Lipid phosphate phosphatases and their roles in mammalian physiology and pathology

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Abstract  Lipid phosphate phosphatases (LPPs) are a group of enzymes that belong to a phosphatase/phosphotransferase family. Mammalian 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, lysophosphatidate (LPA), sphingosine 1-phosphate (S1P), ceramide 1-phosphate, and diacylglycerol pyrophosphate. 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 S1P, thereby attenuating their effects on the activation of surface receptors. LPP3 also exhibits noncatalytic effects at the cell surface. LPP expression on internal membranes, such as endoplasmic reticulum and Golgi, facilitates the metabolism of internal lipid phosphates, presumably on the luminal surface of these organelles. This action probably explains the signaling effects of the LPPs, which occur downstream of receptor activation. The three isoforms of LPPs show distinct and nonredundant effects in several physiological and pathological processes including embryo development, vascular function, and tumor progression. This review is intended to present an up-to-date understanding of the physiological and pathological consequences of changing the expression levels of the different LPPs, especially in relation to cell signaling by LPA and S1P.

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Mammalian lipid phosphate phosphatases (LPPs) were first characterized as Mg$^{2+}$-independent and N-ethylmaleimide-insensitive phosphatidate phosphatases (PAPs) in 1991 (1). These enzymes were called type 2 PAPs (PAP2s) at this time to distinguish them from the type 1 Mg$^{2+}$-dependent PAPs (PAP1s). PAP1 enzymes exist in the cytosol of cells and they translocate to the endoplasmic reticulum where they produce diacylglycerol (DG) for glycerolipid synthesis (2). It was not until 2006 that the structure of the PAP1 enzymes were identified in yeast and were shown to belong to a family of enzymes called lipins (3). All three mammalian lipins have PAP1 activity and are specific for phosphatidate (PA) as a substrate (4).

By contrast, PAP2 enzymes dephosphorylate a wide variety of lipid phosphates including lysophosphatidate (LPA), sphingosine 1-phosphate (SIP), ceramide 1-phosphate (CIP) (5), DG pyrophosphate (6), and N-oleoylthanolamine phosphate (7). Because of this broad specificity, the PAP2 enzymes were renamed as LPPs (8). cDNA was cloned for mammalian LPP1 in 1996 (9) and LPP3 was identified a year later (10). The identities of genes that encode LPP1 and DG pyrophosphate phosphatase (DPP)1 in Saccharomyces cerevisiae were identified in 1998 (11, 12).

Mammalian LPPs consist of three related proteins named LPP1 (and LPP1a, a splice variant), LPP2, and LPP3, which are encoded by three separate genes PPAP2A, PPAP2C, and PPAP2B, respectively. These enzymes belong to a family of phosphatases and phosphotransferases based on the conserved catalytic domains in their structure (13–17)

Abbreviations: ATX, autotaxin; CIP, ceramide 1-phosphate; DG, diacylglycerol; DPP, diacylglycerol pyrophosphate phosphatase; LPA, lysophosphatidic acid, which at physiological pH is in a salt form, i.e., lysophosphatidate; LPC, lysophosphatidylcholine; LPP, lipid phosphate phosphatases; PA, phosphatidate, PAP, phosphatidate phosphatase; PAP1, type 1 Mg$^{2+}$-dependent phosphatidate phosphatase; PAP2, type 2 phosphatidate phosphatase; PLA$_2$, phospholipase A$_2$; PLD, phospholipase D; S1P, sphingosine 1-phosphate; TCF, T cell transcription factor; VEGF, vascular endothelial growth factor.
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external loop contains an N-glycosylation site, which further supports the location of this loop being extracellular (27). The third conserved catalytic domain, C3, is located on the extracellular loop between helices V and VI. C1 is responsible for substrate recognition, whereas C2 and C3 contain the amino acids required for the phosphotransferase reaction (31). The conserved histidine on C3 acts on the phosphate group as a nucleophile to form a phospho-histidine intermediate (31). The C2 histidine is involved in breaking the phosphate bond to release the dephosphorylated lipid product. The conserved lysine and arginine residues on C1, as well as the arginine on C3, help coordinate the substrate in the active site (14).

