Available online at www.jbr-pub.org

Open Access at PubMed Central

The Journal of Biomedical Research, 2016, 30(4):272-284

JBR

Review Article

Recent advances in targeting the autotaxin-lysophosphatidate-lipid phosphate phosphatase axis *in vivo*

Matthew G.K. Benesch, Xiaoyun Tang, Ganesh Venkatraman, Raie T. Bekele, David N. Brindley ✉

Signal Transduction Research Group, Department of Biochemistry, University of Alberta, T6G 2S2, Canada.

Abstract

Extracellular lysophosphatidate (LPA) is a potent bioactive lipid that signals through six G-protein-coupled receptors. This signaling is required for embryogenesis, tissue repair and remodeling processes. LPA is produced from circulating lysophosphatidylcholine by autotaxin (ATX), and is degraded outside cells by a family of three enzymes called the lipid phosphate phosphatases (LPPs). In many pathological conditions, particularly in cancers, LPA concentrations are increased due to high ATX expression and low LPP activity. In cancers, LPA signaling drives tumor growth, angiogenesis, metastasis, resistance to chemotherapy and decreased efficacy of radiotherapy. Hence, targeting the ATX-LPA-LPP axis is an attractive strategy for introducing novel adjuvant therapeutic options. In this review, we will summarize current progress in targeting the ATX-LPA-LPP axis with inhibitors of autotaxin activity, LPA receptor antagonists, LPA monoclonal antibodies, and increasing low LPP expression. Some of these agents are already in clinical trials and have applications beyond cancer, including chronic inflammatory diseases.

Keywords: cancer, chronic inflammation, cytokines, monoclonal antibodies, wound repair

Introduction

As the simplest phospholipid, lysophosphatidate (LPA) is by no means a simple biological molecule. Extracellular LPA, via signaling through at least six G-protein-coupled receptors, mediates a plethora of physiological and pathological processes including embryogenesis, wound healing, chronic inflammatory diseases, and cancer progression and therapy resistance. LPA has an ester-linked fatty acid at the *sn*-1 or *sn-2* position of the glycerol backbone and a hydroxyl group at the other position, and a phosphate head group at the *sn*-3 position (***Fig. 1***). Normally, LPA concentrations in plasma are about 100 nmol/L and this LPA is bound to albumin[1-2]. LPA can rise to about 1 μmol/L in serum as a result of platelet activation[3-5]. The majority of extracellular LPA is generated from unsaturated and polyunsaturated lysophosphatidylcholine (LPC) by the lysophospholipase D activity (lysoPLD) of autotaxin (ATX), which hydrolyzes choline from the phosphate head group (***Fig. 1***). LPC is the most abundant phospholipid in human plasma where it reaches concentrations of greater than 200 μmol/L[6-7]. Historically, LPC was believed to be a bioactive molecule. However, more recent work has shown that ATX inhibition blocks the stimulatory effects of LPC on cell migration and survival[8-9], demonstrating that the biological actions of LPC are mediated through LPA *via* ATX activity. LPA signaling is terminated by its hydrolysis to inorganic phosphate and monoacylglycerol (MAG) by catalytic activity of three related proteins called the lipid phosphate phosphatases (LPP1-3) (***Fig. 1***)[10]. These enzymes were originally characterized by our group as type 2 phosphatidate phosphatases because of their insensitivity

✉Corresponding author: Dr. David Brindley, 357 Heritage Medical Research Centre, Department of Biochemistry, University of Alberta, Edmonton, Alberta, T6G 2S2, Canada, Tel/Fax: 780-492-2078/780-492-3383; E-mail: david.brindley@ualberta.ca.

Received 11 April 2015, Revised 12 May 2015, Accepted 20 May 2015, Epub 28 August 2015

CLC number: R589.2, Document code: A

The authors reported no conflict of interests.

doi: 10.7555/JBR.30.20150058

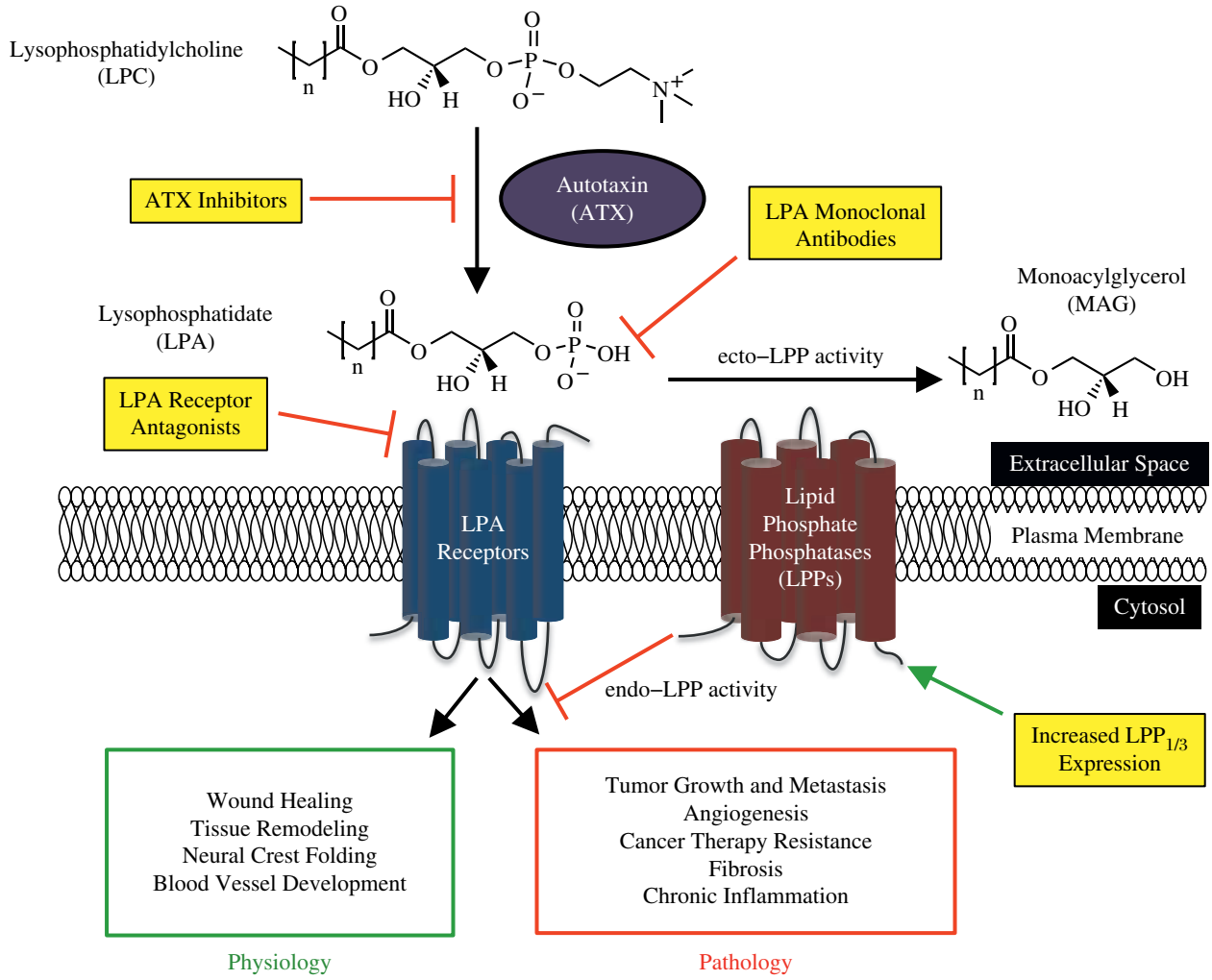

***Fig. 1* Overview of the ATX-LPA-LPP axis and points of therapeutic intervention within the axis.** Extracellular LPA is generated from LPC by the enzymatic lysophospholipase D activity of ATX. LPA can signal through at least 6 G-protein-coupled receptors to mediate both physiological and pathological processes. Extracellular LPA is degraded by the ecto-activity of a family of three enzymes called the LPPs, which hydrolyzes the phosphate head group from LPA to produce MAG. LPPs expressed on the membranes of cellular organelles can also block LPA signaling downstream of receptor activation by endo-LPP activity. LPA signaling can be disrupted by blocking LPA production through ATX activity inhibition, treatment with monoclonal antibodies against LPA, blocking LPA binding to LPA receptors through LPA receptor antagonists, and by increasing expression of $LPP_{1/3}$.

to inhibition by N-ethylmaleimide and lack of Mg^{2+}-requirement[11]. However, it became evident that they also hydrolyze a wide variety of lipid phosphates including LPA and sphingosine 1-phosphate (S1P) and so they were renamed LPPs[12]. All three LPPs are integral membrane proteins containing six transmembrane helices with the C- and N-termini facing the cytosolic side of the plasma membrane[13]. At the plasma membrane, the catalytic site of the LPPs faces the extracellular environment, enabling them to access and hydrolyze extracellular LPA and other phospholipids[10,13].

