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Role of the LDL Receptor-Related Protein 1 in Regulating Protease Activity and Signaling Pathways in the Vasculature

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Abstract

Aortic aneurysms represent a significant clinical problem as they largely go undetected until a rupture occurs. Currently, an understanding of mechanisms leading to aneurysm formation is limited. Numerous studies clearly indicate that vascular smooth muscle cells play a major role in the development and response of the vasculature to hemodynamic changes and defects in these responses can lead to aneurysm formation. The LDL receptor-related protein 1 (LRP1) is major smooth muscle cell receptor that has the capacity to mediate the endocytosis of numerous ligands and to initiate and regulate signaling pathways. Genetic evidence in humans and mouse models reveal a critical role for LRP1 in maintaining the integrity of the vasculature. Understanding the mechanisms by which this is accomplished represents an important area of research, and likely involves LRP1's ability to regulate levels of proteases known to degrade the extracellular matrix as well as its ability to modulate signaling events.

Keywords

Lipoprotein receptors; LRP1; proteases; aneurysms; smooth muscle cells; extracellular matrix

1. INTRODUCTION

Aortic aneurysms result from the progressive enlargement of the aorta and render the vessel wall unstable, prone to life-threatening dissections and ruptures. This disease was the primary cause of 9,863 deaths in the United States in 2015 [1]. Currently, the two major

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treatments for aortic aneurysms include drugs to lower the hemodynamic stress on the vessel wall and surgery to replace the injured section of the vessel. However, most aortic aneurysms go undetected until they rupture. Unfortunately, our current understanding of the molecular mechanisms leading to aneurysm formation is limited.

It is becoming clear that vascular smooth muscle cells (SMC) play a major role in both the appropriate development and maintenance of the vasculature. Exactly how SMC function to maintain the vasculature and their response to injury is an active area of investigation. SMC are responsible for the production, mechanosensing and mechanoregulation of the extracellular matrix [2]. The major extracellular matrix components in large elastic arteries are elastin and collagen [3]. Elastin provides reversible extensibility during the cardiac cycle, while collagen provides strength and prevents failure at high pressure [3]. Elastin does not turn over in normal, healthy arteries [4], thus damage or degradation of elastin results in severe consequences, including aortic dissections and aneurysms. The direct connection between vascular SMC and elastin fibers is provided by the “elastin-contractile unit” (reviewed in [5]) in which the elastic fibers of the aortic medial wall are surrounded by microfibrils which are attached to the dense plaques and focal adhesions in SMC. Failure of appropriate mechanosensing of SMC, defined as the communication of SMC with the surrounding extracellular matrix, can lead to aortic aneurysms and dissections [2]. Recent investigations have identified the LDL receptor-related protein 1 (LRP1) as a major SMC receptor that functions to maintain the integrity of the vasculature [6–9].

Originally identified as the hepatic receptor responsible for the catabolism of α_2 M-protease complexes [10, 11], LRP1 is now known to bind and mediate the internalization of numerous ligands and to also function in signaling pathways [12, 13]. Deletion of the *Lrp1* gene in mice results in early embryonic lethality at E13.5 [14, 15] due to extensive hemorrhaging resulting from a failure to recruit and maintain SMC and pericytes in the vasculature. Genetic studies have revealed that selective deletion of LRP1 in neurons [16], macrophages [17–21], hepatocytes [22, 23], SMC [6–9], or endothelial cells [24, 25] all lead to significant phenotypic alterations revealing critical roles for LRP1 in regulating physiological processes. For example, selective deletion of LRP1 in SMC has revealed that LRP1 protects against the development of atherosclerosis by controlling platelet-derived growth factor (PDGF) receptor activation and prevents aneurysm formation by mechanisms that are not currently well defined. This review will briefly summarize the features of LRP1 and then discuss its role in regulating the integrity of the vasculature.

2. LRP1 IS A MEMBER OF A HIGHLY CONSERVED RECEPTOR FAMILY

LRP1 is a member of the LDL receptor family which includes the LDL receptor, the VLDL receptor, apoE receptor 2, LRP4, LRP1, LRP1b and LRP2 as its core members (Fig. 1). These receptors are composed of clusters of ligand binding repeats, EGF-repeats, β -propeller domains, a transmembrane domain as well as a cytoplasmic domain. In addition, the LDL receptor, VLDL receptor and apoE receptor 2 contain an additional O-linked sugar domain. Members of this family are highly conserved both at the DNA and protein levels. Utilizing the NCBI HomoloGene database, we compared the DNA and protein sequences of LDL receptor family members with their putative homologs in 12 eukaryotic species (Fig.

2A). Although homolog annotations are incomplete in some species, as indicated by blank tiles, the DNA and protein sequences of the receptor family are remarkably well conserved in vertebrate animals.

