for their functions in different physiological and pathophysiological conditions (1). LPA is synthesized mainly from lysophosphatidylcholine by autotaxin with a phospholipase D (lysoPLD) activity (2,3) and act via cognate G-protein-coupled receptors (GPCRs) to exert their effects via remodeling actin cytoskeleton or altering gene expression (1). S1P can be generated via phosphorylation of sphingosine by sphingosine kinases and its S1P level can be modulated by additional enzymes like sphingosine phosphatases, and sphingosine lyase (4,5). S1P was initially found to be a sphingolipid that elevates intracellular calcium and cell proliferation (6). Similar to LPA receptors, S1P receptors also function via cognate GPCRs (7,8). Here, the synthesis and receptor signaling will be described for both LPA and S1P. We will also discuss their roles *in vivo* with an emphasis on those recently accumulated zebrafish data.

Autotaxin (ATX)

ATX, also named ectonucleotide pyrophosphatase/phosphodiesterase 2 (ENPP2), is one of seven members in the ENPP family. They hydrolyze nucleotides *in vitro* and are classified as ecto- and exo-enzymes (9). ATX is a 125 *KDa* glycoprotein first isolated in a conditioned medium of

A2058 melanoma cells. It is a secreted protein with a high cancer cell motility stimulating activity that is pertussis toxin (PTX)-sensitive and thus should be mediated via receptor(s) coupled to PTX—dependent G protein (10). ATX increases tube formation in matrigel assays (11-13) and enhances metastasis and angiogenesis (13). It was hypothesized that ATX may provide microenvironments favoring invasion and/or angiogenesis for malignant cells to promote tumor progression. However, the biochemical activity of ATX was not known until it was found to contain lysoPLD activity that can hydrolyze lysophosphatidylcholine into LPA (3,14).

ATX is initially synthesized as a pre-pro-enzyme. Its N-terminal signal peptide is first removed and then followed by its cleavage using convertases and secretion into the extracellular space where it exerts lysoPLD activity (15-17). An active ATX contains two N-terminal somatomedin B-like (SMB) domains, a central catalytic phosphodiester domain (PDE) and a C-terminal nuclease-like (NUC) domain. The PDE domain has a lipid binding pocket and a flanking tunnel that may serve as a lysophospholipid entry and/or exit site (18). The NUC domain helps the rigidity of the PDE domain, while the SMB domains assist in the binding of ATX to integrins (19) that brings ATX to the vicinity of LPA cognate receptors and then those receptors can be activated by LPA synthesized by ATX (18,20,21). Although the biochemical and structural properties ATX have been intensively studied, the regulatory mechanisms of ATX activity and the control of LPA release are still illusive. Intriguingly, despite their similarity between catalytic domains, ATX is the only ENPP family member containing intrinsic lysoPLD activity. But LPA can also be synthesized by other ecto/exo-phospholipases, including phosphatidic acid (PA)-specific phospholipase A1 (22) and sphingomyelinases D (23).

LPA and LPA receptors

Lysophospholipids are minor membrane components and also extracellular signaling molecules found in many tissues and biological fluids. LPA is one of the major lysophospholipids (24). Diverse LPA activities have been reported in different cellular and developmental studies. LPA can affect cell survival, proliferation, migration, adhesion, morphology and other cellular functions of various cell types via GPCR-mediated signaling (25,26). Cell types affected include neuronal cells like neural progenitor cells, astrocytes and oligodendrocytes (27), vascular endothelial cells (28), osteoblasts and osteoclasts (29,30) proliferating pre-adipocytes (31), reproductive-related cells (32) and immune cells (33,34).

LPA is a metabolite derived from the synthesis of membrane phospholipids. It is ubiquitously present in many tissues examined. LPAs have various forms with different acyl chain lengths, saturation, and position. The most commonly used LPA is 18:1 oleoyl-LPA (1-acyl-2-hydroxy-sn-glycero-3-phosphate). LPA is present in significant amount in blood fractions from 0.1 μM to more than 10 μM in plasma and serum, respectively (35,36). LPA concentrations at those levels are higher than the Kd values of LPA receptors for LPA, and thus should have enough LPA to exert their functions.