The prototypical pathology associated with ATX-LPA-LPP axis is best illustrated in cancer. ATX protein expression is increased leading to higher LPA levels in many different tumors. Cancer cells also have increased expression of LPA receptors on their cell surface compared to normal and benign cells, and downregulated expression of LPPs[7,14]. Thus, a triad of increased LPA production by ATX, increased response to LPA by increased LPA receptor expression and decreased LPP activity to degrade LPA on the cell surface creates the perfect storm for cancer cell proliferation, migration, metastasis and therapy resistance[7,10,14-15]. Other inflammatory diseases including asthma, rheumatoid arthritis, lung and liver fibrosis, and inflammatory bowel diseases are also distinguished from normal physiology by increased ATX/LPA signaling. Hence, the importance of studying LPA signaling within the ATX-LPA-LPP axis is now gaining more recognition[16-17]. In this review, we will summarize current knowledge of the ATX-LPA-LPP axis in pathology and describe the approaches being taken to target this axis to improve therapeutic interventions.

Overview of the ATX-LPA-LPP axis

ATX - The predominant producer of extracellular LPA

Enzymatic generation of LPA in plasma by lysoPLD activity was first described in 1986[18]. ATX is a 125-kDa secreted glycoprotein that was first isolated in 1992 from A2058 melanoma cells and described as an "autocrine motility factor"[19]. At the time, its enzymatic activity and homology to other growth or motility factors were unknown. Two years later, sequencing the cDNA clone of ATX showed that it had significant homology to the plasma cell glycoprotein-1 (PC-1)[20], which was known to have nucleotide pyrophosphatase and phosphodiesterase (NPP) activity[21-22]. PC-1 was later named NPP1 and ATX as NPP2, the first two members of a family of seven enzymes that hydrolyze phosphodiester and pyrophosphate bonds to remove phosphates from ADP and ATP[6]. As a secreted enzyme, NPP2 was later referred to as ecto-NPP2 (ENPP2), which has since become the gene symbol for ATX[23]. However, it was not clear how this extracellular nucleotide activity could explain the motility-stimulating capability of ATX described in melanoma cell culture. This mystery remained until 2002, when the plasma lysoPLD activity described in 1986 was purified and found to be identical to ATX[24-26]. Subsequent work showed that ATX/ENPP2 was unique among the NPPs/ENPPs because of its lysoPLD activity, which converts LPC to LPA[24]. The affinity of ATX for LPC was shown to be higher than for nucleotides, suggesting that LPC is the preferred physiological substrate for ATX [24]. The motility-stimulating function of ATX was enhanced by addition of LPC to cell culture experiments, and this action could be mimicked by direct addition of LPA[8,24]. Furthermore, deletion of LPA_1 receptors in fibroblasts abolished the effects of ATX on cell migration[27]. Thus, LPA, the product of ATX, is the actual “motility factor”.

ATX is a vital enzyme that is needed for proper early embryological development. ATX knockout (KO) ($ENPP2^{-/-}$) embryos die *in utero* on day 9.5 with vascular and neural tube defects[28-31]. In these mice, malformations in the allantois, neural tube and headfold are detected by day 8.5, and at day 10.5 embryos become necrotic and are reabsorbed[32]. Normally, extra-embryonic endothelial cells remodel from day 8.5 to 9.5 to create a vascular network that connects with the embryo, allowing the yolk sac to function as the main nutrient source. $ENPP2^{-/-}$ embryos have increased expression of VEGF mRNA, consistent with hypoxic conditions in the absence of a functional vascular system[28,33].

Neural tube closure typically begins at day 8.5. The neural tube closure defects in $ENPP2^{-/-}$ embryos have been attributed to a local deficiency in ATX expression[28]. In $ENPP2^{-/-}$ embryo explants, these folding abnormalities are abrogated by exogenous addition of LPA[34]. The role of ATX in vascular and neural development has also been confirmed in zebrafish[32,35]. ATX regulates oligodendrocyte differentiation in the developing zebrafish hindbrain[36] and the correct left-right asymmetry for normal organ morphogenesis through Wnt-dependent pathways[37]. $ENPP2^{+/-}$ mice are viable, and express half the levels of both ATX and LPA compared to normal mice[38]. However, they are hyper-responsive to hypoxia-induced vasoconstriction and remodeling, and they develop pulmonary hypertension[38].

One of the important roles of ATX after birth is in wound healing and tissue remodeling. LPA is a potent activator of platelet aggregation and it stimulates the division and migration of fibroblasts, vascular smooth muscle cells, endothelial cells and keratinocytes[39]. Increased ATX activity is found in blister fluid where local production of LPA promotes re-epithelialization[40]. ATX expression and LPA production are also increased in rabbit aqueous humor following corneal freeze wounds[41]. Recently discovered physiological roles for ATX include hair follicle morphogenesis[42], bone mineralization[43] and myeloid differentiation in human bone marrow[44]. ATX/LPA signaling also remodels luteal tissue in regressing corpora lutea of cycling rats by recruiting phagocytes and proliferating fibroblasts[45]. ATX expression is also upregulated in microglia in response to oxidative stress. This protects microglia cells against damage from H_2O_2, an effect which is partially reversed in the presence of the mixed $LPA_{1/3}$ antagonist Ki16425[46]. A follow-up study showed that ATX overexpression in microglia limited the pro-inflammatory response to lipopolysaccharide exposure, mimicking Gram-negative infection[47]. ATX is expressed in high endothelial venules (HEVs) in lymph nodes and other secondary lymphoid tissues[48] and mediates lymphocyte extravasation, which is crucial for maintaining immune homeostasis[49-51]. However, in chronically inflamed tissues, ATX mediates lymphocyte trafficking and upregulates cytokine production in response to repeated microinjuries and incomplete tissue repair[52-54].

We recently showed that ATX expression is negatively regulated by LPA signaling through increased phosphatidylinositol 3-kinase (PI3K); however, this inhibition is overcome by pro-inflammatory cytokines[55]. The production of inflammatory cytokines,

such as in damaged and inflamed tissue, is a signal for increased ATX expression and LPA production to heal the wound[39,56]. If this process is successful and inflammation subsides, then LPA produced by ATX feeds back and blocks further ATX production. However, if inflammation is unresolved, inflammatory cytokines stimulate further ATX production and consequent LPA formation stimulates more cytokine production in a vicious cycle[56].

The initial relation of ATX/LPA signaling with melanoma cells resulted in much of the early research into ATX being concentrated in the cancer field[39,57-65]. LPA increases vascular endothelial growth factor (VEGF) production, which stimulates angiogenesis[66], a process necessary for tumor progression. LPA decreases the expression of the tumor suppressor p53[67], thus increasing cancer cell survival and division. We discovered that LPA produces resistance to the cytotoxic effects of paclitaxel, a first line treatment for breast cancer[9,15,68]. This was confirmed[69] and extended since LPA produces resistance to the apoptotic effects of carboplatin[70] and radiation-induced cell death[14-15,71]. LPA levels as high as 10 μmol/L have been reported in ascites fluid of advanced ovarian cancer patients[5]. Mice that overexpress ATX in mammary epithelium develop spontaneous metastatic mammary tumors[72]. Further, the ATX gene is among the top 40 most upregulated genes in metastatic cancers[73]. ATX/LPA signaling is positively correlated with the invasive and metastatic potential of several cancers including melanoma, breast cancer, ovarian cancer, thyroid cancer, renal cell cancer, lung cancer, neuroblastoma, hepatocellular carcinoma and glioblastoma multiforme[15,74].

However, the tumor is a heterogeneous environment composed of many different cell types including fibroblasts, endothelial cells and leukocytes in addition to cancer cells. There are also a host of soluble molecules in the tumor and this heterogeneity adds another layer of complexity to ATX/LPA signaling. Popnikolov *et al.* showed that immunohistochemical staining for ATX in stromal cells and LPA_3 and cancer epithelial cells correlate positively with cancer aggressiveness[75]. This study highlights the role of ATX from tumor stroma as a producer of LPA for cancer progression. We have since shown that breast cancer cells are very poor expressers of ATX and instead breast tumors in mice induce ATX in adjacent mammary adipose tissue[76]. More recent work has shown that metastasis of breast cancer cells to bone depends on the interaction of platelet-derived ATX with $\alpha_V\beta_3$ integrins on cancer cells[77]. Hence, in tumors like neuroblastomas, melanomas and thyroid carcinomas, the cancer cells may be abundant producers of ATX whereas in other tumors like breast, the cancer cells may instead rely on ATX produced from other tissues such as adjacent adipose tissue or in platelets for metastatic cells. A more extensive overview of ATX production in cancer and other diseases has been previously reviewed[56,78,79].

LPA receptor diversity and signaling

To date, there are at least six known LPA receptors, all of which are G-protein-coupled (GPCRs) (***Fig. 1***)[80]. GPCRs are integral membrane proteins composed of a single polypeptide with seven transmembrane domains. GPCRs transmit the signals from extracellular stimuli to intracellular signals through activation of a heterotrimeric guanosine triphosphate-binding protein (G-protein) by the receptor, which involves dissociation of the Gα subunit from the Gβγ subunits by exchange of a guanosine diphosphate (GDP) for a guanosine triphosphate (GTP). These subunits, particularly the Gα subunit, interact with a host of downstream effectors to elicit an intracellular response.

