

LRP in Endothelial Cells A Little Goes a Long Way

Dudley K. Strickland, Selen C. Muratoglu

Endothelial cells are highly heterogeneous¹ and participate in angiogenesis, an integrated set of responses in which new blood vessels are formed from existing ones. During angiogenesis, endothelial cells exhibit increased migration and proliferation² and contribute to the reorganization of the extracellular matrix. Angiogenesis occurs during embryonic development and is also initiated during wound healing and pathological conditions, such as ischemia. Although most often the revascularization of ischemic tissues is highly beneficial, under certain conditions, it can be harmful. For example, pathological angiogenesis in the retina during diabetic retinopathy can result in blindness.³ Several molecules influence angiogenesis, including vascular endothelial growth factor,⁴ Notch,⁵ and Wnt.⁶ In this edition of *Atherosclerosis, Thrombosis, and Vascular Biology*, Mao et al⁷ identify another key player in this process: the low-density lipoprotein receptor-related protein 1 (LRP1) by demonstrating a significant role for LRP1 in orchestrating angiogenesis during retinal neovascularization.

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LRP1, is a highly efficient endocytic as well as a signal transducing receptor that binds multiple ligands,^{8–11} and modulates signaling pathways by regulating the extracellular levels of growth factors and binding adaptor molecules to its intracellular domain (ICD). LRP1 plays an important role in the development^{12,13} and maintenance of the vasculature.^{14,15} Deletion of the *Lrp1* gene in mice results in early embryonic lethality¹² because of extensive hemorrhaging occurring around E13.5.¹³ The underlying vascular defect results from a failure to recruit and maintain vascular smooth muscle cells and pericytes of vessels resulting in extreme dilation of the aorta with a thin and disorganized smooth muscle cell layer and discontinuity of the vascular endothelium. Interestingly, the phenotype observed for *Lrp1*^{-/-} embryos resembles that of mice genetically deficient in sphingosine-1-phosphate receptor S1PR1. Like the *Lrp1*^{-/-} embryos, the *S1pr1*^{-/-} embryos also exhibit embryonic hemorrhage due to failure to recruit

vascular smooth muscle cells and pericytes.¹⁶ This suggests that LRP1 might regulate the S1P signaling pathway.¹³ LRP1 is also required for appropriate vascular development in zebrafish, where loss of *Lrp1a* results in a disrupted vascular phenotype associated with excess bone morphogenic protein signaling.¹⁷

LRP1 is ubiquitously expressed in numerous cell types, including brain endothelium, neurons, smooth muscle cells, astrocytes, macrophages, fibroblasts, and hepatocytes.¹⁸ Although LRP1 is expressed in high levels in most cells, protein levels in the endothelium are low. Its expression is regulated by a variety of pathological conditions, such as hypoxia^{19–21} and Alzheimer disease,²² or by changes in physiological conditions, such as aging.²³ The relatively low level of LRP1 expression in endothelium is tightly regulated by physiological conditions reflecting its important role in this tissue. The first role identified for endothelial LRP1 occurs at the blood–brain barrier, where LRP1 functions to avert accumulation of amyloid- β in brain which is a key event in Alzheimer disease pathogenesis.²³ In addition to its endocytic and clearance role of amyloid- β in the brain, in endothelial cells, LRP1 ligands may also undergo transcytosis.^{24,25} Although some studies contradict a role for LRP1 in mediating the efflux of amyloid- β across the blood–brain barrier,^{26,27} an elegant study using a brain endothelial-specific LRP1 knockout mouse model convincingly demonstrated the importance of this function for endothelial LRP1 in transporting amyloid- β across blood–brain barrier.²⁸