LPPs can form homo- and hetero-oligomers, with each subunit capable of functioning independently of the others in the dephosphorylation reactions. These different combinations of oligomeric states could regulate subcellular localization (35). Results from Drosophila melanogaster also show dimerization of Wunen, the homolog of mammalian LPP3, but this is not a requirement for biological function (36).

Dephosphorylation of extracellular LPA by the “ecto-activity” of the LPPs

In order to understand the functions of the LPPs in normal physiology and in pathological conditions, it is necessary to understand how they regulate the turnover of bioactive lipid mediators. A major function of the LPPs is to dephosphorylate extracellular LPA and S1P, which are important regulators of cell division, migration, and survival. LPA is present in extracellular fluids at concentrations of about 100 nM in plasma to 2 μM in serum and other fluids (37, 38). Formation of extracellular LPA is mainly through the hydrolysis of lysophosphatidylcholine (LPC) by autotaxin (ATX) (30), which is a secreted enzyme with lysophospholipase D activity. LPC concentrations in human blood are >200 μM, which provides an abun-
dant supply of LPC for ATX (37). Saturated LPC is pro-
duced mainly by lecithin:cholesterol acyltransferase in
high density lipoproteins (30). Saturated LPA can also be
produced by secretory phospholipase A2 (PLA2), which hy-
drolyzes PA in microvesicles during inflammation (39)
and platelet aggregation (40). However, a large propor-
tion of circulating LPC is polyunsaturated and these LPCs
are secreted by hepatocytes and probably by other cells
(41, 42). ATX preferentially catalyzes the hydrolysis of
mono- and polyunsaturated LPC (42, 43). LPP1 activity is
partly responsible for counter-balancing the signaling ef-
fects of ATX by degrading circulating LPA.

LPA signals to cells by stimulating at least six G protein-
coupled receptors (44, 45). The lack of LPA signaling in
ATX knockout mice is embryonically lethal, because ATX
and LPA are required for vasculogenesis and neural crest
formation in embryos (46, 47). LPA also facilitates wound
repair by stimulating platelet aggregation and the migra-
tion of fibroblasts, vascular smooth muscle cells, endothe-
lium cells, and keratinocytes into the wounded area (30).
LPA mediates lymphocyte extravasation, which is cru-
cial for maintaining immune homeostasis (48, 49). How-
ever, in chronically inflamed tissues, high LPA enhances lymph-
ocyte invasion and increases cytokine production in re-
sponse to repeated micro-injuries and incomplete tissue
repair (50–52). LPA signaling plays a major role in neural
development and repair (53), neuropathic pain (54), rheu-
matoid arthritis (55), fertility (58), obesity (56), and cancer
(57). All of these actions of LPA could be impacted by LPP
action, but this has not yet been investigated fully.

The ecto-activity of LPP1 in vivo has been demonstrated in
LPP1 hypomorph mice (PPAP2A<sup>tr/tr</sup>) (58), which have
increased plasma LPA concentrations and a longer half-
life for circulating LPA relative to the control mice (12
min versus 3 min). However, it is surprising that the circu-
lating LPA concentrations were not significantly decreased
in transgenic mice with LPP1 overexpression (38). Fibro-
blasts from these transgenic mice responded less to LPA-
stimulated migration (59, 60) and showed increased DG
accumulation in the cells after stimulation of PA produc-
tion with phorbol ester (38). These fibroblasts also show
3-fold higher ecto-activity against LPA. Therefore, this un-
expected result of circulating LPA in the transgenic mice
may suggest a more complex mechanism of LPA regula-
tion in the blood. There was no change in the expressions
of LPP2 and LPP3 in the LPP1 hypomorph mice (58).
However, it should be noted that the expression level of
ATX was not measured in the LPP1 transgenic or LPP1
hypomorph mice. This could be important because ATX
expression is controlled partly by negative feedback regu-
lation from LPA (61).