The first three of the LPA receptors, LPA_{1-3}, belong to the Edg (endothelial differentiation gene) family[80]. These are the best understood and most studied of the LPA receptors. LPA_1/EDG2 was the first to be discovered in 1996, followed soon after by LPA_2/EDG4 and LPA_3/EDG7[81-84]. These receptors have a ubiquitous distribution, and the other five GPCRs in the Edg-subfamily are receptors for sphingosine-1-phosphate (S1P) ($S1P_{1-5}$), which is the sphingolipid analog of LPA[80]. Knockout (KO) mice have been generated for all the known LPA receptors, as well as double KOs for combinations of LPA_{1-3} and the triple KO of LPA_{1-3}, all of which are viable[6,85-86]. LPA_1 deletion shows craniofacial deformity while LPA_3 deletion leads to delayed implantation of embryos and impaired embryo spacing[87-88]. No obvious phenotypic defect has been found in LPA_2 KO mice [89]. These findings underscore the redundancy of LPA-receptor signaling pathways and also suggest that there may be other receptors that can mediate LPA signaling.

LPA_{4-6} belong to the P2Y purinergic family of receptors and have specific tissue distributions unlike LPA_{1-3}[80-90]. LPA4/GPR23/p3y9 is found in the embryonically developing brain and in adults largely in the ovaries[91-92]. LPA_5/GPR92 is highly expressed in the spleen, small intestine and dorsal root ganglion cells[93]. In mast cells, LPA_5 is the main LPA receptor responsible for LPA-induced release of chemokine (C-C motif) ligand 4 (CCL4/MIP-1β)[94]. Genetic ablation of either LPA_4 or LPA_5 does not result in obvious phenotypic defects[95-96]. LPA_6/p2y5 appears to be important for

hair growth since genetic truncations of the receptor leads to hypotrichosis simplex, a set of diseases involving familial hair loss[97]. *In vitro*, this receptor only responds to μM concentrations of LPA while $LPA_{1\text{-}5}$ will activate in response to nM concentrations[98]. Finally, there are at least three more proposed LPA receptors (GPR87, P2Y10 and GPR35), but their specificity for LPA requires further experimental validation[80].

Overall, LPA signaling through at least six known G-protein-coupled receptors ($LPA_{1\text{-}6}$) stimulates cell survival and migration through the relative activations of (PI3K), $ERK_{1/2}$, mTOR, Ca^{2+}-transients, Rac, Rho and Ras, and these receptors are often overexpressed in cancer cells[15]. Like for ATX, mice that overexpress LPA_1, LPA_2 or LPA_3 in mammary epithelium develop spontaneous metastatic mammary tumors[72]. LPA_1 and/or LPA_2 receptors are overexpressed in many cancers, particularly gastric, ovarian, breast, colorectal and thyroid cancers compared to healthy tissues[99-104]. In melanoma, LPA_3 is the dominant receptor subtype, and LPA_3 overexpression in rat hepatoma cells enhances tumor growth and cell migration[7,105]. Little cancer research has focused on $LPA_{4\text{-}6}$, however, one study showed an increase in the unmethylated state of LPA_5-encoding DNA in lung and hepatic cancer cell lines compared to normal tissues[106]. KO mice for LPA_1 and LPA_5 have fewer lung metastatic nodules compared to wild-type mice following tail vein injection of cancer cells[107]. This finding suggests that LPA signaling in host tissue is important for establishing a permissible microenvironment for metastatic cancer cells to form new tumors. Targeting LPA receptors as a means of treating disease is discussed later on in this review, and more extensive reviews on LPA receptors are found elsewhere[90,108-109].

LPPs as regulators of extracellular LPA signaling

In mammals, LPPs consist of three isoforms: LPP1, LPP2 and LPP3. They share highly conserved catalytic domains and catalyze the dephosphorylation of a variety of lipid phosphates, including phosphatidate, LPA, S1P, ceramide 1-phosphate and diacylglycerol pyrophosphate[110]. LPPs are integral membrane proteins, which are localized on plasma membranes with the active site on the outer leaflet. This enables the LPPs to degrade extracellular LPA and other signaling lipids, attenuating their effects on surface receptor activation[110]. The importance of this ecto-activity has been demonstrated *in vivo* where the half-life of circulating LPA increases from 3 minutes to 12 minutes in LPP1 hypomorph mice compared to normal control littermates[111]. LPPs are also localized on the internal membranes including the endoplasmic reticulum[112] and Golgi network[113], where presumably the catalytic domains face the lumenal sides of these organelles. As such, intracellular LPPs could potentially regulate signal transduction through dephosphorylation of lipid phosphates inside cells as opposed to the degradation of extracellular LPA or S1P[110]. Evidence for this was first obtained from experiments in which increasing the expression of LPP1, LPP1a (a spice variant) and LPP2 decreased thrombin-induced ERK activation[114]. Increasing LPP1 expression also attenuated fibroblast migration in response to an $LPA_{1/2}$ agonist (wls-31), which cannot be dephosphorylated[115]. Later work with cancer cells showed that increasing LPP1 expression decreased the activation of Ca^{2+}-transients by wls-31 and a protease-activated receptor-1 peptide[116]. These effects depend on the catalytic activity of LPP1, but the lipid phosphate which is degraded by LPP1 is not yet known. Since the substrates of LPPs also exist inside cells, degradation of unidentified intracellular substrates could be one of the explanations for the intracellular functions of LPPs.

The LPPs and their role in cancer biology are emerging fields of study. Significantly, LPP1 expression is decreased in many cancers including ovarian, renal, leukemia, colorectal, melanoma and lung cancers compared to normal tissues[116]. Increasing LPP1 expression in ovarian cancer cells increases extracellular LPA hydrolysis, deceases cell proliferation and colony-forming activity, and increases apoptosis[117]. Virtually identical results in ovarian cancer cell lines have been obtained through overexpression of LPP3[118]. However, there is one report that LPP3 overexpression actually increases tumor growth in glioblastoma models by increasing β-catenin stability and cyclin D1 synthesis. LPP3 is highly expressed in human primary glioblastoma tumors[119]. Rather than targeting LPP signaling in cancer cells, Nakayama *et al.* recently examined the contribution of LPP1 expression in the tumor microenvironment to cancer cell seeding[120]. Intraperitoneal injection of syngeneic ovarian cancer cells into LPP1 knockout mice leads to enhanced cancer cell seeding compared to wildtype mice[120]. Presumably, higher systemic levels of LPA can explain this result as a consequence of decreased LPA turnover in LPP1 knockout mice compared to wildtype controls[111,120]. Other work on targeting LPP expression or investigations to its role in tumor biology is presented later in this review.

Thus far, the relationship between LPP2 expression and cancer is unclear. Work in our laboratory showed

that overexpression of LPP2 in fibroblasts causes premature entry into S-phase of the cell cycle, whereas knockdown of LPP2 delays entry by 1.5 hours[121]. Similar effects could not be obtained with either overexpression or knockdown of LPP1/3, suggesting that LPP2 might have a unique role among the LPPs in cell cycle regulation[121]. Genomic screens showed LPP2 (*PPAP2C*) to be upregulated in transformed human adult mesenchymal stem cells. In the same study, knockdown of LPP2 impaired anchorage-dependent growth of cancers cells and mesenchymal stem cells[122]. This work replicated our S-phase cell cycle findings, and further showed LPP2 to be negatively regulated by p53, a major tumor suppressor[122]. More information on the LPPs and related proteins can be obtained by reference to other reviews[10,39,110,123].

Targeting the ATX/LPA/LPP axis *in vivo*

Currently, several strategies are being investigated to target ATX/LPA signaling at multiple levels: by monoclonal antibody therapy against LPA, antagonizing LPA receptor signaling, attenuating LPA signaling by increasing LPP1 or LPP3 activities and by blocking LPA production through inhibition of ATX activity (***Fig. 1***). We will now summarize current progress in each of these areas.

LPA monocolonal antibodies

Antibody-mediated therapy is advantageous over traditional therapeutics in that it is extremely selective in its targeting and is very stable once delivered intravenously[124]. Normal immunological processes usually only clear antibodies once they bind to their targets and thus have a long bioavailability. For LPA as a target, all published work thus far comes from Lpath Inc. in San Diego. Their first work determined the X-ray crystal structure of LPA bound to the humanized monoclonal anti-LPA antibody LT3015[125]. They found that both heavy and light chain loops of the antibody create eight hydrogen bonds with the glycerophosphate headgroup of LPA[125]. Furthermore, the antibody had no binding affinity to S1P, LPC, PA or PC[125].

Blocking LPA signaling with another monoclonal antibody, called B3, improved spinal cord injury outcomes in both zebrafish and mouse models[126]. When treated with B3, there was reduced glial inflammation and neuronal cell death, leading to increased neuronal survival upstream of the injury and consequently some improvement in function[126]. B3 was later renamed Lpathomab™, and in a follow up study, treatment with Lpathomab™ reduced IL-6 expression and lesion volume, and improved functional outcomes in a mouse model of traumatic brain injury[127] (***Table 1***). The authors also quantified increases in LPA in the cerebrospinal fluid of human patients and mice with traumatic brain injuries relative to controls. This led the authors to conclude that anti-LPA antibody therapy could have neuroprotective effects following injury[127]. Lpath Inc. has begun the process of entering Lpathomab™ into clinical trials[128].

LPA receptor antagonists

Thus far, there are dozens of known LPA receptor antagonists, but few are effective *in vivo*[54]. They can be broadly classified into lipid mimetics or small molecule inhibitors. Virtually all research into LPA receptor antagonist therapy comes from studies of fibrotic models[54,108,129]. LPA accelerates lung fibrosis by differentiating mesenchymal stem cells into myofibroblasts through LPA_1 signaling in idiopathic pulmonary fibrosis (IPF). This is a disease of interstitial infiltrates in the lungs characterized by progressive shortness of breath and worsening pulmonary function[130-131]. This process is slowed in bleomycin-induced IPF mouse models with the LPA_1 antagonist AM966[132]. To date, there are at least three LPA_1 antagonists in phase I/II clinical trials for IPF[108,133-135] (***Table 1***).