A second function for LRP1 expressed in the endothelium is revealed in this study. Using a mouse model of oxygen-induced retinopathy, Mao et al⁷ found that mice in which LRP1 is selectively deleted in endothelial cells display significantly more neovascularization response in the retina under hypoxic stress. To address the potential mechanisms involved, Mao et al⁷ discovered that LRP1 directly interacts with poly(ADP-ribose) polymerase-1 (PARP-1) and colocalizes with this molecule in the nucleus. PARP-1 is a ubiquitous nuclear DNA base repair enzyme²⁹ that is activated in response to DNA damage in eukaryotes.³⁰ In the nucleus, activated PARP-1 catalyzes the transfer of ADP-ribose from nicotinamide adenine dinucleotide⁺ onto nuclear acceptor proteins, including histones and PARP-1 itself.³¹ This process initiates chromatin relaxation and subsequent recruitment of DNA repair proteins. These conformational changes in chromatin lead to diverse biological processes, including chromatin remodeling, transcriptional regulation, DNA repair, cell proliferation, and apoptosis.³²

Because mature forms of LRP1 are located on the plasma membrane and within endosomal compartments, and because PARP-1 is primarily found within the nucleus, the question

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arises as to how these 2 molecules could colocalize. The answer may lie in the fact that LRP1 undergoes regulated intramembrane proteolysis. Regulated intramembrane proteolysis is a process in which sequential proteolysis of a transmembrane protein ultimately leads to release of its ICD. May et al³³ demonstrated that the LRP1-ICD is released by presenilin-1 after shedding of the ectodomain in an event that is enhanced by activation of protein kinase C, but not influenced by LRP1 ligands (Figure, A). Regulated intramembrane proteolysis of LRP1 has at least 2 major consequences that have been well defined. First, release of the LRP1-ICD suppresses inflammation by reducing the transcription of lipopolysaccharide-inducible genes.³⁴ This occurs when the LRP1-ICD translocates to the nucleus and associates with interferon regulatory factor 3 promoting its nuclear export (Figure, B). This pathway is clearly a feedback inhibitory pathway because presenillin-1-mediated cleavage of LRP1 in macrophages is increased by treatment with lipopolysaccharide. The net result of this pathway is attenuation of tumor necrosis factor- α and interleukin-6 secretion in response to lipopolysaccharide.

Mao et al⁷ identifies a second consequence of regulated intramembrane proteolysis in endothelial cells in which LRP1 regulates hypoxia-mediated angiogenesis (Figure, C). The precise mechanism of how this occurs still needs to be established, but the results suggest that LRP1 regulates endothelial cell proliferation by preventing cell cycle progression. During the premitotic stage of the cell cycle, multiple checkpoints ensure the integrity of the genome³⁵ and several crucial proteins play critical roles in these check points. These include

cyclin-dependent kinases (Cdks) including Cdk2, which are Ser/Thr kinases that play a central role in the eukaryotic cell division cycle³⁶ by mediating the phosphorylation of retinoblastoma to facilitate the G1/S transition in cell cycle.³⁷ Mao et al⁷ provided evidence that *LRP1* knockdown in human retinal microvascular endothelial cells significantly increases Cdk2 activity as detected by increased phosphorylation at Thr160. Similarly, *LRP1* knockdown increased retinoblastoma phosphorylation at Ser807/811 leading to retinoblastoma inactivation resulting in release of sequestered E2F transcription factors. The studies also revealed that inhibition of PARP-1 rescues these effects resulting from LRP1 knockdown. The conclusion from this work is that LRP1 expression attenuates the phosphorylation of Cdk2 and retinoblastoma in normoxia conditions by associating with PARP-1. In hypoxic conditions, despite the fact that *LRP1* mRNA expression and protein levels increase,^{19–21} the interaction between LRP1 and PARP-1 is diminished and PARP-1 is freed from the LRP1-ICD leading to its increased activation. This in turn increases Cdk2 and retinoblastoma phosphorylation driving cell proliferation (Figure, C).