The apparent K<sub>s</sub> (concentration needed to attain half
maximum velocity) for the degradation of exogenous LPA
by LPP1 in fibroblasts is about 36 μM (62), which is much
higher than physiological concentrations of LPA (100 nM
to 2 μM). This indicates that the LPPs can degrade LPA in
proportion to its concentration over the physiological and patholog-
ical ranges. In addition, extracellular Ca<sup>2+</sup>
concentrations are about 2 mM and Ca<sup>2+</sup> severely de-
creases LPP1 activity (62). This latter effect could explain
some of the differences between results in vitro and in vivo. Salous et al. (63) found that exogenous LPA injected
into the circulation of mice is rapidly absorbed by non-
parenchymal cells in the liver. Our unpublished results
showed the same phenomenon: after injecting [<sup>32</sup>P]LPA
into the circulation of rats, the radioactivity in plasma de-
creased rapidly and after 2 min, most of the radioactivity
accumulated in the liver. Therefore, the liver probably acts
as a powerful buffering system to regulate LPA levels
in the blood.

**Dephosphorylation of extracellular S1P**

SIP is the sphingolipid analog of LPA, and it signals
through its five G protein-coupled receptors (64). SIP is
present in plasma at concentrations from 100 nM to 1 μM
(65). Circulatory SIP is bound to albumin, low density li-
poproteins, and high density lipoproteins. It can also be
carried by erythrocytes (65). SIP is formed inside cells
through phosphorylation of sphingosine by sphingosine
kinase-1 and -2 (66). Different types of cells, including can-
cer cells and astrocytes, secrete SIP through the ABC
transporters, ABCG1, ABCG2, and ABCA1 (65, 67–69),
and by protein spinster homolog-2 (70). SIP is dephos-
phorylated by two specific SIP phosphatases, which are
expressed mainly on the endoplasmic reticulum rather
than on the plasma membrane (23). Extracellular SIP is
cleared from blood in 15–30 min, and this process de-
pends on a cellular phosphatase activity, but not on SIP-
lyase (71). This means that the LPPs should be a major
regulator of external SIP concentrations.

Overexpression of LPP1 in HEK293 and human pulmo-
mary artery endothelial cells leads to increased hydrolysis
of extracellular SIP (72, 73). The conversion of extracel-
lar SIP to sphingosine by LPP1 facilitates sphingosine
uptake, followed by its intracellular conversion to SIP by
sphingosine kinase-1 (72). LPP3 is required for hydrolyz-
ing thymic and cerebral SIP to maintain the normal func-
tions of these organs (74, 75). Studies on FTY720 also
indicate a role for the LPPs as ecto-enzymes. FTY720 is an
analog of sphingosine that is used as an immunomodula-
tory drug for treating multiple sclerosis (76). FTY720 is
converted to FTY720-P by sphingosine kinase-2 (77).
Results from cells that overexpress LPP1, -2, and -3 showed
that only LPP3 dephosphorylates FTY720-P. LPP3 acted as
an ecto-phosphatase in intact cells to control the equilib-
rium between FTY720 and FTY720-P (77). This result is
surprising compared with the broad substrate preference
of the LPPs for lipid phosphates (31). Other studies show
that LPP1a has the highest activity and affinity for FTY720-
P (78). These results suggest that the first extracellular
loop, which is different in LPP1a compared with LPP1, is
involved in substrate recognition.

**Dephosphorylation of other extracellular lipid phosphates**

In addition to using extracellular LPA and SIP as sub-
strates, LPPs also degrade extracellular PA and C1P (62).
PA and C1P are more hydrophobic than LPA and SIP, and
they are not transported to a significant extent by binding to albumin and other proteins in the circulation. PA in the plasma membrane of neutrophils increases endothelial membrane permeability by stimulating intracellular tyrosine kinases (79); this process is suppressed by LPPs, which hydrolyze the extracellular PA. Overexpression of LPPs in HEK293 cells increases the degradation of extracellular PA and the consequent uptake of DG into the cells (80). Exogenously added C1P increases the intracellular concentrations of C1P (81). This is partly explained because the LPPs dephosphorylate exogenous C1P to produce ceramides, which can be phosphorylated back to C1P once they enter the cells. C1P activates cytosolic PLA2 activity, which leads to arachidonate production and increased prostaglandin E2 synthesis in human alveolar epithelial cells (81).