Scleroderma is also a fibrotic disease characterized by hardening of the skin and is a chronic systemic autoimmune disease[136]. Significantly, C20:4-LPA levels are two-fold higher in the serum of scleroderma patients compared to healthy controls[137]. Studies with LPA_1 KO mice show protection against scleroderma[138], and phase II clinical trials of the $LPA_{1/3}$ inhibitor SAR100842 are underway for systemic sclerosis[108]. We refer the reader to other reviews that discuss LPA antagonists in greater detail[90,108-109].

Most efforts from the pharmaceutical industry have been based on the development of LPA_1-selective, LPA_2-selective, and $LPA_{1/3}$ dual antagonists, since LPA_1 and LPA_3-mediated signaling is overlapping[54]. However, to address the issue of signaling redundancy among the LPA receptors, there is a debate as to whether a more effective approach would be to develop pan-LPA receptor antagonists[54]. Ultimately, drug design approaches need to balance efficacy with safety, and a hypothetical pan-antagonist is more likely to have cross-reactivity with other unintended G-protein-coupled receptors than an antagonist designed specifically to interact with one or two receptors. Such antagonists would likely have to be delivered directly to the organ of treatment to limit otherwise increased systemic side effects

Table 1 **Overview of targeting the autotaxin-lysophosphatidate-lipid phosphate phosphatase axis *in vivo*.**

Chemical/Technique	Function	Stage	Pharmacological/ Dosing Parameters	Applications [Refs]
PF-8380	Autotaxin activity inhibitor (competitive)	Preclinical	IC_{50} 101 nmol/L 30 mg/kg	Inflammatory hyperalgesia[140] Radiotherapy sensitizer in glioblastoma[146]
ONO-8430506	Autotaxin activity inhibitor (competitive)	Preclinical	IC_{50} 5 nmol/L 10-30 mg/kg	Benign prostatic hyperplasia[147] Reduces breast tumor growth and metastasis and increases sensitization to doxorubicin[76,148]
Lpathomab™	LPA monoclonal antibody	Preclinical	25 mg/kg	Traumatic brain injury[127]
AM966	LPA_1 antagonist	Preclinical	IC_{50} 17 nmol/L 10 mg/kg	Idiopathic pulmonary fibrosis[132]
BMS-986020	LPA_1 antagonist	Phase II	600 mg/day (patients)	Idiopathic pulmonary fibrosis[133]
BMS-986202/AM152	LPA_1 antagonist	Phase I	20-40 mg/kg	Idiopathic pulmonary fibrosis[134]
SAR100842	$LPA_{1/3}$ antagonist	Phase II	20-40 mg/kg	Systemic sclerosis[135]
Gene overexpression	Induced *LPP* gene expression	Preclinical	Overexpressed in cancer cells	LPP3 overexpression reduces ovarian cancer cell growth[118] LPP1 overexpression reduces tumor growth and metastasis in breast and thyroid cancers[116]

compared to simpler oral, subcutaneous and/or intravenous administration options[54].

Increasing LPP expression as a cancer therapy

To date little is known about how to increase the low expression of LPP1 and LPP3 in cancer cells. One early study showed gonadotropin-releasing hormone increased LPP activity in ovarian cancer cells expressing the gonadotropin-releasing hormone receptor[139]. This work with cultured cancer cells was validated by measuring tumor growth from SKOV3 ovarian carcinoma cells in nude mice. Cells that over-expressed hLPP3 showed lower tumor growth[118] (***Table 1***). The authors ascribed this result to the ecto-phosphatase activities of LPP3 at a time when the effects that occur downstream of receptor activation were not widely appreciated.

In our laboratory, we showed that overexpression of LPP1 in breast and thyroid cancer cell lines decreases tumor growth and metastasis by up to 80% compared with the expression of catalytically inactive LPP1 in both syngeneic and xenograft mouse models[116] (***Table 1***). We also demonstrated that these effects on the cancer cells cannot be explained by the increased ecto-activity, which degrades exogenous LPA or S1P. Instead, the main action of LPP1 is mediated downstream of receptor activation since LPP1 overexpression also decreased the stimulation of Ca^{2+}-transients by the stable $LPA_{1/2}$ receptor agonist wls-31[116]. LPP1 expression also decreased Ca^{2+}-transients that resulted from activation by a protease-activated receptor-1 agonist peptide, which acts independently of LPA signaling. At present, we do not know what substrate LPP1 acts on intra-cellularly to modify cell signaling and slow tumor progression.

ATX inhibitors

ATX inhibitors hold great potential to treat a multitude of diseases mediated by LPA signaling, particularly cancer and other chronic inflammatory-mediated diseases[56,79]. Because LPA is rapidly turned over, plasma LPA levels fall by >95% upon treatment with a potent ATX inhibitor[140]. There are numerous small non-lipid molecules that have been discovered and modified to inhibit ATX activity through screening large libraries of compounds[141-142]. Unlike classical lipid analogs, these inhibitors tend to have better oral bioavailability due to their decreased hydrophobicity and they are unlikely to be degraded rapidly by endogenous hydrolytic pathways[143]. A more thorough history of ATX inhibitor development has been published previously[56,79,109,141,144], and here we will discuss work on ATX-specific inhibitors that have been used *in vivo*.

PF-8380, a piperazinylbenzoxazolone derivative, was developed by Pfizer from screening a compound library followed by optimization. PF-8380 has an IC_{50} of 2.8 nmol/L against recombinant human ATX and 101 nmol/L for whole human blood. It was the first reported ATX inhibitor to reduce plasma LPA levels *in vivo* for an extended period[140]. In rat air-pouch models, 30 mg/kg PF-8380 inhibited inflammatory hyperalgesia with the same efficacy as 30 mg/kg naproxen, a routinely used nonsteroidal anti-inflammatory drug

(NSAID)[140]. At this concentration, PF-8380 maximally reduced LPA levels in both plasma and at the site of inflammation. PF-8380 also had radio-sensitizing effects in heterotopic mouse models for glioblastoma multiforme, delaying tumor growth by at least 20 days[145-146]. In this study, inhibition of ATX by PF-8380 abrogated radiationinduced activation of Akt and subsequently decreased tumor vascularity and tumor growth[145] (***Table 1***).

Another potent ATX inhibitor, ONO-8430506, currently in development by Ono Pharmaceuticals Ltd. (Osaka, Japan), is a tetrahydrocarboline derivative with an IC_{50} of 5 nmol/L for plasma ATX activity[147]. Dosing rats orally with 30 mg/kg of this compound potently suppressed ATX plasma activity and LPA concentrations for 24 hours and decreased intraurethral pressure in benign prostatic hyperplasia models[147] (***Table 1***).

We demonstrated that ONO-8430506 delays initial tumor growth by about 60% in Balb/c mice with orthotopic 4T1 breast tumors for about 10 days. This growth reduction is synergic with doxorubicin and the combined therapy decreases tumor growth by >70% for about 17 days[76-148] (***Table 1***). Subsequent lung and liver metastases were also reduced by about 50%-60% with ATX inhibition[76,148]. In the same studies, we showed for first time that activation of LPA_1 receptors increases the stabilization of the transcription factor, Nrf2, and induces its nuclear translocation[148]. Nrf2 increases the expression of anti-oxidant genes (for example, NADPH-quinone oxidoreductase-1 and hemoxygenase-1), which protects cancer cells against oxidative damage caused by chemotherapy[149]. Also, LPA through Nrf2 increases the expression of the multi-drug resistant transporters, ABCC1, ABCG2, ABCC2 and ABCC3, which export toxic oxidation products and many chemotherapeutic drugs from cancer cells[148]. We confirmed these results *in vivo* by showing that ATX inhibition with ONO-8430506 decreased Nrf2 levels and the expression of anti-oxidant genes and multi-drug resistant transporters in the 4T1 model of breast cancer[148]. This explains why the doxorubicin treatment was more effective in mice that were simultaneously treated with an ATX inhibitor. These responses could also protect against cell death during chemotherapy with a variety of different agents. Our findings prompt the need for further investigations into combination therapy with other common chemotherapies like paclitaxel, as well as with radiotherapy.

Given the physiological importance of ATX to processes like smooth muscle contraction, platelet aggression and wound healing, we do not know yet if potent ATX inhibition adversely affects tissue repair and remodeling. So far, ATX inhibition studies do not show obvious side effects, but studies using existing wound healing models are warranted[150]. While ATX inhibition is particularly effective at reducing unsaturated and polyunsaturated LPA species, saturated species are decreased less since their production is probably largely mediated by PLA_2 activity[7,151]. Hence, LPA generation by minor sources like PLA_2 may be sufficient to maintain physiological processes, whereas higher concentrations of unsaturated LPA species from induced ATX expression drive pathological processes. Answers to questions like these are required to determine if ATX inhibitors (as by extension, LPA monoclonal antibodies and LPA receptor antagonists) are contra-indicated in patients with pre-existing wound healing problems, for example, in cases of diabetic neuropathy and autoimmune diseases[152].