LRP1 is synthesized as a single chain molecule and is cleaved by furin in the trans-Golgi into a 515 kDa heavy chain and an 85 kDa light chain [26]. The resultant heavy and light chain remain non-covalently associated in the mature receptor. LRP1 is expressed in most cells and tissues and is most abundant in SMC, hepatocytes, fibroblasts, macrophages and neurons [13, 27]. The physiological roles of LRP1 in diverse tissues are in part mediated by the ability of LRP1 to bind and internalize a variety of structurally-diverse ligands. Investigation of LRP1 ligands and their homologs in eukaryotic species reveal that LRP1 trends toward a higher degree of sequence conservation than any single ligand at both the DNA and protein levels (Fig. 2B). We interpret this finding to mean that the functional role of LRP1 is multifaceted and extends beyond the interaction with any single ligand. This conclusion is supported by the association of LRP1 function with numerous diseases based on both clinical studies and in studies employing various mouse models. These include vascular disease (see below), hepatic steatosis [22], insulin resistance (see review [28]) and Alzheimer's disease (see review [29]).

3. AORTIC ANEURYSMS

The pathobiology of aortic aneurysms is complex and largely unsolved. Unbiased whole genome sequencing is now being used to elucidate the genetic basis of aortic aneurysms to uncover the germline genetic variants that cause or influence the risk of disease. The two types of aortic aneurysms, abdominal aortic aneurysms (AAA) and thoracic aortic aneurysms (TAA) have different etiologies. Abdominal aortic aneurysms (AAA) are more common and are linked to multiple genetic, environmental and lifestyle-associated risk factors, including hyperlipidemia, hypertension, sex, smoking and age [30–33]. Several genes and loci have been associated with AAA, including *LRP1*, *CDKN2B-AS1*, *CNTN3*, *LPA*, *IL6R* and *SORT1*, *MMP3* [31, 34, 35]. Thoracic aortic aneurysms (TAA) are much rarer and have a stronger linkage to genetic factors, and the numerous causal genes thus far identified point to the importance of the maintenance of interaction between vascular SMC and the medial ECM. These genes include matrix components (*FBN1*, *EFEMP2*, *COL3A1*), components of the TGF β signaling pathway (*TGFBR1*, *TGFBR2*, *SMAD3*, *TGFB2*), and smooth muscle cell genes that are involved in the contractile process (*ACTA2*, *MYH11*, *MYLK*) [36–39].

4. GENETIC STUDIES IN HUMANS LINK LRP1 TO ANEURYSMS

Genetic studies, including genome-wide association studies (GWAS), exome sequencing, and TaqMan single nucleotide polymorphism (SNP) genotyping assays, have revealed the association of *LRP1* SNPs with aortic aneurysms, aortic dissections, and Marfan Syndrome (Table 1). In a seminal GWAS, Bown *et al.* [40] demonstrated a significant association of a *LRP1* SNP, rs1466535, with AAA. Additional independent association studies of coronary artery disease, blood pressure, hyperlipidemia, or diabetes showed no association with rs1466535 or the 12q13.3 locus, suggesting a specific association of the locus with AAA.

Bown *et al.* [40] also confirmed during the replication stage that the SNP rs1466535 risk allele is the C allele. Association of *LRPI* SNPs with AAA was confirmed in a separate GWAS by Bradley *et al.* [41]. The discovery stage revealed suggestive association (p -value $< 1 \times 10^{-4}$) of two SNPs within the 12q13.3 locus with AAA, rs4759044 ($p = 5.40 \times 10^{-5}$) and rs1466535 ($p = 2.12 \times 10^{-5}$). In contrast to the GWAS by Bown *et al.* [40], the SNP rs1466535 risk allele reported by Bradley *et al.* [41] is the A allele. Interestingly, a separate study [42] involving TaqMan SNP genotyping of an Italian population showed a significant ($p = 0.01$) association of the rs1466535 polymorphism with AAA, but the study reported a T risk allele for the locus. In a recent meta-analysis of GWAS for AAA [35], the previously reported *LRPI* SNPs associated with AAA were not replicated. A newly identified SNP within the 12q13.3 locus, rs1385526, showed border-line association with AAA. Although the SNP reached the discovery threshold of significance ($p = 1.1 \times 10^{-9}$), it failed to reach the genome-wide threshold of significance when combined with the validation cohort ($p = 6.4 \times 10^{-7}$). Although these studies reported different SNPs within the 12q13.3 locus and risk alleles associated with AAA, these results confirm that polymorphisms of the *LRPI* gene is a susceptibility factor for AAA. It is likely that reported differences in association significance (*i.e.* p -values) and risk alleles are largely attributed to population availability and variation. Importantly, biological evidence and association with other vascular diseases support the role of *LRPI* in AAA and warrant further investigation. Variants in *LRPI* have also been shown to be associated with aortic dissections. In a GWAS, Debette *et al.* [43] identified two *LRPI* SNPs associated with cervical artery dissections, rs11172113 ($p = 3.03 \times 10^{-7}$) and rs1466535 ($p = 4.94 \times 10^{-6}$). Interestingly, the same SNP rs11172113, identified through exome sequencing, was significantly associated with a decreased risk for sporadic thoracic aortic dissection (STAD) ($p = 2.74 \times 10^{-8}$) and type A dissection alone ($p = 3.11 \times 10^{-6}$) [44]. These studies demonstrate that different *LRPI* variants can be associated with an increased risk for specific vascular diseases or confer protection.