NONCATALYTIC ACTIONS OF LPPs

In addition to its phosphatase activity, human LPP3 interacts with integrins at the plasma membrane. This interaction relies on an arginine-glycine-aspartate (RGD) recognition motif on LPP3, which is located at the second extracellular loop between transmembrane α-helices III and IV. Endothelial cell-to-cell adhesion is promoted by binding of LPP3 to integrins, especially α5β3 and α3β1 integrins (82). This interaction does not depend on the catalytic LPP activity. Mutation of RGD into arginine-glycine-glutamate (RGE) in human LPP3 can abolish its interaction with integrins. Interestingly, mouse and rat LPP3 contain RGE instead of RGD, but murine LPP3 can also interact with α5β3 and α3β1 integrins (83). LPP1 possesses arginine-glycine-asparagine (RGN) instead of RGD, and is not able to bind to integrins.

INTRACELLULAR FUNCTIONS OF LPPs

LPPs are also localized on the internal membranes, including the endoplasmic reticulum (84) and Golgi network (10), where, presumably, the catalytic domains face the luminal sides of these organelles. As such, intracellular LPPs could potentially regulate signal transduction through dephosphorylation of substrates inside cells (Fig. 2). One report shows that overexpression of LPP1 in fibroblasts suppresses the stimulation of cell migration by wls-31, which is a phosphonate analog of LPA that activates LPA1/2 receptors (60). Wls-31 cannot be hydrolyzed by LPPs and therefore the ecto-activity of LPP1 does not contribute to the inhibition. Another report shows LPPs can regulate ERK activation downstream of thrombin signaling (85). Because thrombin is not a LPP substrate, this inhibition in ERK is also not caused by the ecto-activity of LPPs. Results from our group further confirm the intracellular functions of LPPs. MDA-MB-231 cells overexpressing LPP1 also show an inhibition of migration upon wls-31 stimulation, which involves an inhibitory effect on Ca2+-mobilization. LPP1 expression also decreases Ca2+-transients after stimulation of the protease-activated receptor-1. The mechanism of how LPPs affect receptor downstream signaling is not yet known. Because the substrates of LPPs also exist inside cells, degradation of unidentified intracellular substrates may be one of the explanations for the intracellular functions of LPPs (Fig. 2).

One of the possible substrates for intracellular LPPs is PA, which activates a variety of targets, including Sos, Raf,

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![Fig. 2](https://via.placeholder.com/150)  
**Fig. 2.** Roles of the LPPs in regulating the dephosphorylation of extracellular lipid phosphates at the cell’s surface (ecto-activities) and internal substrates through the intracellular activities. LPPs dephosphorylate extracellular LPA and SIP. LPPs also have intracellular effects, which depend on their catalytic activities. These effects occur downstream of receptor stimulation and they regulate the activations of phospholipase D, ERK, and Ca2+-transients. The lipid targets of these intracellular activities are unknown.
and S1P are involved in inflammation. C1P activates PLA2 to the surface of membranes (93). To provide the capacity for degrading PA on the cytosolic side of the membranes, which is opposite to the orientation of the catalytic domains of LPPs. Therefore, PA should not be hydrolyzed unless it can be efficiently transported to the luminal sides of organelles. Although the evidence listed above strongly suggests a potential role of LPPs in regulating intracellular PA accumulation, it is still not clear how the PA is able to access the intracellular LPPs. Furthermore, increasing the expression of LPP1 in fibroblasts decreases the effects of LPA and platelet-derived growth factor on cell proliferation (96). These S1P-mediated processes are conserved between flies and mammals. Wunen and Wunen-2 express Wunen and Wunen-2 in Drosophila, which causes impaired migration of primordial germ cells. The death of germ cells can be rescued by expression of human and mouse LPP3, but not human LPP1 or mouse LPP2 (102, 103).

Studies on Drosophila also shed light on the differential functions of LPPs in vivo. Drosophila expresses Wunen and Wunen-2, which are homologs of human LPPs with highly conserved phosphatase domains (102). Wunen and Wunen-2 show similar catalytic activity against LPA relative to mammalian LPPs (102). They are required by Drosophila in controlling primordial germ cell migration and maintaining the septate junction paracellular barrier during development of the trachea (102–104). Unlike vertebrate cells, insect cells do not express LPA and SIP receptors. Therefore, these functions of Wunens are independent of these receptors. Furthermore, mutation of Wunens in Drosophila causes impaired migration of primordial germ cells. The death of germ cells can be rescued by expression of human and mouse LPP3, but not human LPP1 or mouse LPP2 (102, 103).