Conclusion

Tremendous progress has been made in recent years by targeting the ATX-LPA-LPP axis and now this work is rapidly being translated into clinical trials. We are hopeful that LPA monoclonal antibodies, LPA receptor antagonists and ATX inhibitors will become viable therapeutic interventions within the next decade. With several different pharmacological options, anti-ATX/LPA therapeutics can target all the way from individual receptors with receptor antagonists to systemic voidance of LPA with antibodies and ATX inhibitors. These strategies should be applicable in cancer therapy by providing adjuvants to improve chemotherapy and radiotherapy. Blocking LPA signaling could also provide a novel treatment for several inflammatory diseases.

Acknowledgements

MGKB is the recipient of a Vanier Canada Graduate Scholarship from the Government of Canada, a Canadian Institutes of Health Research (CIHR) MD/PhD Studentship, a MD/PhD Studentship from Alberta Innovates-Health Solutions (AI-HS), and a Killam Trust Award. XT received a post-doctoral fellowship from the Canadian Breast Cancer Foundation (CBCF). GV and RTB received studentships from the Women and Children's Health Research Institute (WCHRI) at the University of Alberta, and GV from AI-HS. The work was also supported by grants from the CBCF, CIHR with WCHRI, and Ono Pharmaceuticals Ltd. The following conflicts of interest are disclosed: DNB has received consulting fees from Ono

Pharmaceuticals Ltd. The other authors declare no conflicts of interest.

References

[1] Croset M, Brossard N, Polette A, et al. Characterization of plasma unsaturated lysophosphatidylcholines in human and rat[J]. *Biochem J*, 2000, 345(Pt 1): 61-67.

[2] Wiesner P, Leidl K, Boettcher A, et al. Lipid profiling of FPLC-separated lipoprotein fractions by electrospray ionization tandem mass spectrometry[J]. *J Lipid Res*, 2009, 50(3): 574-585.

[3] Eichholtz T, Jalink K, Fahrenfort I, et al. The bioactive phospholipid lysophosphatidic acid is released from activated platelets[J]. *Biochem J*, 1993, 291 (Pt 3): 677-680.

[4] Tigyi G, Miledi R. Lysophosphatidates bound to serum albumin activate membrane currents in Xenopus oocytes and neurite retraction in PC12 pheochromocytoma cells[J]. *J Biol Chem*, 1992, 267(30): 21360-21367.

[5] Baker DL, Morrison P, Miller B, et al. Plasma lysophosphatidic acid concentration and ovarian cancer[J]. *JAMA*, 2002, 287(23): 3081-3082.

[6] Nakanaga K, Hama K, Aoki J. Autotaxin-an LPA producing enzyme with diverse functions[J]. *J Biochem*, 2010, 148(1): 13-24.

[7] Brindley DN, Lin FT, Tigyi GJ. Role of the autotaxin-lysophosphatidate axis in cancer resistance to chemotherapy and radiotherapy[J]. *Biochim Biophys Acta*, 2013, 1831(1): 74-85.

[8] Gaetano CG, Samadi N, Tomsig JL, et al. Inhibition of autotaxin production or activity blocks lysophosphatidylcholine-induced migration of human breast cancer and melanoma cells[J]. *Mol Carcinog*, 2009, 48(9): 801-809.

[9] Samadi N, Gaetano C, Goping IS, et al. Autotaxin protects MCF-7 breast cancer and MDA-MB-435 melanoma cells against Taxol-induced apoptosis[J]. *Oncogene*, 2009, 28(7): 1028-1039.

[10] Kok BP, Venkatraman G, Capatos D, et al. Unlike two peas in a pod: lipid phosphate phosphatases and phosphatidate phosphatases[J]. *Chem Rev*, 2012, 112(10): 5121-5146.

[11] Jamal Z, Martin A, Gomez-Munoz A, et al. Plasma membrane fractions from rat liver contain a phosphatidate phosphohydrolase distinct from that in the endoplasmic reticulum and cytosol[J]. *J Biol Chem*, 1991, 266(5): 2988-2996.

[12] Brindley DN, Waggoner DW. Mammalian lipid phosphate phosphohydrolases[J]. *J Biol Chem*, 1998, 273(38): 24281-24284.

[13] Jasinska R, Zhang QX, Pilquil C, et al. Lipid phosphate phosphohydrolase-1 degrades exogenous glycerolipid and sphingolipid phosphate esters[J]. *Biochem J*, 1999, 340 (Pt 3): 677-686.

[14] Bekele R, Brindley DN. Role of autotaxin and lysophosphatidate in cancer progression and resistance to chemotherapy and radiotherapy[J]. *Clin Lipidol*, 2012, 7: 313-328.

[15] Samadi N, Bekele R, Capatos D, et al. Regulation of lysophosphatidate signaling by autotaxin and lipid phosphate phosphatases with respect to tumor progression, angiogenesis, metastasis and chemo-resistance[J]. *Biochimie*, 2011, 93(1): 61-70.

[16] Panchatcharam M, Salous AK, Brandon J, et al. Mice with targeted inactivation of ppap2b in endothelial and hematopoietic cells display enhanced vascular inflammation and permeability[J]. *Arterioscler Thromb Vasc Biol*, 2014, 34(4): 837-845.

[17] Smyth SS, Mueller P, Yang F, et al. Arguing the case for the autotaxin-lysophosphatidic acid-lipid phosphate phosphatase 3-signaling nexus in the development and complications of atherosclerosis[J]. *Arterioscler Thromb Vasc Biol*, 2014, 34(3): 479-486.

[18] Akira T, Kengo H, Kenji F, et al. Involvement of lysophospholipase D in the production of lysophosphatidic acid in rat plasma[J]. *Biochim Biophys Acta*, 1986, 875(1): 31-38.

[19] Stracke ML, Krutzsch HC, Unsworth EJ, et al. Identification, purification, and partial sequence analysis of autotaxin, a novel motility-stimulating protein[J]. *J Biol Chem*, 1992, 267(4): 2524-2529.

[20] Murata J, Lee HY, Clair T, et al. cDNA cloning of the human tumor motility-stimulating protein, autotaxin, reveals a homology with phosphodiesterases[J]. *J Biol Chem*, 1994, 269(48): 30479-30484.

[21] Buckley MF, Loveland KA, McKinstry WJ, et al. Plasma cell membrane glycoprotein PC-1. cDNA cloning of the human molecule, amino acid sequence, and chromosomal location[J]. *J Biol Chem*, 1990, 265(29): 17506-17511.

[22] Funakoshi I, Kato H, Horie K, et al. Molecular cloning of cDNAs for human fibroblast nucleotide pyrophosphatase[J]. *Arch Biochem Biophys*, 1992, 295(1): 180-187.

[23] Kawagoe H, Soma O, Goji J, et al. Molecular cloning and chromosomal assignment of the human brain-type phosphodiesterase I/nucleotide pyrophosphatase gene (PDNP2)[J]. *Genomics*, 1995, 30(2): 380-384.

[24] Umezu-Goto M, Kishi Y, Taira A, et al. Autotaxin has lysophospholipase D activity leading to tumor cell growth and motility by lysophosphatidic acid production[J]. *J Cell Biol*, 2002, 158(2): 227-233.

[25] Tokumura A, Majima E, Kariya Y, et al. Identification of human plasma lysophospholipase D, a lysophosphatidic acid-producing enzyme, as autotaxin, a multifunctional phosphodiesterase[J]. *J Biol Chem*, 2002, 277(42): 39436-39442.

[26] Moolenaar WH. Lysophospholipids in the limelight: autotaxin takes center stage[J]. *J Cell Biol*, 2002, 158(2): 197-199.

[27] Hama K, Aoki J, Fukaya M, et al. Lysophosphatidic acid and autotaxin stimulate cell motility of neoplastic and non-neoplastic cells through LPA1[J]. *J Biol Chem*, 2004, 279(17): 17634-17639.

[28] van Meeteren LA, Ruurs P, Stortelers C, et al. Autotaxin, a secreted lysophospholipase D, is essential for blood vessel formation during development[J]. *Mol Cell Biol*, 2006, 26(13): 5015-5022.

[29] Koike S, Keino-Masu K, Masu M. Deficiency of autotaxin/lysophospholipase D results in head cavity formation in mouse embryos through the LPA receptor-Rho-ROCK pathway[J]. *Biochem Biophys Res Commun*, 2010, 400(1): 66-71.

[30] Koike S, Keino-Masu K, Ohto T, et al. Autotaxin/lysophospholipase D-mediated lysophosphatidic acid signaling is required to form distinctive large lysosomes in the visceral endoderm cells of the mouse yolk sac[J]. *J Biol Chem*, 2009, 284(48): 33561-33570.

[31] Koike S, Yutoh Y, Keino-Masu K, et al. Autotaxin is required for the cranial neural tube closure and establishment

of the midbrain-hindbrain boundary during mouse development[J]. *Dev Dyn*, 2011, 240(2): 413-421.

[32] Moolenaar WH, Houben AJ, Lee SJ, et al. Autotaxin in embryonic development[J]. *Biochim Biophys Acta*, 2013, 1831(1): 13-19.

[33] Tanaka M, Okudaira S, Kishi Y, et al. Autotaxin stabilizes blood vessels and is required for embryonic vasculature by producing lysophosphatidic acid[J]. *J Biol Chem*, 2006, 281(35): 25822-25830.