Connective tissue disorders, such as Marfan syndrome, often involve cardiovascular complications including aortic aneurysms and dissections, mitral valve prolapse, and aortic regurgitation. Exome sequencing of two Marfan syndrome patients from a four-generation Marfan syndrome family identified a missense mutation ($G > A$) in exon 15 of *LRPI* [45]. This study further supports the involvement of the *LRPI* gene in aortic aneurysms and dissections and provides an additional target for further pathobiological research.

The human *LRPI* gene is located on chromosome 12 and contains 89 exons (Ensembl Release 89 – May 2017; Gene ENSG00000123384; Transcript ID ENST00000243077.7). A majority of the *LRPI* variants cataloged in *Homo sapiens* [46], occur within introns of the gene. Furthermore, *LRPI* SNPs associated with AAA or aortic dissections are located within the first two introns (Fig. 3). Gene expression studies by Bown *et al.* [40] showed a trending increase in *LRPI* expression with the rs1466535 CC genotype in sampled arterial tissues and a significant ($p = 0.029$) increase (1.19-fold [1.04–1.36]) in *LRPI* expression in the aortic adventitia of CC homozygotes compared to TT homozygotes. Additional functional studies suggested that rs1466535 may alter a SREBP-1 binding site and influence enhancer activity [40]. In contrast to the *LRPI* SNP locations associated with AAA or aortic dissections, the *LRPI* SNP associated with Marfan syndrome was mapped to exon 15. Exon 15 corresponds to an EGF repeat located between a β -propeller (YWTD) domain and cluster II on the *LRPI*

ectodomain, and a missense mutation at this location may affect ligand binding. Collectively, genetic studies have demonstrated a significant association of *LRP1* SNPs with AAA, aortic dissections, and Marfan syndrome; however, additional functional studies are necessary to elucidate the effect of these polymorphisms on ligand binding and/or signaling pathways.

Of interest in this regard are the studies of Chan *et al.*, [47] who reported a significant reduction in protein levels of LRP1 in human AAA samples associating LRP1 with AAA pathogenesis. Recently, Chan *et al.* [48] demonstrated that translational inhibition by microRNA-205 was responsible for driving the lower expression levels of LRP1. Furthermore, lower levels of LRP1 were speculated to result in accumulation of excess matrix metalloproteinase (MMP)-9, a well-documented protease that contributes to the degradation of the extracellular matrix proteins leading to AAA [49].

5. A PARADOXICAL ROLE FOR TGF β SIGNALING IN THE VASCULATURE

The role of TGF β signaling in vascular development and its subsequent contribution to the development of vascular disease is still not fully understood. First of all, studies in mice have revealed that TGF β signaling is absolutely required for appropriate vascular development, and global loss of TGF β signaling or selective ablation of TGF β signaling in SMC during development leads to embryonic lethality due to severe vascular defects [50–53]. An important role for TGF β signaling during vascular development is to facilitate SMC differentiation. Interestingly, conditional deletion of *Tgfb2* [54, 55] or *Smad4* [56] in SMC following birth results in aneurysm formation, revealing that TGF β signaling is not only required for vascular development, but is also essential for vessel wall homeostasis in postnatal mice.

In apparent contradiction to these results, mouse models have revealed that excess TGF β signaling promotes TAA and dissections in a mouse model of Marfan syndrome [57–59] as well as in mouse models of Loeys-Dietz syndrome [60]. This occurs *via* non-canonical TGF β signaling resulting in activation of the ERK1/2 and JNK1 pathways [61]. While all of the complexities of TGF β signaling in the vasculature are far from being resolved, an elegant study of Cook *et al.* [62], provides significant insight into the essential role of TGF β signaling during vascular development along with the harmful effects of excess signaling following development of the vasculature. This study found that neutralizing TGF β signaling in mice prior to postnatal day 16 exacerbated TAA formation, while neutralizing TGF β signaling following postnatal day 45 reduced TAA formation. Taken together, these studies reveal that tight regulation of TGF β signaling pathways is essential for vascular development as well as for maintenance of the vessel wall, and that deregulation of TGF β signaling leads to disease.