LPA receptors exert their effect via remodeling actin cytoskeleton or altering gene expression (37). At least six GPCRs for LPA, named LPA1-LPA6 (or LPAR1-LPAR6), have been identified. Similar to S1P receptors, but only three of those LPA receptors (LPA1-3) belong to the endothelial-derived growth factor (Edg) receptor family (38,39). LPA receptors are more closely related to the purinergic GPCRs (38-41). They are widely expressed with overlapping and distinct signaling and tissue distributions (38,40).

ATX–LPA signaling in zebrafish

The so called "ATX-LPA signaling axis" (13) is implicated in a variety of physiological and patho-physiological processes, including vascular and neural development (13,42-45), tumor progression and metastasis (46,47), lymphocyte trafficking (21), bone development (48), neuropathic pain (49), pulmonary fibrosis (50), fat mass regulation (51), cholestatic pruritus (52), fetal hydrocephalus (53) and chronic inflammation (54). Those studies were mainly carried out in mammals. Not until recently, LPA and its close relative S1P have attracted intensive research in zebrafish.

Zebrafish as a vertebrate model

Zebrafish is a versatile vertebrate model to investigate early embryogenesis and organogenesis including vascular and neuronal development. The major advantages of using zebrafish as a model are as following: (I) excellent embryology: external fertilization, rapid development and optical transparency allow easy observation and manipulation; (II) small size (about an inch in length) and relative short generation (3 months): allow genetic study in large quantity; (III) accessibility of genome manipulation:

tools available for transgenesis and mutagenesis. Zebrafish is particularly suitable for cardiovascular studies because the optical clarity and their embryos can develop in the absence of blood circulation within 7 days post fertilization. These characteristics make zebrafish a superior model than mammalian species. In fact, many novel discoveries have been made in zebrafish for our understanding in vascular development (55).

Vascular development in zebrafish

Vertebrate vasculature is mainly composed of two systems, blood and lymphatic vasculatures, that develop in parallel. Blood vessels mediate the transportation and exchange of gas, nutrients and metabolites. Lymphatic vessels mainly function to regulate immunity via immune cells and to absorb gut lipids and body fluids. Endothelial cells shape the most interior layer of both blood and lymphatic vessels. Two waves of angiogenic sprouting occur in zebrafish (56). Blood endothelial cells (BECs) of the primary wave sprout from the dorsal aorta (DA) at 22 hours post fertilization (hpf) to form the segmental arteries (SA). These BECs grow dorsally, reach the dorsal neural tube and connect with their neighbors from anterior and posterior segments to form the dorsal longitudinal anastomotic vessel (DLAV). The second wave is the development of lymphatic endothelial cells (LECs), which come from the posterior cardinal vein (PCV) to form lymphatic vessel (57).

Lymphangiogenesis is a process of the formation of new lymphatic vessels from pre-existing lymphatics (58). In zebrafish, lymphatic vessel development starts at 32 hpf (57). The lymphatic vessels are distributed into four regions: facial lymphatic network, intestinal lymphatic network, lateral lymphatic network and trunk lymphatic network (59). The trunk lymphatic network, which consists of thoracic duct, intersomitic lymphatic vessels (ISLVs) and dorsal longitudinal lymphatic vessels (DLLVs), has been most widely studied (60). Among trunk lymphatic vessels, the thoracic duct has often been used as a model to study lymphangiogenesis because of its big size and better visibility (60,61). The thoracic duct is connected to an existing SA and is transformed into segmental veins (SV). They can also reach the horizontal myoseptum (HM) to form parachordal lymphangioblasts (PLs). Between 60 and 84 hpf, PLs migrate ventrally to give rise to the ventral part of the ISLVs or dorsally to form the DLLVs (57,61). The majority of PLs eventually migrate away from the horizontal myoseptum and contribute to the lymphatic vasculature (61).