[34] Fotopoulou S, Oikonomou N, Grigorieva E, et al. ATX expression and LPA signalling are vital for the development of the nervous system[J]. *Dev Biol*, 2010, 339(2): 451-464.

[35] Yukiura H, Hama K, Nakanaga K, et al. Autotaxin regulates vascular development via multiple lysophosphatidic acid (LPA) receptors in zebrafish[J]. *J Biol Chem*, 2011, 286(51): 43972-43983.

[36] Yuelling LW, Waggener CT, Afshari FS, et al. Autotaxin/ENPP2 regulates oligodendrocyte differentiation in vivo in the developing zebrafish hindbrain[J]. *Glia*, 2012, 60(10): 1605-1618.

[37] Lai SL, Yao WL, Tsao KC, et al. Autotaxin/Lpar3 signaling regulates Kupffer's vesicle formation and left-right asymmetry in zebrafish[J]. *Development*, 2012, 139(23): 4439-4448.

[38] Cheng HY, Dong A, Panchatcharam M, et al. Lysophosphatidic acid signaling protects pulmonary vasculature from hypoxia-induced remodeling[J]. *Arterioscler Thromb Vasc Biol*, 2012, 32(1): 24-32.

[39] Brindley DN. Lipid phosphate phosphatases and related proteins: signaling functions in development, cell division, and cancer[J]. *J Cell Biochem*, 2004, 92(5): 900-912.

[40] Mazereeuw-Hautier J, Gres S, Fanguin M, et al. Production of lysophosphatidic acid in blister fluid: involvement of a lysophospholipase D activity[J]. *J Invest Dermatol*, 2005, 125(3): 421-427.

[41] Tokumura A, Taira S, Kikuchi M, et al. Lysophospholipids and lysophospholipase D in rabbit aqueous humor following corneal injury[J]. *Prostaglandins Other Lipid Mediat*, 2012, 97(3-4): 83-89.

[42] Grisanti L, Rezza A, Clavel C, et al. Enpp2/Autotaxin in dermal papilla precursors is dispensable for hair follicle morphogenesis[J]. *J Invest Dermatol*, 2013, 133(10): 2332-2339.

[43] Mebarek S, Abousalham A, Magne D, et al. Phospholipases of mineralization competent cells and matrix vesicles: roles in physiological and pathological mineralizations[J]. *Int J Mol Sci*, 2013, 14(3): 5036-5129.

[44] Evseenko D, Latour B, Richardson W, et al. Lysophosphatidic acid mediates myeloid differentiation within the human bone marrow microenvironment[J]. *PLoS One*, 2013, 8(5): e63718.

[45] Masuda K, Haruta S, Orino K, et al. Autotaxin as a novel, tissue-remodeling-related factor in regressing corpora lutea of cycling rats[J]. *FEBS J*, 2013, 280: 6600-6612.

[46] Awada R, Rondeau P, Gres S, et al. Autotaxin protects microglial cells against oxidative stress[J]. *Free Radic Biol Med*, 2012, 52(2): 516-526.

[47] Awada R, Saulnier-Blache JS, Gres S, et al. Autotaxin downregulates LPS-induced microglia activation and pro-inflammatory cytokines production[J]. *J Cell Biochem*, 2014, 115(12): 2123-2132.

[48] Umemoto E, Hayasaka H, Bai Z, et al. Novel regulators of lymphocyte trafficking across high endothelial venules[J]. *Crit Rev Immunol*, 2011, 31(2): 147-169.

[49] Bai Z, Cai L, Umemoto E, et al. Constitutive lymphocyte transmigration across the basal lamina of high endothelial venules is regulated by the autotaxin/lysophosphatidic acid axis[J]. *J Immunol*, 2013, 190(5): 2036-2048.

[50] Zhang Y, Chen YC, Krummel MF, et al. Autotaxin through lysophosphatidic acid stimulates polarization, motility, and transendothelial migration of naive T cells[J]. *J Immunol*, 2012, 189(8): 3914-3924.

[51] Knowlden S, Georas SN. The autotaxin-LPA axis emerges as a novel regulator of lymphocyte homing and inflammation[J]. *J Immunol*, 2014, 192(3): 851-857.

[52] Nakasaki T, Tanaka T, Okudaira S, et al. Involvement of the lysophosphatidic acid-generating enzyme autotaxin in lymphocyte-endothelial cell interactions[J]. *Am J Pathol*, 2008, 173(5): 1566-1576.

[53] van Meeteren LA, Moolenaar WH. Regulation and biological activities of the autotaxin-LPA axis[J]. *Prog Lipid Res*, 2007, 46(2): 145-160.

[54] Budd DC, Qian Y. Development of lysophosphatidic acid pathway modulators as therapies for fibrosis[J]. *Future Med Chem*, 2013, 5(16): 1935-1952.

[55] Benesch MGK, Zhao YY, Curtis JM, et al. Regulation of autotaxin expression by lysophosphatidate and sphingosine 1-phosphate[J]. *J Lipid Res*, 2015: doi:10.1194/jlr.M057661.

[56] Benesch MGK, Ko YM, McMullen TPW, et al. Autotaxin in the crosshairs: Taking aim at cancer and other inflammatory conditions[J]. *FEBS Lett*, 2014,588(16): 2712-2727.

[57] Bollen M, Gijsbers R, Ceulemans H, et al. Nucleotide pyrophosphatases/phosphodiesterases on the move[J]. *Crit Rev Biochem Mol Biol*, 2000, 35(6): 393-432.

[58] Debies MT, Welch DR. Genetic basis of human breast cancer metastasis[J]. *J Mammary Gland Biol Neoplasia*, 2001, 6(4): 441-451.

[59] Moolenaar WH, van Meeteren LA, Giepmans BN. The ins and outs of lysophosphatidic acid signaling[J]. *Bioessays*, 2004, 26(8): 870-881.

[60] Gendaszewska-Darmach E. Lysophosphatidic acids, cyclic phosphatidic acids and autotaxin as promising targets in therapies of cancer and other diseases[J]. *Acta Biochim Pol*, 2008, 55(2): 227-240.

[61] Liu S, Murph M, Panupinthu N, et al. ATX-LPA receptor axis in inflammation and cancer[J]. *Cell Cycle*, 2009, 8(22): 3695-3701.

[62] Panupinthu N, Lee HY, Mills GB. Lysophosphatidic acid production and action: critical new players in breast cancer initiation and progression[J]. *Br J Cancer*, 2010, 102(6): 941-946.

[63] Houben AJ, Moolenaar WH. Autotaxin and LPA receptor signaling in cancer[J]. *Cancer Metastasis Rev*, 2011, 30 (3-4): 557-565.

[64] Gotoh M, Fujiwara Y, Yue J, et al. Controlling cancer through the autotaxin-lysophosphatidic acid receptor axis[J]. *Biochem Soc Trans*, 2012, 40(1): 31-36.

[65] Peyruchaud O, Leblanc R, David M. Pleiotropic activity of lysophosphatidic acid in bone metastasis[J]. *Biochim Biophys Acta*, 2013, 1831(1): 99-104.

[66] So J, Wang FQ, Navari J, et al. LPA-induced epithelial ovarian cancer (EOC) in vitro invasion and migration

are mediated by VEGF receptor-2 (VEGF-R2)[J]. *Gynecol Oncol*, 2005, 97(3): 870-878.

[67] Murph MM, Hurst-Kennedy J, Newton V, et al. Lysophosphatidic acid decreases the nuclear localization and cellular abundance of the p53 tumor suppressor in A549 lung carcinoma cells[J]. *Mol Cancer Res*, 2007, 5(11): 1201-1211.

[68] Samadi N, Bekele RT, Goping IS, et al. Lysophosphatidate induces chemo-resistance by releasing breast cancer cells from taxol-induced mitotic arrest[J]. *PLoS One*, 2011, 6(5): e20608.

[69] Zhang R, Wang J, Ma S, et al. Requirement of Osteopontin in migration and the protection against Taxol-induced apoptosis via ATX-LPA axis in SGC7901 cells[J]. *BMC Cell Biol*, 2011, 12(1): 11.

[70] Vidot S, Witham J, Agarwal R, et al. Autotaxin delays apoptosis induced by carboplatin in ovarian cancer cells[J]. *Cell Signal*, 2010, 22(6): 926-935.

[71] Deng W, Shuyu E, Tsukahara R, et al. The lysophosphatidic acid type 2 receptor is required for protection against radiation-induced intestinal injury[J]. *Gastroenterology*, 2007, 132(5): 1834-1851.

[72] Liu S, Umezu-Goto M, Murph M, et al. Expression of autotaxin and lysophosphatidic acid receptors increases mammary tumorigenesis, invasion, and metastases[J]. *Cancer Cell*, 2009, 15(6): 539-550.

[73] Euer N, Schwirzke M, Evtimova V, et al. Identification of genes associated with metastasis of mammary carcinoma in metastatic versus non-metastatic cell lines[J]. *Anticancer Res*, 2002, 22(2A): 733-740.

[74] St-Coeur PD, Ferguson D, Morin P, Jr., et al PF-8380 and closely related analogs: synthesis and structure-activity relationship towards autotaxin inhibition and glioma cell viability[J]. *Arch Pharm*, 2013, 346(2): 91-97.

[75] Popnikolov NK, Dalwadi BH, Thomas JD, et al. Association of autotaxin and lysophosphatidic acid receptor 3 with aggressiveness of human breast carcinoma[J]. *Tumour Biol*, 2012, 33(6): 2237-2243.

