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Review

Lipid phosphate phosphatase (LPP3) and vascular development ☆

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ABSTRACT

Lipid phosphate phosphatases (LPP) are integral membrane proteins with broad substrate specificity that dephosphorylate lipid substrates including phosphatidic acid, lysophosphatidic acid, ceramide 1-phosphate, sphingosine 1-phosphate, and diacylglycerol pyrophosphate. Although the three mammalian enzymes (LPP1-3) demonstrate overlapping catalytic activities and substrate preferences in vitro, the phenotypes of mice with targeted inactivation of the *Ppap2* genes encoding the LPP enzymes reveal nonredundant functions. A specific role for LPP3 in vascular development has emerged from studies of mice lacking *Ppap2b*. A meta-analysis of multiple, large genome-wide association studies identified a single nucleotide polymorphism in *PPAP2B* as a novel predictor of coronary artery disease. In this review, we will discuss the evidence that links LPP3 to vascular development and disease and evaluate potential molecular mechanisms. This article is part of a Special Issue entitled Advances in Lysophospholipid Research.

1. The LPP family

Lipid phosphate phosphatases are integral membrane proteins with broad substrate specificity that dephosphorylate lipid substrates including phosphatidic acid (PA), lysophosphatidic acid (LPA), ceramide 1-phosphate (C1P), sphingosine 1-phosphate (S1P), and diacylglycerol pyrophosphate (DGPP) [1]. They belong to a broader class of structurally-unrelated phosphatidic acid-phosphatases (PAP) that comprise both membrane and soluble family members [2]. In humans, three genes, *PPAP2A*, *PPAP2C*, and *PPAP2B*, encode the enzymes LPP1, LPP2 and LPP3, respectively [3,4]. In this review, we refer to the gene names using the *PPAP* nomenclature but the corresponding proteins as LPPs.

The predicted topology of the LPPs suggests that they possess six transmembrane domains, an active site comprised from at least 3 regions of the protein that localizes to the extracellular or luminal surface of the membrane, and a glycosylation site on a hydrophilic loop between the first and second active site domains (Fig. 1) [2]. Mammalian LPPs form homo- and hetero-oligomers [5]. The *Drosophila* homolog of mammalian LPP, wunen, forms homodimers via the last C-terminal 35 amino acids, but cannot form heterodimers with wunen2 or mammalian LPP1 or LPP3 [6]. The functional significance of these interactions is not known.

LPPs localize to both the plasma membrane and intracellular membrane organelles, in particular the endoplasmic reticulum and Golgi apparatus [1,2,7,8]. Subcellular localization of these enzymes is both dynamic and cell-specific. LPP1 and LPP3 appear to have distinct subcellular localization [9], between lipid rafts and the apical and basolateral membranes of polarized cells, which could account for their observed differences in biological functions despite their essentially identical catalytic activities. Evidence that LPPs can act on both extracellular and intracellular substrates has come from studies in which these enzymes are over expressed or inactivated in cell culture systems coupled with measurements of their substrates and products using radiolabeling or mass spectrometry based approaches.

Although the three mammalian LPP enzymes demonstrate overlapping catalytic activities and substrate preferences in vitro, the phenotypes of mice with targeted inactivation of the *Ppap2* genes indicate that they have nonredundant functions. The *Ppap2a* gene encoding murine LPP1 has been disrupted using an exon trap insertion strategy. Mice harboring the exon trap inactivated allele appear phenotypically unremarkable [10]. Multiple tissues, including heart, kidney, lung, liver and spleen, isolated from the animals display a reduced ability to dephosphorylate exogenously provided LPA, indicating a role for LPP1 as a widely expressed LPA phosphatase. Decreased dephosphorylation of exogenous LPA by thymocytes from these LPP1 deficient mice indicates that endogenously expressed LPP1 can function as an “ecto” LPA phosphatase, at least in these cells. Mice homozygous for an insertionally inactivated allele of the *Ppap2c* gene encoding murine LPP2 are phenotypically unremarkable [11]. By contrast, inactivation of *Ppap2b* results in early embryonic lethality in part to due to failure of extra-embryonic vascular development [12].

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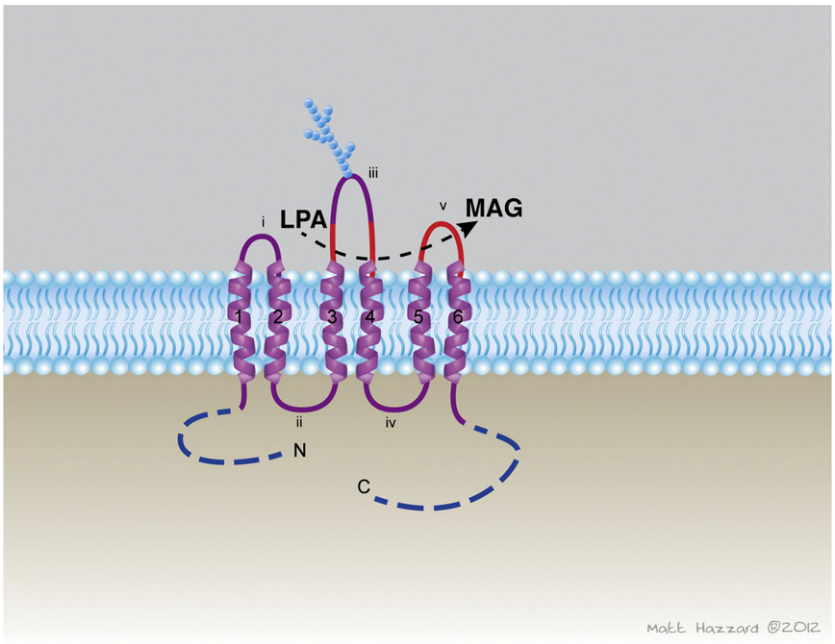

