Lipid Phosphate Phosphatase 3 (LPP3) TerminatesLysophosphatidic Acid (LPA) Pathologic Signaling in Vascular Wall Cells but Does not Mediate Systemic Elimination of LPA from Plasma

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Bioactive Lipid signaling and metabolism in the Vasculature

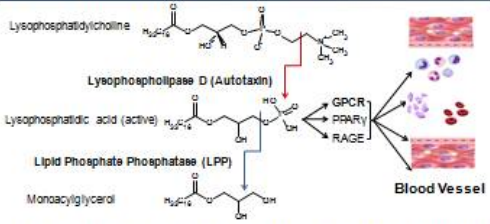
Introduction
The bioactive lipid lysophosphatidic acid (LPA) is present in most biological fluids including plasma and cerebrospinal fluid at a concentration of ~0.1-1 uM. LPA has a number of developmental, physiological, and pathophysiological actions in the vasculature including cellular differentiation, atherothrombosis, inflammation, response to injury, and vascular permeability. LPA has pleiotropic effects in vascular wall cells including vascular endothelial and smooth muscle cell functions, with potential roles in cerebrovascular disease. This research reports on the role of the enzyme termed lipid phosphate phosphatase 3 (LPP3) in the termination of pathologic LPA signaling in vascular models of injury and its role LPA metabolism.

Methods
We used animal models of targeted genetic inactivation of LPP3 in vascular smooth muscle and vascular endothelial cells and a modified surgical model to study the mechanisms underlying the elimination of LPA from the plasma. Our methods included loss and gain of function approaches, *in vitro* expression systems, synthetic lysophospholipid mimetics, and HPLC/tandem mass spectrometry assays.

Results
LPP3 plays a critical role in attenuating LPA signaling mediating the pathological processes of intimal hyperplasia and vascular permeability/leak in mouse models of vascular disease. Enzymatic inactivation contributed by LPP3 or related enzymes does not play a key role in the rapid elimination of plasma LPA. Alternatively, transcellular uptake by hepatic nonparenchymal cells emerges as a predominant mechanism for elimination of LPA and related analogs from the plasma

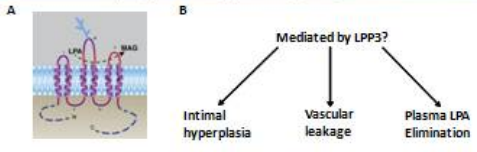
Conclusions
These findings provide potential mechanistic insights for recent genome wide association studies reporting on the association of a polymorphism of LPP3 with coronary artery disease. This polymorphism may explain a potential role of LPP3 allele in modifying susceptibility of intracranial vessels to vascular injury following carotid endarterectomy, coiling of aneurysms, and embolization of arteriovenous malformations. Additionally, the previously established LPA signaling pathways in brain gliomas, traumatic brain injury, and hydrocephalus coupled with rapid elimination of LPA mimetics from the plasma (reported here), suggest that bypassing the circulation to deliver LPA antagonists or autotaxin inhibitors may be a promising and beneficial approach to advance the treatment modalities of these conditions.

Figure 1. Synthesis and Degradation Pathways of LPA



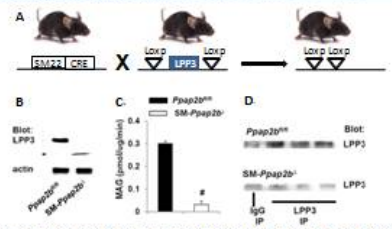
Synthesis, signaling, and inactivation of LPA. In the vasculature, LPA actions on blood and vascular wall cells are implicated in promoting vascular pathology.

Figure 2. LPP3 topology and role(s) in LPA signaling and metabolism



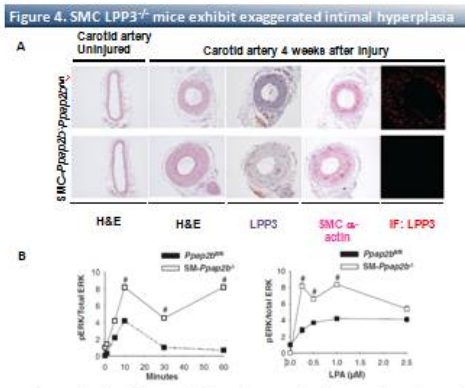
A. LPP3 is a unique membrane-bound lipid phosphatase that uniquely possess ectophosphatase activity, expresses highly in the blood and vascular endothelial cells, and results in embryonic lethality in LPP3$^{-/-}$ mice. B. Exploring the role of LPP3 in modulating intimal hyperlpasia, vascular leakage, and systemic elimination of LPA.

Figure 3. Generation of smooth muscle cell specific LPP3$^{-/-}$ mice



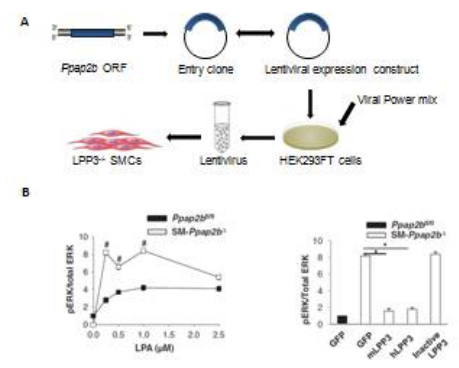
A. Using the cre-lox system to generate smooth muscle cell specific LPP3 deficiency. Validation of LPP3 inactivation in isolated SMCs using B. western blotting, C. phosphatase activity assay, and D. immunoprecipitation

Figure 4. SMC LPP3$^{-/-}$ mice exhibit exaggerated intimal hyperplasia



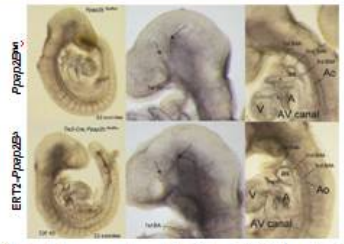
A. Characterization of LPP3 and alpha actin expression in WT and LPP3$^{-/-}$ SMC mice after induction of intimal hyperplasia. B. SMC proliferation (quantitated by measuring the phosphorylation of extracellular signal regulated kinase (pERK) significantly increased in a time- and dose-dependent manner in LPP3$^{-/-}$ SMCs vs. WT littermates.

