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Review

Biological roles of lysophospholipid receptors revealed by genetic null mice: An update

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ABSTRACT

Two lysophospholipids (LPs), lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P), are known to affect various cellular events. Their actions are mediated by binding to at least ten bona fide high-affinity G protein-coupled receptors referred to as LPA_{1-5} and $S1P_{1-5}$. These LPs are expressed throughout the body and are involved in a range of biological activities including normal development, as well as functioning in most organ systems. A growing number of biological functions have been uncovered in vivo using single- or multiple-null mice for each LP receptor. This review will focus on findings from in vivo as well as in vitro studies using genetic null mice for the LP receptors, $LPA_{1,2,3}$ and $S1P_{1,2,3,5}$, and for the LP producing enzymes, autotaxin and sphingosine kinase 1/2.

1. Introduction

Lysophospholipids (LPs) are a quantitatively minor lipid species that have been known for decades as components in the biosynthesis of cell membranes [1]. Two of the best characterized LPs are lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P). LPA and S1P are both abundant in blood (0.2–5 μM) as well as tissues (0.2–100 nmol/g) [2–6], and are produced by activated platelets and other cell types, including erythrocytes [5,7,8].

In addition to their roles as metabolic intermediates, these lipids also function as extracellular signals through G protein-coupled receptors (GPCRs) and there are currently 10 identified lysophospholipid receptors, named LPA_{1-5} and $S1P_{1-5}$ [9–12]. Many of the LP receptors are expressed in various cell types and activate signaling pathways to be coupled with different G-proteins [9,12,13]. Consequently, LPA and S1P trigger a variety of biological activities under physiological and pathological situations, including vascular/nervous system development, reproduction, angiogenesis, immunity/transplantation, asthma, autoimmune diseases, cancer, cardiovascular diseases [14,15], hearing loss [16–18], and pain transmission [19–27].

LPA and S1P receptors are expressed and function widely throughout the body, but the specific function of each receptor still needs to be elucidated. Using genetic null mice for individual LP receptors allows direct examination of their systemic roles in vivo and further study of LP-receptor specific signaling pathways in receptor-disrupted primary cells. Targeted gene disruption in mice has been utilized to uncover the biological functions of each lipid receptor-mediated signaling pathway in vivo and to identify the specific signaling pathways for each LP receptor in primary cells. In this review, we will focus on LP receptor functions from numerous studies that have created and studied genetic null mice. To date, single- or multiple-null mice have been reported for most of the known LPA and S1P receptors, including LPA_1-, LPA_2-, LPA_3-, $S1P_1$-, $S1P_2$-, $S1P_3$-, and $S1P_5$-null mice. In addition, as signaling mediators in vivo, the production and degradation of lysophospholipids is enzymatically controlled, and two LP producing enzymes, sphingosine kinase 1/2 (Sphk1/2) and autotaxin (ATX), play a role in tuning the level of S1P and LPA, respectively [28–34]. Therefore, we will also discuss biological functions that have been revealed using genetic null mice for those enzymes.

2. LPA_1

LPA_1 was the first receptor identified for LPA [35]. It shows broad gene expression in organ tissues such as brain, heart, lung, stomach, small intestine, spleen, thymus, testis, and skeletal muscle in adult mice [36] and has also been detected in human tissues including brain, heart, placenta, spleen, kidney, colon, small intestine, prostate, testis, ovary, pancreas, skeletal muscle, and thymus [37]. LPA_1 has been broadly studied using a heterologous expression system [36,38]. Its signaling induces cell proliferation, serum-response element (SRE) activation, MAPK activation, adenylyl cyclase (AC) inhibition, PLC/PKC

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activation, Akt activation, and Rho activation through three types of G proteins, $G_{i/o}$, G_q, and $G_{12/13}$ [39,40].