[76] Benesch MGK, Tang X, Maeda T, et al. Inhibition of autotaxin delays breast tumor growth and lung metastasis in mice[J]. *FASEB J*, 2014, 28(6): 2655-2666.

[77] Leblanc R, Lee SC, David M, et al. Interaction of platelet-derived autotaxin with tumor integrin alphaVbeta3 controls metastasis of breast cancer cells to bone[J]. *Blood*, 2014, 124(20): 3141-3150.

[78] Brindley DN, Benesch MGK, Murph MM. Autotaxin - An Enzymatic Augmenter of Malignant Progression Linked to Inflammation. *Melanoma - Current Clinical Management and Future Therapeutics*: InTech Open. 2015: 297-324. doi:210.5772/59013.

[79] Barbayianni E, Kaffe E, Aidinis V, et al. Autotaxin, a secreted lysophospholipase D, as a promising therapeutic target in chronic inflammation and cancer[J]. *Prog Lipid Res*, 2015, 58: 76-96.

[80] Chun J, Hla T, Lynch KR, et al. International Union of Basic and Clinical Pharmacology. LXXVIII. Lysophospholipid receptor nomenclature[J]. *Pharmacol Rev*, 2010, 62(4): 579-587.

[81] Hecht JH, Weiner JA, Post SR, et al. Ventricular zone gene-1 (vzg-1) encodes a lysophosphatidic acid receptor expressed in neurogenic regions of the developing cerebral cortex[J]. *J Cell Biol*, 1996, 135(4): 1071-1083.

[82] An S, Bleu T, Huang W, et al. Identification of cDNAs encoding two G protein-coupled receptors for lysosphingolipids[J]. *FEBS Lett*, 1997, 417(3): 279-282.

[83] An S, Bleu T, Hallmark OG, et al. Characterization of a novel subtype of human G protein-coupled receptor for lysophosphatidic acid[J]. *J Biol Chem*, 1998, 273(14): 7906-7910.

[84] Bandoh K, Aoki J, Hosono H, et al. Molecular cloning and characterization of a novel human G-protein-coupled receptor, EDG7, for lysophosphatidic acid[J]. *J Biol Chem*, 1999, 274(39): 27776-27785.

[85] Ye X, Skinner MK, Kennedy G, et al. Age-Dependent Loss of Sperm Production in Mice via Impaired Lysophosphatidic Acid Signaling[J]. *Biol Reprod*, 2008, 79(2): 328-336.

[86] Contos JJA, Ishii I, Fukushima N, et al. Characterization of lpa2 (Edg4) and lpa1/lpa2 (Edg2/Edg4) Lysophosphatidic Acid Receptor Knockout Mice: Signaling Deficits without Obvious Phenotypic Abnormality Attributable to lpa2[J]. *Mol Cell Biol*, 2002, 22(19): 6921-6929.

[87] Contos JJ, Fukushima N, Weiner JA, et al. Requirement for the lpA1 lysophosphatidic acid receptor gene in normal suckling behavior[J]. *Proc Natl Acad Sci U S A*, 2000, 97(24): 13384-13389.

[88] Ye X, Hama K, Contos JJ, et al. LPA3-mediated lysophosphatidic acid signalling in embryo implantation and spacing[J]. *Nature*, 2005, 435(7038): 104-108.

[89] Contos JJ, Ishii I, Fukushima N, et al. Characterization of lpa(2) (Edg4) and lpa(1)/lpa(2) (Edg2/Edg4) lysophosphatidic acid receptor knockout mice: signaling deficits without obvious phenotypic abnormality attributable to lpa(2)[J]. *Mol Cell Biol*, 2002, 22(19): 6921-6929.

[90] Yung YC, Stoddard NC, Chun J. LPA receptor signaling: pharmacology, physiology, and pathophysiology[J]. *J Lipid Res*, 2014, 55(7): 1192-1214.

[91] Ohuchi H, Hamada A, Matsuda H, et al. Expression patterns of the lysophospholipid receptor genes during mouse early development[J]. *Dev Dyn*, 2008, 237(11): 3280-3294.

[92] Choi JW, Herr DR, Noguchi K, et al. LPA receptors: subtypes and biological actions[J]. *Annu Rev Pharmacol Toxicol*, 2010, 50: 157-186.

[93] Meyer zu Heringdorf D, Jakobs KH. Lysophospholipid receptors: signalling, pharmacology and regulation by lysophospholipid metabolism[J]. *Biochim Biophys Acta*, 2007, 1768(4): 923-940.

[94] Lundequist A, Boyce JA. LPA5 is abundantly expressed by human mast cells and important for lysophosphatidic acid induced MIP-1beta release[J]. *PLoS One*, 2011, 6(3): e18192.

[95] Lee Z, Cheng CT, Zhang H, et al. Role of LPA4/p2y9/GPR23 in negative regulation of cell motility[J]. *Mol Biol Cell*, 2008, 19(12): 5435-5445.

[96] Lin ME, Rivera RR, Chun J. Targeted deletion of LPA5 identifies novel roles for lysophosphatidic acid signaling in development of neuropathic pain[J]. *J Biol Chem*, 2012, 287(21): 17608-17617.

[97] Pasternack SM, von Kugelgen I, Al Aboud K, et al. G protein-coupled receptor P2Y5 and its ligand LPA are involved in maintenance of human hair growth[J]. *Nat Genet*, 2008, 40(3): 329-334.

[98] Lee M, Choi S, Hallden G, et al. P2Y5 is a G(alpha)i, G(alpha)12/13 G protein-coupled receptor activated

by lysophosphatidic acid that reduces intestinal cell adhesion[J]. *Am J Physiol Gastrointest Liver Physiol*, 2009, 297(4): G641-654.

[99] Furui T, LaPushin R, Mao M, et al. Overexpression of edg-2/vzg-1 induces apoptosis and anoikis in ovarian cancer cells in a lysophosphatidic acid-independent manner[J]. *Clin Cancer Res*, 1999, 5(12): 4308-4318.

[100] Pustilnik TB, Estrella V, Wiener JR, et al. Lysophosphatidic acid induces urokinase secretion by ovarian cancer cells[J]. *Clin Cancer Res*, 1999, 5(11): 3704-3710.

[101] Shida D, Kitayama J, Yamaguchi H, et al. Lysophosphatidic acid (LPA) enhances the metastatic potential of human colon carcinoma DLD1 cells through LPA1[J]. *Cancer Res*, 2003, 63(7): 1706-1711.

[102] Schulte KM, Beyer A, Kohrer K, et al. Lysophosphatidic acid, a novel lipid growth factor for human thyroid cells: over-expression of the high-affinity receptor edg4 in differentiated thyroid cancer[J]. *Int J Cancer*, 2001, 92(2): 249-256.

[103] Kitayama J, Shida D, Sako A, et al. Over-expression of lysophosphatidic acid receptor-2 in human invasive ductal carcinoma[J]. *Breast Cancer Res*, 2004, 6(6): R640-646.

[104] Shida D, Watanabe T, Aoki J, et al. Aberrant expression of lysophosphatidic acid (LPA) receptors in human colorectal cancer[J]. *Lab Invest*, 2004, 84(10): 1352-1362.

[105] Okabe K, Hayashi M, Kato K, et al. Lysophosphatidic acid receptor-3 increases tumorigenicity and aggressiveness of rat hepatoma RH7777 cells[J]. *Mol Carcinog*, 2013, 52(4): 247-254.

[106] Okabe K, Hayashi M, Yamawaki Y, et al. Possible involvement of lysophosphatidic acid receptor-5 gene in the acquisition of growth advantage of rat tumor cells[J]. *Mol Carcinog*, 2011, 50(8): 635-642.

[107] Lee SC, Fujiwara Y, Liu J, et al. Autotaxin and LPA1 and LPA5 Receptors Exert Disparate Functions in Tumor Cells versus the Host Tissue Microenvironment in Melanoma Invasion and Metastasis[J]. *Mol Cancer Res*, 2015, 13(1): 174-185.

[108] Kihara Y, Mizuno H, Chun J. Lysophospholipid receptors in drug discovery[J]. *Exp Cell Res*, 2014: doi:10.1016/j.yexcr.2014.1011.1020.

[109] Stoddard NC, Chun J. Promising pharmacological directions in the world of lysophosphatidic Acid signaling[J]. *Biomol Ther (Seoul)*, 2015, 23(1): 1-11.

[110] Tang X, Benesch MG, Brindley DN. Lipid phosphate phosphatases and their roles in mammalian physiology and pathology[J]. *J Lipid Res*, 2015: doi:10.1194/jlr.R058362.

[111] Tomsig JL, Snyder AH, Berdyshev EV, et al. Lipid phosphate phosphohydrolase type 1 (LPP1) degrades extracellular lysophosphatidic acid in vivo[J]. *Biochem J*, 2009, 419(3): 611-618.

[112] Barila D, Plateroti M, Nobili F, et al. The Dri 42 gene, whose expression is up-regulated during epithelial differentiation, encodes a novel endoplasmic reticulum resident transmembrane protein[J]. *J Biol Chem*, 1996, 271(47): 29928-29936.

[113] Kai M, Wada I, Imai S, et al. Cloning and characterization of two human isozymes of Mg2+-independent phosphatidic acid phosphatase[J]. *J Biol Chem*, 1997, 272(39): 24572-24578.