Lymphangiogenesis is balanced between pro- and anti-lymphangiogenic factors to maintain its homeostasis. VEGF, HGF and FGF2 signaling are well known pro-lymphangiogenic factors (62). They affect collagen and calcium binding EGF domains 1 (CCBE 1), vascular endothelial growth factor C (VEGFC), prospero-related homeobox gene 1 (PROX1), and lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1) to regulate lymphangiogenesis (63). VEGFC and its receptor VEGFR3 play a key role during multiple developmental stages of lymphatic vessel. The sprouting of LECs from veins is VEGFC-dependent. VEGFR3 receptors are expressed in endothelial cells, including in PROX1-positive lymphatic precursors and blood vessel precursors (64). The VEGFR3 co-receptor neuropilin 2 (NRP2) modulates the signaling pathways that are activated in response to VEGFC and VEGFD (65). PROX1 is the major regulator of LECs fate and the most reliable marker of LEC identity (66). Its activity is crucial for the appearance of LEC progenitors to leave the cardinal vein during lymphatic development (67,68). PROX1 and its regulators COUP-TFII and SOX18 drive LECs specification in mice. The cooperative control of early LEC fate induction is intriguing during this process (69). LYVE1 is one of the most specific and commonly used mammalian lymphatic endothelial markers. A LYVE1 orthologue has also been found in zebrafish (70,71).

ATX-LPA signaling in vascular development

The role of ATX in vascular development has been well documented in mice. At embryonic day 9.5 (E9.5), ATX-knockout mice die and suffer with sever yolk sac vascular defects and other defects. The appearance of abnormalities matches the timing of elevated expression of ATX and LPA receptors in wild-type embryos (45). Since ATX is the main synthesizing enzyme for LPA, it suggests that the vascular abnormality in ATX-knockout embryos may be caused by deficiency of LPA signaling through LPA receptors. However, the loss of function assay of LPA receptors initially failed to demonstrate their importance in vascular development. Mice lacking LPA1, LPA2 or LPA3 do not show notable vascular defects except hemorrhage in the frontal head of LPA1 or LPA1/2 double-knockout mice. Neonatal LPA1-deficient mice show abnormal suckling behavior presumably due to defects in olfactory system. LPA2-knockout mice appear normal. LPA1/2 double-knockout mice show no additional defects but a higher

incidence (26%) of frontal hematoma was observed in the neonatal mice as compared to that (2.5%) of LPA1 knockout mice (72). LPA3-deficiency results in abnormal embryonic spacing in uterus and impaired implantation in mice (32). Collectively, those studies in mice do not support a role of LPA receptors in mediating vascular development *in vivo*, therefore how ATX/LPA mediates vascular development *in vivo* remains unresolved.

LPA receptor signaling in lymphangiogenesis

To clarify the unresolved issue, we decided to further study the *in vivo* functions of LPA receptors in zebrafish because its advantages to study vascular development *in vivo*. We have cloned and analyzed expression patterns of *lpa1-lpa3* genes in zebrafish (73,74). The LPA1-LPA3 proteins are highly homologous to their mammalian homologues with at least 60% identity. We knocked down *lpa1* by antisense morpholino oligonucleotides (MO) and found that the development of early blood vasculature is only mildly delayed at the dosages tested. However, edema gradually appears in pericardium and mid-trunk regions. Further examination revealed that the formation of the major lymphatic vessel, thoracic duct, is inhibited and thus tissue fluid cannot be drained via lymphatic vessels (74). This is the first *in vivo* evidence to document the importance of any LPA receptors in vascular development. Mechanistically, LPA1 may act via its regulation on VEGFC, one of the key regulators of lymphatic vessels (75). We demonstrated that VEGFC rescues lymphatic defects in *lpa1*-deficient embryos. It suggested that LPA1 exerts its effects on lymphangiogenesis via VEGFC. To explore the link between LPA and VEGFC signaling, Lin *et al*. showed that LPA stimulates the expression of *VEGFC* and other lymphatic marker genes, including *PROX-1*, *LYVE-1* and *Podoplanin*, and tube formation in human umbilical vein endothelial cells (HUVECs). Furthermore, these LPA-mediated events depend on LPA1/LPA3 signaling and EGF transactivation to activate NF-kB mediated *VEGFC* expression (76,77). HUVECs are blood vessel endothelial cells that may not fully support a role of LPA in lymphangiogenesis. Using human lymphatic endothelial cells LPA was shown to induce lymphangiogenesis and IL-8 production *in vitro* (78). More interestingly, LPA was shown to enhance *VEGFC* expression in human prostate cancer PC-3 cells. It implied that LPA-dependent *VEGFC* expression and lymphangiogenesis may be involved in cancer metastasis (79).