The original LPA_1-null mice were generated by deletion of exon 3 which contains the transmembrane domains I–VI [41]. About 50% of the LPA-null mice show perinatal lethality, and survivors exhibited abnormal phenotypes, such as reduced body size, craniofacial dysmorphism with shorter snouts and wider-spaced eyes, and reduced brain mass [12,41]. The majority of LPA_1-null mice pups also exhibited a suckling defect, which was evidenced by little or no milk in their stomachs. Impaired suckling behavior from defective olfaction, may explain to neonatal lethality and reduced body size [41].

Since LPA was known to induce Schwann cell survival through the G_i and phosphoinositide 3-kinase/Akt signaling pathway and overexpression of LPA_1 decreases Schwann cell apoptosis in response to serum deprivation [42], the effects of LPA_1 deficiency on Schwann cell survival was examined [41]. Young adult mouse sciatic nerve sections were examined for cellular apoptosis using in situ end labeling ($ISEL^+$), which labels fragmented DNA [43]. There was an 80% increase in the percentage of $ISEL^+$-positive cells in LPA_1-null mice compared to that of wild-type, although overall, this represented a low frequency of 18% of Schwann cell apoptosis in LPA_1-null mice compared to 10% in wild-type mice [41]. No grossly abnormal movement was observed [41], reflecting a need for more profound nerve fiber loss to reveal an overt phenotype. These data indicated in vivo effects on Schwann cells while indicating that loss of LPA1 is not sufficient to produce a more pronounced myelination defect.

LPA_1 was identified in the cortical neurogenic region of the embryonic cerebral wall, the ventricular zone, which was reflected in its original name, "ventricular zone gene-1 (vzg-1)" [35]. When neural progenitor cells (NPCs) are exposed to LPA they show cell rounding, process retraction and retraction fibers. These cytoskeletal changes bear similarity to interkinetic nuclear migration in the ventricular zone (VZ) that is associated with neurogenesis [44]. LPA_1 has been studied to understand the role of LPA in cortical development [44,45]. No obvious abnormalities in the cerebral cortex were observed in LPA_1-null mice, except for sporadic reductions in cerebral wall thickness in embryonic and neonatal LPA_1-null mice [41,46]. However, a recently identified variant of LPA_1-null mice (called "Málaga variant" or maLPA_1-null mice) has been reported [47]. These maLPA_1-null mice were spontaneously and stably generated during extended breeding of the original LPA_1-null mice [41,47]. Like their predecessors, similar phenotypic abnormalities were observed in the maLPA_1-null mice including reduced size and body mass, craniofacial defects such as shorter snouts and wider-spaced eyes, and reduced brain volume and mass [41,47]. Most notably, the absence of LPA_1 in the maLPA_1-null mice results in defects of cortical development including reduced proliferative populations, and increased cortical cell death that results in a loss of cortical layer cellularity in adult mice [47].

Since LPA is known to be a lipid metabolite released following tissue injury, LPA_1 plays an important role in the initiation of neuropathic pain [48,49]. LPA_1, unlike LPA_2 or LPA_3, is expressed in both dorsal root ganglion (DRG) and dorsal root neurons [48]. Using the antisense oligodeoxynucleotide (AS-ODN) for LPA_1 and LPA_1-null mice, it was found that LPA-induced mechanical allodynia and hyperalgesia is mediated in an LPA_1-dependent manner [48]. Neuropathic pain is mediated by the Rho-ROCK pathway. Pretreatment with Clostridium botulinum C3 exoenzyme (BoTXC3, Rho inhibitor) or Y-27632 (ROCK inhibitor) completely abolished the allodynia and hyperalgesia in nerve-injured mice [48]. LPA also induced demyelination of the dorsal root, which was prevented by BoTXC3. The dorsal root demyelination by injury was not observed in LPA_1-null mice or AS-ODN injected wild-type mice [48]. However, the precise relationship between demyelination and LPA-initiated neuropathic pain is still being determined. LPA signaling appears to induce important neuropathic pain markers such as protein kinase Cγ (PKCγ) and a voltage-gated calcium channel $\alpha_2\delta_1$ subunit ($Ca\alpha_2\delta_1$) in an LPA_1 and Rho-dependent manner [48].