Although the potential of LRP1 to regulate PARP-1 is an exciting new discovery, this finding raises several important questions that need to be further examined. First, as the binding site on the LRP1-ICD for PARP-1 has been localized to the last 33 amino acids within the ICD, a deletion mutant of LRP1 would be instrumental in teasing out the molecular mechanisms of LRP1/PARP-1 interactions in endothelial cell biology. Second, the role of LRP1/PARP-1 interactions

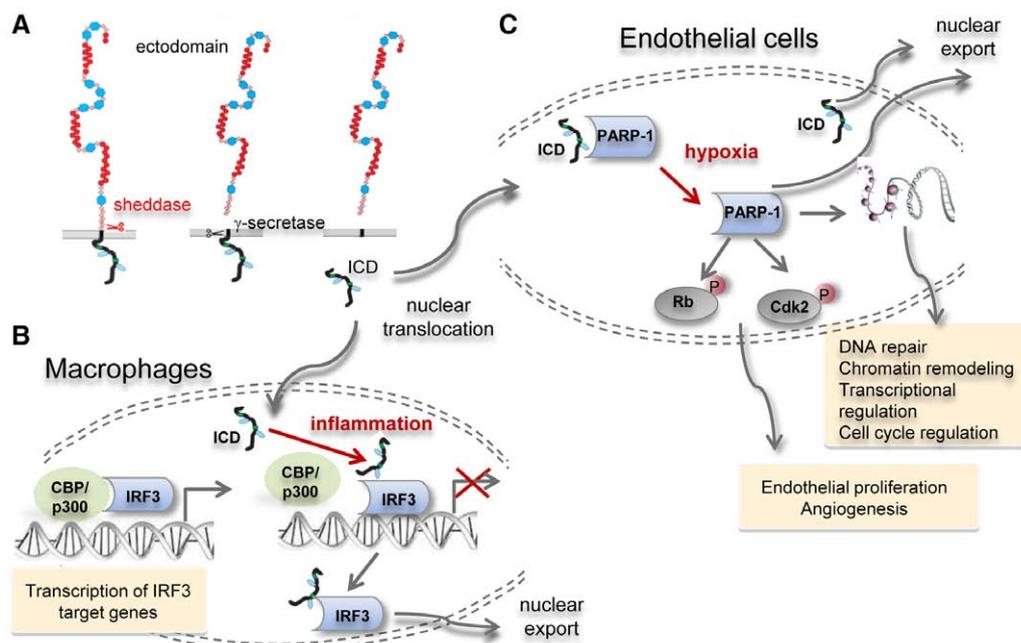


Figure. Regulated intramembrane proteolysis (RIP) of low-density lipoprotein receptor-related protein 1 (LRP1) regulates inflammation and endothelial cell proliferation. **A**, RIP is a sequential process that first involves shedding of the LRP1 ectodomain, likely via an ADAM-like protease. This is followed by presenilin-1-mediated cleavage of shed LRP1 releasing the LRP1-intracellular domain (ICD), which can diffuse to the nucleus. **B**, In macrophages, the LRP1-ICD attenuates lipopolysaccharide-mediated inflammation by associating with interferon regulatory factor 3 (IRF3) resulting in nuclear export of this transcription factor. **C**, In endothelial cells, the LRP1-ICD associates with poly(ADP-ribose) polymerase-1 (PARP-1), attenuating its function. Under conditions of hypoxia, the interaction between LRP1-ICD and PARP-1 is weakened, and PARP-1 is freed to mediate the activation of cyclin-dependent kinase 2 (Cdk2) and inhibition of retinoblastoma (Rb), leading to angiogenesis.

in other pathological and physiological situations needs to be investigated. For example, does this interaction play any role in cancer biology? In addition, through detailed studies of PARP-1 inhibitors additional roles of PARP-1 are emerging, including regulation of inflammatory mediators,³⁸ regulation of transcription factor activity³⁹ and regulation of sex hormone signaling⁴⁰ and cell division.⁴¹ What is the potential role of LRP1 in regulating PARP-1 activation in these scenarios? Furthermore, PARP-1 has been reported to undergo activation during long-term memory,⁴² and it is interesting to consider the possibility that LRP1 may influence this pathway as well. The answers to these questions will prove to be exciting areas for further investigation.

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Disclosures

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