Figure 5. Re-expression of WT LPP3 reverses LPP3$^{-/-}$ SMC phenotype



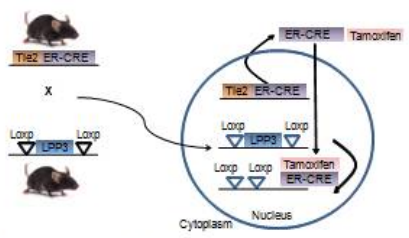
A. Generation of lentiviral LPP3 variants. B. Re-expression of catalytically active human and mouse LPP3 in LPP3$^{-/-}$ SMCs reversed ERK phosphorylation

Figure 6. Deletion of vascular endothelial LPP3 is lethal *in utero*



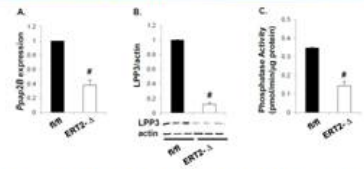
Inactivation of whole body or vascular endothelial specific LPP3 results in embryonic lethality and defects in blood vessels and patterning

Figure 7. Induction of endothelial LPP3 inactivation in adult mice



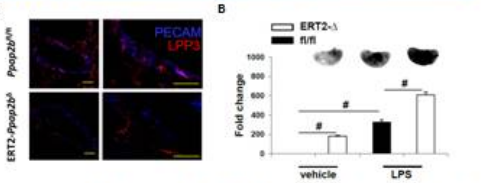
A. Schematic of specific inducible inactivation of mLPP3 in vascular endothelial cells using tamoxifen-driven estrogen receptor-cre (ER-CRE) fusion protein under the control of the Tie2 promoter.

Figure 8. Vascular endothelial LPP3$^{-/-}$ following tamoxifen treatment



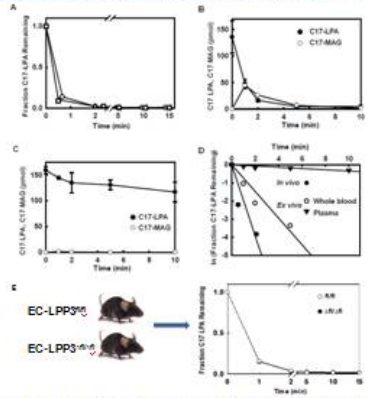
Validation of tamoxifen driven deletion of vascular endothelial LPP3 using A. quantitative PCR, B. protein expression, and C. phosphatase activity in IP:LPP3 from homogenized lung tissue following treatment with tamoxifen.

Figure 9. EC LPP3 deficiency promotes vascular leak in lungs



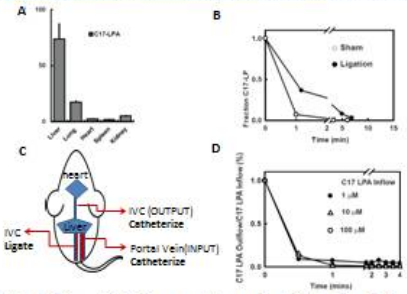
A. Immunostaining of representative blood vessels sections show co-localization (magenta) of endothelial cell marker PECAM (blue) and LPP3 (red) in wild type but not LPP3$^{-/-}$ vascular endothelial cells. B. Extravasation of Evans blue dye in EC LPP3$^{-/-}$ mice and their control littermates show significantly increased vascular leak that is more pronounced following lipopolysaccharide (LPS) mediated sepsis.

Figure 10. LPP3 does not mediate the rapid elimination of plasma LPA



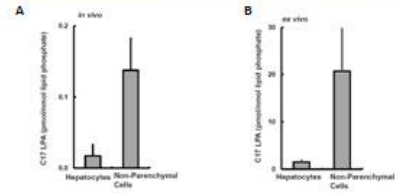
A. LPA is eliminated rapidly from the plasma with a half-life of ~30 sec. B.&C. Plasma LPA elimination does not involve in situ degradation. D. Semi-log plot of data in A-C. E. C17-LPA elimination from the plasma in LPP3$^{-/-}$ EC mice vs. control littermates.

Figure 11. Plasma LPA elimination is mainly mediated by the liver



A. IV administered C17-LPA accumulates massively in the liver. B. The elimination process is attenuated by ligation of the hepatic blood supply. C. Single pass hepatic perfusion model. D. The liver extracts 85-90% of administered C17-LPA at first pass.

Figure 12. Hepatic non-parenchymal cells mediate LPA elimination



A. Association of C17-LPA with hepatocytes and non-parenchymal liver cells after a single bolus intravenous administration. B. Association of C17 LPA with isolated hepatocytes and non-parenchymal liver cells cultured *ex vivo*.

Acknowledgements

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