Pulmonary fibrosis has been studied in LPA_1-null mice because the level of LPA remarkably increases in bronchoalveolar lavage (BAL) fluid after bleomycin-induced lung injury [50]. LPA_1 is the most highly expressed LPA receptor in lung fibroblasts among the 5 known LPA receptors (LPA_{1-5}), and LPA-induced chemotaxis of mouse embryonic fibroblast (MEF) cells are mediated by LPA_1 signaling [50,51]. Migration of fibroblasts into the fibrin wound matrix is an essential step in the wound healing process in injured tissues [52]. Tager et al. [50] showed that bleomycin-challenged LPA_1-null mice were markedly protected from pulmonary fibrosis. The mortality of wild-type mice at 21 days after administration of bleomycin was 50%, whereas LPA_1-null mice were 0%. In addition, the accumulation of fibroblasts was dramatically reduced in the injured lungs of LPA_1-null mice, and the persistent vascular leak produced by bleomycin-induced injury was notably attenuated in LPA_1-null mice [50]. Thus, LPA_1-mediated signaling was shown to have an important role between lung injury and the progression to pulmonary fibrosis. However, no significant differences in the numbers of total leukocytes, macrophages, or neutrophils were observed in the BAL of wild-type and LPA_1-null mice [50].

Astrocytes have a response to cAMP-elevating reagents that changes morphology and induces the glial fibrillary acidic protein (GFAP), indicating astrocyte differentiation. LPA has been studied in cultured astrocytes which express the five known LPA receptors (LPA_{1-5}) [53–55]. In the differentiated astrocytes induced by cAMP-elevating reagents, the levels of LPA_{2-4} were markedly reduced, whereas LPA_1 was not affected [55]. In fact, LPA-induced DNA synthesis was notably reduced in the astrocytes derived from LPA_1-null mice, indicating that LPA-induced astrocyte proliferation is mediated by LPA_1 [55].

3. LPA_2

LPA_2 was identified from sequence homology searches using LPA_1 [12,56]. LPA_2 is expressed in the embryonic brain, testis, kidney, lung, thymus, spleen, and stomach in mice [36]. In humans it is detected in the testis, pancreas, prostate, thymus, spleen, and peripheral blood leukocytes [37]. LPA_2 induces cellular signaling through three G proteins, $G_{i/o}$, G_q, and $G_{12/13}$, similar to LPA_1 [36,38].

LPA_2-null mice were generated by deletion of exon 2, containing putative transmembrane domains I to VI [57]. LPA_2-null mice were born normally, at the expected Mendelian frequency, and showed no obvious phenotypic abnormalities [57]. However, LPA-induced PLC activation and Ca^{2+} mobilization are notably reduced in MEF cells derived from LPA_2-null mice [57]. When LPA_1/LPA_2 double-null mice were generated, no additional phenotypic abnormalities were detected when compared to LPA_1 single-null mice [57]. Thus, LPA_1 and LPA_2 may have redundant functions in mediating LPA signaling, such as PLC activation, Ca^{2+}mobilization, proliferation, JNK activation, Akt activation, and stress fiber formation at least within MEFs [57]. Use of the double-null mutants in an ex vivo cerebral cortical culture system for studying embryonic neural functions of LPA signaling identified these receptors as important for LPA effects. In wild-type embryos, LPA exposure increased NPC terminal mitosis and decreased cell death resulting in induced cortical folding and thickening [46]. However, LPA_1/LPA_2 double-null mice lost these cortical growth responses [46].

Recently, cerebral cortical astrocytes have been studied towards understanding possible indirect effects of LPA on neuronal differentiation of cortical NPCs through requisite LPA_1 and LPA_2 signaling in astrocytes [58]. When neural progenitor cells were co-cultured with astrocytes that had been previously primed by LPA, the population of β-tubulin III positive neuron-like cells were increased by 41% without changing the overall cell number. Additionally, comparable effects