These combined results demonstrate that individual LPPs have distinct functions that cannot be replaced by other family members. The work also shows that the LPPs appear to control cell migration through a signaling system that may be conserved between flies and mammals.

THE ROLE OF LPPs IN PATHOLOGY

This section will concentrate on the LPPs in mammals, while recognizing that the LPPs have a significant role in ERK, mTOR, Akt, and sphingosine kinase-1 (86–88). The LPPs have the potential to convert intracellular PA into DG. DG produced from PA can activate the classical and novel protein kinase Cs and Ras guanyl-releasing protein 1 (29). Increasing LPP1 and LPP2 expression can decrease intracellular PA/DG ratios (89). Overexpression of LPP3 in Swiss 3T3 and HEK293 cells increases the conversion of PA to DG (90). LPP3 depletion decreases the levels of de novo synthesized DG and the Golgi-associated DG content, which impairs protein trafficking in the early secretory pathway (91). Furthermore, LPP2 and LPP3 decrease cell survival by regulating the intracellular levels of PA (92).

It should also be noted that a large proportion of intracellular PA is formed by phospholipase D (PLD) activation. PLD produces PA on the cytosolic sides of the membranes, which is opposite to the orientation of the catalytic domains of LPPs. Therefore, PA should not be hydrolyzed unless it can be efficiently transported to the luminal sides of organelles. Although the evidence listed above strongly suggests a potential role of LPPs in regulating intracellular PA accumulation, it is still not clear how the PA is able to access the intracellular LPPs. Furthermore, increasing the expression of LPP1 in fibroblasts decreases the effects of LPA and platelet-derived growth factor on cell proliferation (96). These S1P-mediated processes are conserved between flies and mammals. Wunen and Wunen-2 express Wunen and Wunen-2 in Drosophila, which causes impaired migration of primordial germ cells. The death of germ cells can be rescued by expression of human and mouse LPP3, but not human LPP1 or mouse LPP2 (102, 103).

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LPP3 and the vasculature

Studies have already indicated the close relationship between LPP3 and the vascular system. Strong evidence comes from the phenotype of PPAP2B/LPP3 knockout mice. The mice have a deletion of exon 5 in the LPP3 gene that encodes one of its catalytic domains (101). Knockout mice die between E7.5 and E9.5, and do not form a chorioallantoic placenta and yolk sac vasculature. A subset of embryos show a shortening of the anterior-posterior axis that was similar to that in axin deficiency, a critical regulator of Wnt signaling. Loss of LPP3 activity results in decreased transcription through the β-catenin-mediated T cell transcription factor (TCF), whereas elevated levels of LPP3 produced the opposite effect (101). This action did not appear to depend totally upon the phosphatase activity of LPP3 because a catalytically inactive LPP3 mutant was partially effective in inhibiting TCF/β-catenin transcription in HEK293 cells. Additional support for a role of LPP3 in axis patterning was provided from the observations that ectopic expression of LPP3 in dorsal blastomeres of Xenopus embryos causes a mild ventralizing effect (101). Furthermore, axis duplication by Xwnt8 or Xwnt3a mRNA is inhibited by coinjection of LPP3 mRNA.

Further work was performed using heterozygous embryos carrying a β-galactosidase reporter gene to monitor the expression pattern of LPP3 in different stages (106). The earliest expression of LPP3 in vessels is seen on vascular mesodermal cells of the yolk sac in E8.5 embryos, and then on blood vessel endothelial cells by E10.5. LPP3 is expressed in thalamus ventricular areas at E12.5. This dynamic expression pattern of LPP3 in vasculature could reflect its critical role during vascular development (106). A mouse model with conditional knockout of LPP3 in endothelial and hematopoietic cells shows similar defects in vasculogenesis and embryo death to those in mice with global knockout of LPP3 (107). The function of LPP3 knockout in endothelial cells of mice was revealed using a tamoxifen-inducible Cre-recombination. LPP3 deficiency in endothelial cells increases vascular permeability and enhanced sensitivity of inflammation-induced vascular leak, which is restored by blocking LPA production or signals through LPA receptors (107). LPP3 also attenuates injury-induced proliferation of carotid artery smooth muscle cells. This effect is partially through hydrolyzing LPA, which inhibits the LPA stimulation of smooth muscle cell migration and Rho activation (108). Furthermore, LPP3 is involved in suppressing abnormal accelerated lymphangiogenesis. Knockdown of LPP3 significantly increases capillary formation of human lymphatic endothelial cells (109). A reporter-null allele of PPAP2B/LPP3 was used to make a heterozygous genotype in mice, and this showed that LPP3 is dynamically expressed in limb buds, mammary gland primordia, and heart cushions and valves, among others (106). This suggests that LPP3 plays a key role in regulating multiple signaling pathways during development.