The lack of notable vascular phenotype in LPA1-LPA3-deficient mice is in clear contrast to that of zebrafish embryos. One possibility is that different or multiple LPA receptors are involved. Indeed, it may be the case. Less than 20% of LPA4-deficient mouse embryos died during gestation showing hemorrhages and/or edema in various organs at different stages. Both blood and lymphatic vessel were often dilated that might be attributed to defects in recruitment of mural cells and/or pericytes (80). Taken together, the ATX/LPA axis is critical in the establishment of vasculature via different LPA receptors in different species.

Biochemical properties of zebrafish ATX and LPA have been comprehensively characterized (81). Similar to its mammalian homologues, zebrafish ATX also contains lysophospholipase D activity to produce LPA. We also observed similar results (73). All zebrafish LPA receptors were shown to react to LPA but not LPA5a and LPA5b. The ATX-MO causes abnormal vascular branching that is similar to that observed in the ATX-null mice (45) and a hampered blood circulation in zebrafish embryos (*Table 1*). Zebrafish embryos injected with MOs against individual LPA receptors have normal vasculature. In contrast, intersegmental arteries sprout normally from the dorsal aorta but stop at horizontal myotectum with aberrant vascular connection in a portion of embryos treated with MOs against both LPA1 and LPA4 (81). These results further demonstrate the necessity of ATX-LPA-LPAR axis for proper vascular development.

ATX-LPA signaling in left-right asymmetry

The abnormal vasculature and circulation are caused by severe stretching of atria to ventricle and weak heart contraction in *atx* morphants. Interestingly, cardiac jogging and looping were often observed in those morphants which is a sign of an interference of establishing left–right (L-R) patterning (82). The L-R axis is one of the body axes that are fundamental to embryogenesis. L-R asymmetry occurs in vertebrates at different stages and has been studied extensively. The node is a key embryonic structure, which is essential for establishing L-R patterning. The equivalent structure of the node is the Kupffer's vesicle (KV) in zebrafish (83,84). The midline, mainly consisting of the floor plate and notochord, is a barrier which expresses lefty1to prevent signals from intermingling between left and right (85). Dorsal forerunner cells (DFCs), a group of non-involuting cells at the front of the dorsal

Table 1 Knockdown of *lpar3* cause circulation defects

Treatment [n]	% of death	Heart beat [%]				Head circulation [%]				Axial circulation [%]				Intersegment circulation [%]			
		Treatment [n]	Normal	Slow	Barely	Treatment [n]	Normal	Sluggish	Absent	Treatment [n]	Normal	Sluggish	Absent	Treatment [n]	Normal	Sluggish	Absent
Std MO	7.3±1.6	Std MO	98.5±1.5	0.0±0.0	1.5±1.5	Std MO	97.0±3.0	1.5±1.5	1.5±1.5	Std MO	98.5±1.5	0.0±0.0	1.5±1.5	Std MO	96.9±0.1	1.6±1.6	1.5±1.5
[69]		[63]				[63]				[63]				[63]			
lpar3	12.5±4.0*	lpar3	98.3±0.7	1.4±0.7	0.4±0.4	lpar3	81.3±4.7	16.0±5.1	2.1±1.7	lpar3	93.4±4.2	4.9±3.7	1.6±1.3	lpar3	42.3±8.1 **	54.5±7.9**	3.2±1.8
tMO1		tMO1				tMO1				tMO1				tMO1			
[260]		[230]				[230]				[230]				[230]			
Buffer	5.9±2.8	Buffer	100.0±0.0	0.0±0.0	0.0±0.0	Buffer	100.0±0.0	0.0±0.0	0.0±0.0	Buffer	100.0±0.0	0.0±0.0	0.0±0.0	Buffer	100.0±0.0	0.0±0.0	0.0±0.0
[114]		[107]				[107]				[107]				[107]			
lpar3	20.7±7.6*	lpar3	77.3±8.4*	16.9±5.7*	5.7±4.5	lpar3	46.3±9.4**	15.1±6.7*	38.6±13.5*	lpar3	57.5±11.5**	6.2±3.3	36.3±12.3*	lpar3	41.2±8.2 **	20.2±8.0 *	38.6±13.5 *
tMO2		tMO2				tMO2				tMO2				tMO2			
[120]		[94]				[94]				[94]				[94]			