[114] Pyne S, Kong KC, Darroch PI. Lysophosphatidic acid and sphingosine 1-phosphate biology: the role of lipid phosphate phosphatases[J]. *Semin Cell Dev Biol*, 2004, 15(5): 491-501.

[115] Pilquil C, Dewald J, Cherney A, et al. Lipid phosphate phosphatase-1 regulates lysophosphatidate-induced fibroblast migration by controlling phospholipase D2-dependent phosphatidate generation[J]. *J Biol Chem*, 2006, 281(50): 38418-38429.

[116] Tang X, Benesch MG, Dewald J, et al. Lipid phosphate phosphatase-1 expression in cancer cells attenuates tumor growth and metastasis in mice[J]. *J Lipid Res*, 2014, 55(11): 2389-2400.

[117] Tanyi JL, Hasegawa Y, Lapushin R, et al. Role of decreased levels of lipid phosphate phosphatase-1 in accumulation of lysophosphatidic acid in ovarian cancer[J]. *Clin Cancer Res*, 2003, 9(10 Pt 1): 3534-3545.

[118] Tanyi JL, Morris AJ, Wolf JK, et al. The human lipid phosphate phosphatase-3 decreases the growth, survival, and tumorigenesis of ovarian cancer cells: validation of the lysophosphatidic acid signaling cascade as a target for therapy in ovarian cancer[J]. *Cancer Res*, 2003, 63(5): 1073-1082.

[119] Chatterjee I, Humtsoe JO, Kohler EE, et al. Lipid phosphate phosphatase-3 regulates tumor growth via beta-catenin and CYCLIN-D1 signaling[J]. *Mol Cancer*, 2011, 10(1): 51.

[120] Nakayama J, Raines TA, Lynch KR, et al. Decreased peritoneal ovarian cancer growth in mice lacking expression of lipid phosphate phosphohydrolase 1[J]. *PLoS One*, 2015, 10(3): e0120071.

[121] Morris KE, Schang LM, Brindley DN. Lipid phosphate phosphatase-2 activity regulates S-phase entry of the cell cycle in Rat2 fibroblasts[J]. *J Biol Chem*, 2006, 281(14): 9297-9306.

[122] Flanagan JM, Funes JM, Henderson S, et al. Genomics screen in transformed stem cells reveals RNASEH2A, PPAP2C, and ADARB1 as putative anticancer drug targets[J]. *Mol Cancer Ther*, 2009, 8(1): 249-260.

[123] Brindley DN, Pilquil C. Lipid phosphate phosphatases and signaling[J]. *J Lipid Res*, 2009, 50 Suppl: S225-230.

[124] Beck A, Wurch T, Bailly C, et al. Strategies and challenges for the next generation of therapeutic antibodies[J]. *Nat Rev Immunol*, 2010, 10(5): 345-352.

[125] Fleming JK, Wojciak JM, Campbell M-A, et al. Biochemical and Structural Characterization of Lysophosphatidic Acid Binding by a Humanized Monoclonal Antibody[J]. *J Mol Biol*, 2011, 408(3): 462-476.

[126] Goldshmit Y, Matteo R, Sztal T, et al. Blockage of Lysophosphatidic Acid Signaling Improves Spinal Cord Injury Outcomes[J]. *The American Journal of Pathology*, 2012, 181(3): 978-992.

[127] Crack PJ, Zhang M, Morganti-Kossmann MC, et al. Anti-lysophosphatidic acid antibodies improve traumatic brain injury outcomes[J]. *J Neuroinflammation*, 2014, 11: 37.

[128] ClinicalTrials.gov. A Phase 1a, Double-Blind, Placebo-Controlled, Single Ascending Dose Study to Evaluate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of Lpathomab in Subjects With Neuropathic Pain[J] 2015, https://clinicaltrials.gov/show/NCT02341508.

[129] Rancoule C, Pradere JP, Gonzalez J, et al. Lysophosphatidic acid-1-receptor targeting agents for fibrosis[J]. *Expert Opin Investig Drugs*, 2011, 20(5): 657-667.

[130] Gross TJ, Hunninghake GW. Idiopathic Pulmonary Fibrosis[J]. *N Engl J Med*, 2001, 345(7): 517-525.

[131] Tang N, Zhao Y, Feng R, et al. Lysophosphatidic acid accelerates lung fibrosis by inducing differentiation of mesenchymal stem cells into myofibroblasts[J]. *J Cell Mol Med*, 2014, 18(1): 156-169.

[132] Swaney JS, Chapman C, Correa LD, et al. A novel, orally active LPA(1) receptor antagonist inhibits lung fibrosis in the mouse bleomycin model[J]. *Br J Pharmacol*, 2010, 160(7): 1699-1713.

[133] ClinicalTrials.gov. Safety and Efficacy of a Lysophosphatidic Acid Receptor Antagonist in Idiopathic Pulmonary Fibrosis[J] 2015, https://clinicaltrials.gov/ct2/show/NCT01766817.

[134] Bradford WZ. Pirfenidone and anti-fibrotic therapy in selected patients [J], 2012: Patent (WO2012162592).

[135] ClinicalTrials.gov. Proof of Biological Activity of SAR100842 in Systemic Sclerosis[J] 2014, https://clinicaltrials.gov/ct2/show/NCT01651143.

[136] Pattanaik D, Postlethwaite AE. A role for lysophosphatidic acid and sphingosine 1-phosphate in the pathogenesis of systemic sclerosis[J]. *Discov Med*, 2010, 10(51): 161-167.

[137] Tokumura A, Carbone LD, Yoshioka Y, et al. Elevated serum levels of arachidonoyl-lysophosphatidic acid and sphingosine 1-phosphate in systemic sclerosis[J]. *Int J Med Sci*, 2009, 6(4): 168-176.

[138] Castelino FV, Seiders J, Bain G, et al. Amelioration of dermal fibrosis by genetic deletion or pharmacologic antagonism of lysophosphatidic acid receptor 1 in a mouse model of scleroderma[J]. *Arthritis Rheum*, 2011, 63(5): 1405-1415.

[139] Imai A, Furui T, Tamaya T, et al. A gonadotropin-releasing hormone-responsive phosphatase hydrolyses lysophosphatidic acid within the plasma membrane of ovarian cancer cells[J]. *J Clin Endocrinol Metab*, 2000, 85(9): 3370-3375.

[140] Gierse J, Thorarensen A, Beltey K, et al. A novel autotaxin inhibitor reduces lysophosphatidic acid levels in plasma and the site of inflammation[J]. *J Pharmacol Exp Ther*, 2010, 334(1): 310-317.

[141] Barbayianni E, Magrioti V, Moutevelis-Minakakis P, et al. Autotaxin inhibitors: a patent review[J]. *Expert Opin Ther Pat*, 2013, 23(9): 1123-1132.

[142] Albers HM, Ovaa H. Chemical evolution of autotaxin inhibitors[J]. *Chem Rev*, 2012, 112(5): 2593-2603.

[143] North EJ, Howard AL, Wanjala IW, et al. Pharmacophore development and application toward the identification of novel, small-molecule autotaxin inhibitors[J]. *J Med Chem*, 2010, 53(8): 3095-3105.

[144] Teo K, Brunton VG. The role and therapeutic potential of the autotaxin-lysophosphatidate signalling axis in breast cancer[J]. *Biochem J*, 2014, 463(1): 157-165.

[145] Bhave SR, Dadey DY, Karvas RM, et al. Autotaxin Inhibition with PF-8380 Enhances the Radiosensitivity of Human and Murine Glioblastoma Cell Lines[J]. *Front Oncol*, 2013, 3(236): 00236.

[146] Schleicher SM, Thotala DK, Linkous AG, et al. Autotaxin and LPA receptors represent potential molecular targets for the radiosensitization of murine glioma through effects on tumor vasculature[J]. *PLoS One*, 2011, 6(7): e22182.

[147] Saga H, Ohhata A, Hayashi A, et al. A novel highly potent autotaxin/ENPP2 inhibitor produces prolonged decreases in plasma lysophosphatidic acid formation in vivo and regulates urethral tension[J]. *PLoS One*, 2014, 9(4): e93230.

[148] Venkatraman G, Benesch MG, Tang X, et al. Lysophosphatidate signaling stabilizes Nrf2 and increases the expression of genes involved in drug resistance and oxidative stress responses: implications for cancer treatment[J]. *FASEB J*, 2015, 29(3): 772-785.

[149] Wang XJ, Sun Z, Villeneuve NF, et al. Nrf2 enhances resistance of cancer cells to chemotherapeutic drugs, the dark side of Nrf2[J]. *Carcinogenesis*, 2008, 29(6): 1235-1243.

[150] Dunn L, Prosser HC, Tan JT, et al. Murine model of wound healing[J]. *J Vis Exp*, 2013(75): e50265.

[151] Ma L, Uchida H, Nagai J, et al. Evidence for de novo synthesis of lysophosphatidic acid in the spinal cord through phospholipase A2 and autotaxin in nerve injury-induced neuropathic pain[J]. *J Pharmacol Exp Ther*, 2010, 333(2): 540-546.

[152] Thomas Hess C. Checklist for Factors Affecting Wound Healing[J]. *Advances in Skin & Wound Care*, 2011, 24(4): 192 110.1097/1001.ASW.0000396300.0000304173.ec.