Consistent with data from animal models, genome-wide association studies with a meta-analysis, which includes over 86,000 individuals, identified SNPs in the PPAP2B gene. These represent novel loci, which are strongly associated with coronary artery disease (110). Erbilgin et al. (111) performed expression quantitative locus mapping in normal and pathologically changed vascular tissues to characterize the roles of candidate genes identified from the genome-wide association studies. PPAP2B is one of seven genes, which play a causal role in atherosclerosis. These results support the hypothesis that LPP3 is required for maintaining blood vessel function and LPP3 deficiency increases the risk of coronary disease. It should be noted that some chronic diseases could affect the role of PPAP2B as a predictor. One analysis based on CaTS, power calculator for two stage association studies, did not find any association between PPAP2B and cardiovascular disease in 2,140 rheumatoid arthritis patients (112).

LPA and S1P are two substrates of LPP3 and both play important roles in vascular development. Mice lacking ENPP2, the gene encoding ATX, die in utero at E9.5 with severe defects in the vasculature of the yolk sac and embryo and in neural crest formation (46). These results demonstrate the importance of LPA in tissue remodeling and vasculogenesis. ENPP2−/− embryos have increased expression of vascular endothelial growth factor (VEGF) mRNA, consistent with hypoxic conditions as a consequence of the absence of a functional vascular system (46, 47). The requirement of ATX in vascular development has also been established in zebrafish models (113, 114), demonstrating that LPA or other products derived from ATX activity are required for embryonic blood vessel formation.

SIP increases endothelial cell migration and angiogenesis (64, 115). It does this directly through SIP receptors (116), by promoting transactivation of VEGF receptors (117) and by increasing VEGF release (118–121). SIP also shows a critical role in maintaining endothelial barrier integrity by stabilizing tight and adherent junctions between cells (122). Decreases in SIP or SIP receptor antagonists induce vascular leakage (123). It is possible that LPP3 also contributes to vascular function in a phosphatase-independent manner, as illustrated with a catalytically inactive LPP3 mutant, which was partially effective in inhibiting β-catenin-mediated TCF transcription in HEK293 cells (101). This possibility is also supported by a report from Huntse et al. (124). In their study, LPP3 stimulated β-catenin/lymphoid enhancer binding factor-1 to induce endothelial cell migration and formation of branching point structures, which regulated endothelial homeostasis. Catalytically inactive LPP3 shows the same effect as the wild-type LPP3 (124).

Role of the ATX-LPA-LPP axis in cancer

LPPs have the potential to regulate tumor progression because LPA signaling is involved at multiple levels in this
process. To understand the potential regulatory effects of the LPPs, we will first discuss the relation of ATX activity and LPA production in cancer. Increased ATX activity and LPA signaling are positively correlated to the invasive and metastatic potential of many cancers (57, 125). ATX is among the top 40 most upregulated genes in patients with highly metastatic cancers (126). The importance of LPA in cancer progression was demonstrated because transgenic mice that overexpress ATX, LPA\textsubscript{1}, LPA\textsubscript{2}, or LPA\textsubscript{3} receptors in mammary cells develop spontaneous metastatic mammary tumors (127). LPA receptors differentially stimulate cell survival and migration through the relative activations of phosphatidylinositol 3-kinase, ERK1/2, mTOR, Ca\textsuperscript{2+}-transients, Rac, Rho, and Ras. LPA increases VEGF production, which stimulates angiogenesis (128), a process necessary for tumor progression. LPA also decreases the expression of the tumor suppressor, p53, thus promoting cancer cell survival and division (129).

High concentrations of LPA are well-documented in the peritoneal ascites fluid and plasma of patients with ovarian cancer (130, 131), and LPA stimulates ovarian tumor growth and metastasis (132). In a colon cancer cell line, LPA increases the synthesis of macrophage migration inhibitory factor, which also promotes tumor growth (133). Women with breast carcinomas that express high levels of LPA\textsubscript{3} receptors in epithelial cells, or ATX in stromal cells, have larger tumors, nodal involvement, and higher stage disease (134).