Embryos were injected with buffer, 5 ng Std MO, 2.5 ng *lpa3* tMO1 or 5 ng *lpa3* tMO2, incubated to 72 hpf and observed for death and circulation in the head, axial and intersegmental vessels. *Note*: the number of embryos used in each treatment is shown in parenthesis; values were compared to respective buffer or Std MO-injected control treatments (N=8). *, P<0.05; **, P<0.01.

shield, are precursors of KV (86-88). DFCs first form a rosette-like structure and then a cavity is created within it to become a vesicle. Cilia are inside the lumen of vesicles and asymmetrically placed along the anterior-posterior axis. The beating of cilia generates a counter-clockwise fluid movement called nodal flow. The nodal flow then drives the asymmetrical expression of genes, such as nodal-related southpaw (*spaw*), *pitx2* and *lefty*, in the left lateral plate mesoderm to direct the laterality of organogenesis (83,89,90).

The formation of KV or node appears to be one of the earliest signals for establishing L-R asymmetry. However, the mechanism regulating KV formation is largely unknown until recently. At the mid-epiboly, the dorsal shield has an increased intracellular calcium level that was demonstrated to be essential for the collective migration of DFCs and formation of KV in the tail bud later on. Perturbing calcium signaling using thapsigargin, a calcium ATPase inhibitor, blocks KV formation and disrupts subsequent L-R patterning (91). However, the mechanism regulating the calcium elevation at the shield remained unclear at that time. The defects in cardiac jogging and looping observed in *atx* or *lpa3* MO-injected embryos might be due to the early perturbation of L-R patterning. Furthermore, one of the LPA downstream signals is the activation of phospholipase C and elevation of intracellular calcium (25). So we reasoned that LPA is the key trigger of the dorsal shield calcium. Using antisense MO, we showed that the blockade of ATX-LPA3 receptor axis inhibits the formation of KV, and later the expression of asymmetric genes, *lefty1/2*, *pitx2* and *spaw*. The dorsalized shield calcium may be induced by LPA synthesized by the enriched ATX therein. It is also associated with Wnt signaling, as shown by the accumulation of β-catenin at the dorsal nuclei of both *atx* and *lpa3* morphants (60).

LPA receptors in neuronal development

The roles of LPARs in neural development have been suggested by the initial discovery of the enrichment of LPAR1 in the brain ventricular that earned its original name, ventricular zone gene-1 (VZG1) (92). Different LPAR subtypes are expressed at different stage and cell types in the developing and mature cerebral cortex. They are also expressed in ependyma, blood-brain barrier, and meninges. The LPARs expression patterns change while progenitors appear in the ventricular zone (VZ) and differentiate during their migration to the subventricular

