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

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Abstract
Extracellular lysophosphatidate (LPA) is a potent bioactive lipid that signals through at least 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...
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 LPA 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.

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 LPP1/3.

| Physiology                          | Pathology                        |
|-------------------------------------|----------------------------------|
| Wound Healing                       | Tumor Growth and Metastasis      |
| Tissue Remodeling                   | Angiogenesis                     |
| Neural Crest Folding                | Cancer Therapy Resistance        |
| Blood Vessel Development            | Fibrosis                         |
|                                     | Chronic Inflammation             |
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[23]. 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 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, abnormalities are abrogated by exogenous addition of LPA[32]. The role of ATX in vascular and neural development has also been confirmed in zebrafish[32,33]. ATX regulates oligodendrocyte differentiation in the developing zebrafish hindbrain[34] and the correct left-right asymmetry for normal organ morphogenesis through Wnt-dependent pathways[35]. ENPP2-/- mice are viable, and express half the levels of both ATX and LPA compared to normal mice[36]. 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 H2O2, an effect which is partially reversed in the presence of the mixed LPA1,3 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[60].

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[60], a process necessary for tumor progression. LPA decreases the expression of the tumor suppressor p53[67,73], 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[60] 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 up-regulated 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 LPA receptors and LPA in stromal cells and LPA receptor diversity and signaling

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 Ga subunit from the Gβγ subunits by exchange of a guanosine diphosphate (GDP) for a guanosine triphosphate (GTP). These subunits, particularly the Ga 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_2_ deletion leads to delayed implantation of embryos and impaired embryo spacing[87-89]. No obvious phenotypic defect has been found in LPA_2_ KO mice[90]. 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_ and LPA_5_ belong to the P2Y purinergic family of receptors and have specific tissue distributions unlike LPA_1-3_. LPA_4/GPR23/p3y9 is found in the embryonically developing brain and in adults largely in the ovaries[91-92]. LPA_4/GPR92 is highly expressed in the spleen, small intestine and dorsal root ganglion cells[93]. In mast cells, LPA_3_ 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_{1-5} 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[99].

Overall, LPA signaling through at least six known G-protein-coupled receptors (LPA_{1-6}) 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[102]. Like for ATX, mice that overexpress LPA_{1}, LPA_{3} or LPA_{5} in mammary epithelium develop spontaneous metastatic mammary tumors[72]. LPA_{1} and/or LPA_{3} 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_{1} overexpression in rat hepatoma cells enhances tumor growth and cell migration[7,105]. Little cancer research has focused on LPA_{4-6}, however, one study showed an increase in the unmethylated state of LPA_{3}-encoding DNA in lung and hepatic cancer cell lines compared to normal tissues[106]. KO mice for LPA_{1} and LPA_{3} 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 permissive 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 splice variant) and LPP2 decreased thrombin-induced ERK activation[114]. Increasing LPP1 expression also attenuated fibroblast migration in response to an LPA_{12} 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[105]. Increasing LPP1 expression in ovarian cancer cells increases extracellular LPA hydrolysis, decreases 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]. Intrapерitoneal 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\cite{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\cite{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\cite{122}. This work replicated our S-phase cell cycle findings, and further showed LPP2 to be negatively regulated by p53, a major tumor suppressor\cite{122}. More information on the LPPs and related proteins can be obtained by reference to other reviews\cite{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 monoclonal 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\cite{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\cite{125}. They found that both heavy and light chain loops of the antibody create eight hydrogen bonds with the glycerophosphate headgroup of LPA\cite{125}. Furthermore, the antibody had no binding affinity to S1P, LPC, PA or PC\cite{125}.

Blocking LPA signaling with another monoclonal antibody, called B3, improved spinal cord injury outcomes in both zebrafish and mouse models\cite{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\cite{129}. B3 was later renamed Lpathomab\textsuperscript{TM}, and in a follow up study, treatment with Lpathomab\textsuperscript{TM} reduced IL-6 expression and lesion volume, and improved functional outcomes in a mouse model of traumatic brain injury\cite{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\cite{127}. Lpath Inc. has begun the process of entering Lpathomab\textsuperscript{TM} into clinical trials\cite{128}.

**LPA receptor antagonists**

Thus far, there are dozens of known LPA receptor antagonists, but few are effective in vivo\cite{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\cite{54,108,129}. LPA accelerates lung fibrosis by differentiating mesenchymal stem cells into myofibroblasts through LPA\textsubscript{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\cite{130-131}. This process is slowed in bleomycin-induced IPF mouse models with the LPA\textsubscript{1} antagonist AM966\cite{122}. To date, there are at least three LPA\textsubscript{1} antagonists in phase I/II clinical trials for IPF\cite{108,133-135} (Table 1).

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

Most efforts from the pharmaceutical industry have been based on the development of LPA\textsubscript{1}-selective, LPA\textsubscript{2}-selective, and LPA\textsubscript{1/3} dual antagonists, since LPA\textsubscript{1} and LPA\textsubscript{3}-mediated signaling is overlapping\cite{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\cite{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.
comparing 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). 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.

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 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 LPA1 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 piperezinylbenzoxazolone 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.
of breast cancer\textsuperscript{[148]}. This explains why the doxorubicin and multi-drug resistant transporters in the 4T1 model Nrf2 levels and the expression of anti-oxidant genes that ATX inhibition with ONO-8430506 decreased tor, Nrf2, and induces its nuclear translocation\textsuperscript{[148]}. Dosing rats orally with 30 mg/kg of this compound potently suppressed ATX plasma activity and ATX concentrations for 24 hours and decreased intraurethral pressure in benign prostatic hyperplasia models\textsuperscript{[147]} (\textbf{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\textsuperscript{[76-148]} (\textbf{Table 1}). Subsequent lung and liver metastases were also reduced by about 50%-60\% with ATX inhibition\textsuperscript{[76,148]}. In the same studies, we showed for first time that activation of LPA\textsubscript{1} receptors increases the stabilization of the transcription factor, Nrf2, and induces its nuclear translocation\textsuperscript{[148]}. Nrf2 increases the expression of anti-oxidant genes (for example, NADPH-quinone oxidoreductase-1 and hemoxxygenase-1), which protects cancer cells against oxidative damage caused by chemotherapy\textsuperscript{[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\textsuperscript{[148]}. We confirmed these results \textit{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\textsuperscript{[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\textsuperscript{[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\textsubscript{2} activity\textsuperscript{[151]}. Hence, LPA generation by minor sources like PLA\textsubscript{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\textsuperscript{[152]}.\textbf{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.

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