LPA also adversely affects cancer therapies by producing resistance to the cytotoxic effects of Taxol (125, 135, 136), a common first line treatment for breast cancer. This work was confirmed (137) and extended to LPA-induced resistance to carboplatin (138) and radiation-induced cell death (125, 139, 140). LPA also attenuates doxorubicin-induced killing of breast cancer cells. This results partly from the LPA-induced increase in the stability of the transcription factor, Nrf2, which stimulates the anti-oxidant response element to increase the expression of the multidrug resistant transporters, ABCG1 and ABCG2, and anti-oxidant genes (141). The anti-oxidant enzymes decrease the extent of oxidative damage produced by many chemotherapeutic agents. Increased expression of multidrug resistant transporters facilitates the export of several chemotherapeutic agents, including doxorubicin, and also toxic oxidation products, thus contributing to chemoresistance. ABCG1 and ABCG2 might also facilitate the secretion of S1P, especially if the S1P transporter, spinster homolog-2, is absent (142). This S1P secretion contributes to chemo-resistance (143) and it stimulates angiogenesis, which is required by the growing tumor. Thus LPA and S1P coordinate the tumor growth (125, 139) and the invasion of breast cancer cells into surrounding tissue to form metastases (121). Blocking signaling by LPA (144) or S1P (65, 145) has, therefore, been proposed as an important therapeutic target for the treatment of cancer. We recently showed that inhibition of ATX and LPA formation decreases breast tumor growth and metastasis and increases the efficacy of doxorubicin therapy (141, 146). Blocking the formation of S1P using inhibitors of sphingosine kinase-1 (147), or increasing S1P breakdown through S1P lyase (148) also has therapeutic potential for cancer treatment.

An alternative approach to targeting LPA signaling is to increase LPA and S1P degradation through the LPPs, which could also decrease their abilities to signal downstream of receptor activation in cancer cells. This can be achieved by increasing the low levels of expression of LPP1 and LPP3, which are observed in several types of cancer from the analysis of gene arrays published in (149–151) and accessed through Oncomine\textsuperscript{TM} (152, 153) (Fig. 3). In support of this hypothesis, overexpressing LPP1 in ovarian cancer cell lines increases LPA hydrolysis. This is associated with a marked inhibition of cell proliferation and colony-forming activity and increased apoptosis (154). LPP3 shows similar effects in SKOV3 and OVCAR-3 ovarian cancer cells. LPP3 promotes LPA hydrolysis in the medium and decreases LPA-stimulated colony formation. This effect of LPP3 depends on its ecto-activity and this effect was diminished by using a nonhydrolyzable LPA analog instead of LPA (99). Gonadotropin-releasing hormone increases ecto-LPP expression in ovarian cancer cells and this explains its anti-proliferative effects (155). These studies with cultured cancer cells were validated by measuring tumor growth from SKOV3 ovarian carcinoma cells in nude mice. Cells with overexpressed human LPP3 showed much lower tumor growth (99). The authors ascribed this result to the ecto-phosphatase activities of LPP3 at a time when the effects, which occur downstream of receptor activation, were not widely appreciated.

We expressed LPP1 and a catalytically inactive mutant, LPP1(R217K), using a doxycycline-inducible promoter in various cancer cells and determined their phenotypes (73). Cells that overexpressed LPP1 show increased hydrolysis of LPA and S1P. Expression of wild-type LPP1, but not the catalytically inactive LPP1, suppresses cell growth in 3D culture and LPA-stimulated cell migration. This latter effect on migration could be related to a decrease in LPA-induced Ca\textsuperscript{2+}-transients and activation of Rho. LPP1 expression also decreased the stimulation of Ca\textsuperscript{2+}-transients and migration by wls-31. This phosphonate analog of LPA, which activates LPA\textsubscript{1/2} receptors, cannot be degraded by LPPs. LPP1 expression also attenuated the stimulation of Ca\textsuperscript{2+}-transients by the protease-activated receptor-1 peptide, which does not involve LPA receptors. Therefore, the major effects of LPP1 cannot be explained by the degradation of exogenous LPA. Instead, the results put emphasis on the postreceptor signaling effects of LPP1 (Fig. 2).