Fig. 1. Predicted topology of lipid phosphate phosphatases. The LPP members are predicted to have six transmembrane spanning regions and an active site composed of regions on the extracellular or abluminal surface of the membrane.

2. The role of LPP3 in blood vascular development

In mice, LPP3 is first expressed in the anterior visceral endoderm, and the extra-embryonic membranes at E7.5 [12,13]. As gastrulation proceeds, LPP3 appears around the node and the tip of the allantois at E8.0, and allantois, the developing gut, the pericardio-peritoneal canal and somites at E8.5. LPP3 is absolutely required in these tissues, as chorio-allantoic placenta do not form in its absence. By E9.5, LPP3 is present in umbilical cord and the chorionic region, and later in mid-gestation, in the apical ectodermal ridge, mesenchyme of the limb buds, and nervous system. In adult mice, expression of LPP3 is particularly prominent in lung, cerebellum and heart atrium. The dynamic and tissue-specific expression pattern may reflect the importance of LPP3 in specific tissues during development.

A critical role for LPP3 in vasculogenesis is indicated by the phenotype of mice with inherited deficiency in *Ppap2b*, created by deleting exon 5 that encodes a domain of the protein essential for its catalytic activity [12]. *Ppap2b*-null embryos die between E7.5 and 9.5 as a consequence of an inability to form extra-embryonic vasculature. The *Ppap2b*-null embryos fail to form chorio-allantoic placenta, and their abnormal yolk sac vascular network results in accumulation of blood cells in the yolk sac cavity. The embryos also demonstrate abnormalities in embryonic axis formation, with shortening of the anterior–posterior axis, anterior truncation and frequent duplication of axial structures [13]. We have targeted LPP3 in the vasculature by breeding mice containing a *Ppap2b*-floxed allele with mice expressing Cre recombinase under the control of the *Tie2* promoter (*Tie2-Cre*) to delete exons 3 and 4 of the floxed *Ppap2b* gene in hematopoietic and endothelial cells [14]. The excised exons encode the second and third transmembrane domains, the first intracellular and the second outer loop, and 12 amino acids of the fourth transmembrane segment. Global Cre-mediated deletion in mice phenocopies complete *Ppap2b* deletion [13]. Mice with *Tie-Cre* mediated deletion, which lack LPP3 in endothelial and some hematopoietic cells, die embryonically with a milder but similar defect in vasculogenesis as is observed in mice with global lack of *Ppap2b* [12]. Consistent with the observations that LPP3 is essential for normal vascular development, allantois explants from *Ppap2b*-null embryos fail to organize endothelial cells into cords. Finally, LPP3 expression is also upregulated as lymphatic endothelial cells organize into capillary-like structures in collagen matrix in vitro. siRNA-targeted knock-down of LPP3 expression enhances capillary formation, suggesting that the protein negatively regulates the process [15]. At the present time, it is not known if LPP3 catalytic activity is required for normal vessel development although in the absence of other well defined non-catalytic functions for the protein this seems likely. In the following sections, we will discuss potential mechanisms by which LPP3 may affect endothelial cell function. We will focus attention on pathways that may be regulated by LPP3 catalytic activity and ways that LPP3 may influence development in phosphatase independent manners.

3. LPP3 as a critical regulator of lysophospholipid signaling

The bioactive lysophospholipids LPA and S1P elicit cell responses by binding to and activating distinct G-protein coupled receptors, initially classified as *Edg* (endothelial differentiation gene family) receptors but subsequently rationally re-named as LPA and S1P receptors. LPP3-catalyzed removal of the phosphate group of LPA and S1P renders them inactive at their receptors. Substantial evidence from cell culture experiments indicates that LPP3 can regulate extracellular signaling by lysophospholipids [2,16,17]. LPP3 overexpression decreases tumorigenesis and colony forming ability of ovarian cancer cells and the effects of LPP3 on colony-forming activity are substantially reversed by an LPP-resistant LPA analog, O-methylphosphothionate and a series of additional phosphatase resistant LPA analogs which have been developed as synthetic LPA receptor selective ligands [18]. These results imply that the inhibitory effects of LPP3 on tumor growth and survival are mediated at least in part by hydrolysis and inactivation of bioactive LPA. Consistent with these reports, cultures of embryonic fibroblasts derived from LPP3 knockout mice exhibit significantly increased extracellular LPA [12].

4. LPA in the vasculature

Extracellular, bioactive LPA is generated by the lysophospholipase D autotaxin, which removes the choline group from lysophosphatidylcholine (LPC) and other lysophospholipids (Fig. 2) [19]. Plasma LPA levels are <1 μM, but increase with platelet activation, due to