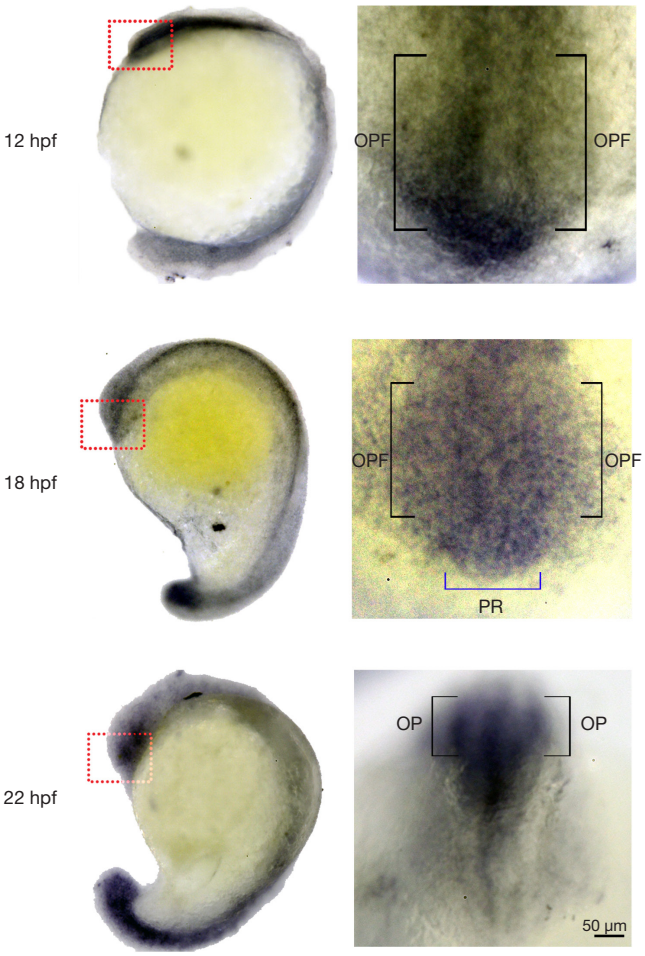

Figure1 lpar1 was expressed in pre-placodal region and olfactory placodal field (OPF). Zebrafish embryos were fixed at designated hour post fertilization (hpf), subjected to whole-mount *in situ* hybridization against *lpar1* and photographed. Representative photographs are shown in lateral view (ventral to the left and dorsal to the right) or frontal view of the dorsal most region (boxed, anterior to the top and posterior to the bottom). The lateral views show that *lpar1* was expressed along the dorsal axis in particular head and tail region. At 12-18 hpf, *lpar1* was expressed in pre-placodal region (PR) and OPF. At 22 hpf, *lpar1* was expressed in olfactory placodes (OP) at the future telecephalon region. Scale bar = 50 μm.

zone (SVZ) and intermediate zone (IZ), and then situate in cortical plate (CP). During the formation of embryonic brain, LPA mediates proliferation, interkinetic nuclear migration, cell migration, neurite retraction and survival. After birth, LPA signaling regulates neuron myelination, responses of microglial and astrocytic, vascular integrity, and cognition (93). Not much has been done to examine the role of LPA signaling in zebrafish neuronal development. Our preliminary data revealed that the formation of brain ventricle is impaired in ATX morphants. In addition, we found that *lpa1* is expressed in the olfactory pre-placodal region and olfactory placodal field (OPF) of developing zebrafish embryos (*Figure 1*). This is intriguing because the postnatal LPA1 null mice die because of the difficulty of milk suckling and presumably harboring an impaired olfaction (94). To examine this notion, we tested the olfactory response of *lpar1* morphants and observed that they have reduced response to the L-alanine, an appetitive odorant. However, how the loss of LPA1 hampers the olfaction in zebrafish remains to be elucidated.

S1P and S1P receptors

S1P is produced via the metabolism of sphingomyelin on cell membrane. Sphingomyelin is hydrolyzed by sphingomyelinase to form phosphocholine and ceramide. Ceramide is then catalyzed by ceramidase to become sphingosine, an immediate precursor of S1P. Sphingosine is then phosphorylated by sphingosine kinases to generate S1P (4,5). S1P is found in all cells examined, however, erythrocytes and endothelial cells are in general considered to be the major sources of S1P in plasma under normal physiology (95). The homeostasis of S1P is regulated by several enzymes, including sphingosine kinases, lysophospholipid phosphatases, and S1P lyase (4,5). S1P like LPA is secreted extracellularly for its actions. The secretion of S1P to the extracellular space is via transporters like ATP-binding cassette (ABC)-type transporters, ABCC1, ABCA1 and ABCG1 (96-98). The regulatory mechanism for S1P transportation via the ABC transporters is still unclear. But recent works in zebrafish have revealed that the *Spns2* gene encodes a specific S1P transporter (99,100). Upon entering circulation, S1P is associated with high-density lipoproteins and other plasma proteins like albumin to provide a stable reservoir of S1P for its subsequent activation of five known S1P receptors (S1PRs, S1P1-S1P5) (101).

S1P receptors have distinct but overlapping intracellular signaling cascades (102). S1P1-S1P3 are expressed in different tissues, including the central nervous system (CNS), immune cells, heart and vasculature (103). S1P4 is not only found in spleen CD4 and CD8 T cells (104) but in cells of the hematopoietic and lymphoid lineages. S1P5 is expressed on natural killer cells and oligodendrocytes (105). S1PRs mainly function in the immune, cardiovascular and

CNS systems.