Zhao et al. (156) also showed that LPP1 expressed in human bronchial epithelial cells inhibits Ca\textsuperscript{2+}-transients and phosphorylation of inhibitory kappa B, followed by NF-κB activation and decreased interleukin-8 production. This work identified a potential role of LPP1 in the regulation of LPA-induced inflammation, which is a major driving stimulus in tumor growth, metastasis, and chemo-resistance (57, 144).

We tested the effects of LPP1 expression in breast and thyroid cancer cells on their abilities to form tumors in
Lipid phosphate phosphatases decreased LPA turnover in LPP1 knockout mice compared with wild-type controls (58, 157). Another study showed that mice lacking LPA1 or LPA5 receptors had decreased melanoma-derived lung metastasis compared with wild-type mice following tail-vein injection of B16F10 melanoma cells (158). These experiments demonstrate the importance of LPA signaling for establishing a suitable environment for cancer cell seeding and subsequent growth.

The effects of LPP2 on tumor growth appear to be quite different from LPP1 and LPP3. Flanagan et al. (100) compared the data from a genomic screen between normal and transformed mesenchymal stem cells and identified PPAP2C/LPP2 as a target whose expression is elevated in numerous carcinomas and sarcomas (151). This increase in LPP2 is seen in breast, lung, and ovarian cancers where the expressions of LPP1 and LPP3 are decreased (Fig. 3). Overexpressing LPP2 leads to premature S-phase entry, mice. Increasing the expression of LPP1 decreased tumor growth and metastasis by up to 80% compared with expression of the inactive LPP1 (R217K) mutant or green fluorescent protein (73). Increasing LPP1 expression in the cancer cells alone did not change the concentrations of LPA in either the tumor or plasma. This work demonstrates in vivo that targeting LPPs could be a beneficial strategy for cancer therapy and that the major effects of LPP1 could be mediated downstream of receptor activation.

Rather than targeting LPP signaling in cancer cells, Nakayama et al. (157) recently examined the contribution of LPP1 expression in the tumor microenvironment to cancer cell seeding. Intraperitoneal injection of syngeneic ovarian cancer cells into LPP1 knockout mice leads to enhanced cancer cell seeding compared with wild-type mice (157). Presumably, higher systemic levels of LPA can explain this result as a consequence of decreased LPA turnover in LPP1 knockout mice compared with wild-type controls (58, 157). Another study showed that mice lacking LPA1 or LPA5 receptors had decreased melanoma-derived lung metastasis compared with wild-type mice following tail-vein injection of B16F10 melanoma cells (158). These experiments demonstrate the importance of LPA signaling for establishing a suitable environment for cancer cell seeding and subsequent growth.

The effects of LPP2 on tumor growth appear to be quite different from LPP1 and LPP3. Flanagan et al. (100) compared the data from a genomic screen between normal and transformed mesenchymal stem cells and identified PPAP2C/LPP2 as a target whose expression is elevated in numerous carcinomas and sarcomas. This increase in LPP2 is seen in breast, lung, and ovarian cancers where the expressions of LPP1 and LPP3 are decreased (Fig. 3). Overexpressing LPP2 leads to premature S-phase entry,
and this effect is not seen with LPP1 and LPP3 (98). Knockdown of LPP2 delays entry into S-phase (98) and impairs anchorage-dependent growth (100). This combined work provides preliminary evidence that increasing the low expressions of LPP1 and LPP3 and decreasing the high expression of LPP2 in cancer cells could provide novel targets for cancer therapy.

CONCLUSION

LPPs play an important role in regulating LPA/S1P signaling through the degradation of these bioactive lipids. LPPs are widely involved in many pathophysiological processes, especially in embryo development, vasculogenesis, and tumorigenesis. It is clear that LPP1, -2, and -3 have distinct and nonredundant functions besides their common phosphatase activities in these processes. The different actions of the LPPs may be attributed to their different preference for substrates, different localizations, effects on postreceptor signaling (159), and their noncatalytic functions. The different functions of the LPPs are not completely understood and they warrant further investigation. The development of activators or inhibitors for the LPPs could provide novel therapies for the treatment of vascular diseases and cancers.

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