Lymphocytes circulate between the blood and lymphatic systems that is essential for immune function. It is known that the S1P-S1P1 signal is critical for the circulation of naïve B and T lymphocytes. Lymphatic vascular cells expressing lymphatic vessel endothelium receptor-1 can secrete S1P to activate S1P1 on lymphocytes. This results in the exit of lymphocytes from lymph nodes and concomitant transient downregulation of lymphocyte expression of S1P1 such that the lymphocytes fail to be activated by S1P and thus remain in lymph nodes. Activation of lymphocytes and following clonal expansion allows S1P1 to be is re-expressed on lymphocytes and regain its responsiveness to S1P and ability of egression from lymph nodes (95,106-108).

S1PRs are expressed on the membranes of neurons, astrocytes, oligodendrocytes, and microglial cells in the CNS (109,110). However, the roles of S1PRs for the CNS are just beginning to be understood. *In vitro* and animal models studies revealed that S1P is critical for conditions like migration of neuronal progenitor cells toward injured areas; astrocyte migration and communication with other CNS cells; oligodendrocyte survival, myelination following injury; regulation of microglial number and activation; and the integrity of the blood-brain barrier (40,111-119).

S1P1 and S1P3 collaboratively induce an acute but transient decrease in heart beats (120,121). S1P1 is highly expressed in cardiomyocytes of ventricles, septa, and atria and in endothelial cells of cardiac vessels (122). S1P1 but not S1P3 is the major regulator of atrial myocyte contraction and heart rate. Collectively, it suggests that S1P may be involved in the regulation of heart rate, but the relative importance of S1P1 and S1P3 may depend on experimental models and animal species (123).

Smooth muscle cells of S1P1-null mice develop nascent endothelial tubes. However, the process of vascular maturation is disrupted (124,125). *In vivo* and *in vitro* human studies in conjunction with animal studies suggest that the S1P1palys a significant role in guiding vascular development (126). In addition, the S1P2 receptor has also be implicated in atherosclerosis (127).

Genetic ablation revealed essential roles of S1prs during development in mice (128). *S1pr1*-knockout mice have severe hemorrhage and die between E12.5 and E14.5 (129), and *S1pr1*, *S1pr2* and *S1pr3*-triple mutants have more profound vascular phenotypes (130). It implies a pivotal role of S1pr1 together with S1pr2 and S1pr3 in vascular development in mice.

S1P signaling in zebrafish

Sphingosine kinases (SPHK1 and SPHK2) are S1P synthesizing enzymes by phosphorylating sphingosine. But their roles *in vivo* have been obscure until recently. Two independent studies in zebrafish generated transcription activator-like effector nuclease (TALEN)-targeted mutations in *sphk1* and *sphk2* (131,132). Both groups found that the *sphk2* zygotic mutants grow normally to adulthood, but the *sphk2* maternal-zygotic mutant showed cardiac bifida phenotypes as that observed in the mutants carrying S1P transporter *spns2* or S1P receptor *s1pr2* mutations (99,100,133). In contrast, no cardiac defect was observed in *sphk1* maternal-zygotic mutants (132). These results clearly demonstrated that the Sphk2-Spns2-S1pr2 axis mediates the cardiac progenitor migration in zebrafish through maternal and zygotic regulation.

Seven S1PRs have been identified and isolated in zebrafish. Comprehensive gene expression and functional analyses had been done for those zebrafish S1PRs (134). Those *s1prs* have unique and overlapping expression domains during early embryogenesis. Using TALEN-mediated mutagenesis, all *s1pr* mutant zebrafish had been generated and analyzed for their phenotypes during early embryogenesis. *S1pr2* mutant show cardiac bifida phenotype that is consistent with a previous identified mutants against the same gene (133,135). Both zygotic and maternal zygotic mutants for other s1prs normally develop to adulthood. It can be occurrence of gene compensation in these deleterious mutations (136) or gene redundancy among S1PRs. The later has been suggested by preliminary data reporting early embryonic defects including vasculogenesis in *s1pr3b* and *s1pr4*-double mutants (134).

Concluding remarks

Despite of comprehensive analyses of ATX and receptors for LPA and S1P in mice, their studies in zebrafish are just beginning to be unraveled. Currently, many functions appear to be conserved in zebrafish. With the ease in gene functional analysis in zebrafish, it provides a great platform for further studies for LPA and S1P signaling. In particular, the feasibility of targeted genome editing like TALEN and clustered, regularly interspaced, short palindromic repeats (CRISPR) (137) technologies in zebrafish provides us more critical tools and strengthen our ability to understand the field of lysophospholipid signaling.

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