A pivotal discovery employing a Marfan mouse model was the finding that administration of the angiotensin II type 1 receptor antagonist, losartan corrected ascending aortic dilatation through its ability to attenuate TGF β signaling [58]. Interestingly, losartan was much more effective than β -adrenergic blocking agents, such as propranolol in this capacity [58]. These exciting results have led to clinical trials examining the effectiveness of losartan for the treatment of Marfan syndrome including the “Atenolol *versus* losartan in children and young

adults” trial [63]. The results of this study revealed no significant difference between these two treatments, and thus the dramatic results of losartan therapy over those of β -blockers observed in Marfan mice were not duplicated inpatient studies, perhaps for a variety of reasons [64].

6. LRP1 PROTECTS THE VASCULATURE

A protective role for LRP1 is well documented in numerous vascular beds. Under hypercholesterolemic conditions in cholesterol-fed LDL receptor-deficient mice, Boucher *et al.* showed an increase in vascular occlusion in mice in which the *Lrp1* gene was specifically deleted in vascular SMC. This effect was attributed to excess activation of the PDGF-signaling pathway [6]. Selective deletion of LRP1 in macrophages also resulted in the development of more extensive atherosclerosis [17–20]. Further, extensive vascular remodeling in these mice was noted employing a carotid ligation model [21]. At present, it is not clear how macrophage LRP1 modulates these processes. It does appear, however, that macrophage LRP1 regulates the TGF β signaling pathway [21], macrophage migration [65] and inflammation [66]. In the brain, LRP1 plays an important role as a key clearance receptor for amyloid- β by endothelial cells as well as SMC of the cerebrovasculature [67–69].

Finally, studies have identified LRP1 as a major SMC receptor that plays a critical role in maintaining the integrity of the vessel wall to prevent aneurysm formation [8, 70]. In mice, genetic deletion of *LRP1* in smooth muscle cells results in the development of spontaneous thoracic aneurysms, as well as progressive aortic root growth and aberrant thickening of the aortic media with fragmentation and disarray of elastic fibers [8, 70, 71]. While the mechanisms are currently not fully understood, it is clear that LRP1’s ability to regulate protease activity and to modulate signaling pathways contribute to this protective role.

7. LRP1 PLAYS A MAJOR ROLE IN REGULATING THE LEVELS OF PROTEASES

Studies have revealed that LRP1 plays a major role in regulating the levels of numerous proteases, protease inhibitors, protease cofactors and complexes of proteases with their inhibitors (Table 2). For certain proteases, their regulation by LRP1 occurs when the protease directly binds LRP1 on the cell surface and is then transported into the cell where it is delivered to lysosomal compartments for subsequent degradation. Interestingly, proteases thus far known to directly bind to LRP1 include those involved in the degradation of the extracellular matrix as well as those involved in blood coagulation and fibrinolysis. Secreted metalloproteinases that degrade the matrix whose levels are regulated by LRP1 include ADAMTS-4 and ADAMTS-5, MMP-13, MMP9 and MMP2. ADAMTS-4 and ADAMTS-5 degrade proteoglycans including Aggrecan, a major component of cartilage. Excess activity of these proteases leads to degradation of Aggrecan, which is a significant event in early stages of osteoarthritis [72, 73]. MMP-13 is thought to play a critical role in the degradation of cartilage and also contributes to the early phases of osteoarthritis [74]. MMP-9 and MMP-2 are involved in tissue remodeling and inflammation [75] and evidence suggests that both proteases can contribute to the development of AAA [76]. Interestingly, MMP-2 is

upregulated in tissue from patients with TAA and aortic dissections [77], and serum levels of MMP-2 correlate with ascending aortic diameter in patients with ascending aortic aneurysms [78]. MMP-2 does not directly bind LRP1, but does bind tightly to thrombospondin 2, which then can interact with LRP1 to mediate the cellular internalization of the complex [78]. Finally, LRP1 also regulates the levels of high temperature requirement A1 (HtrA1) [8], a secreted serine protease that attenuates TGF β signaling and also degrades components of the extracellular matrix [80]. HtrA1 levels increase dramatically in mice in which LRP1 is genetically deleted in SMC [8].

In addition to binding and mediating the cellular internalization of matrix-degrading proteases, LRP1 also regulates the levels of several proteases and co-factors involved in blood coagulation and fibrinolysis. These include factor fVIII, a co-factor protein whose deficiency leads to hemophilia A. Upon activation of fVIII to fVIIIa by the enzyme thrombin, fVIIIa associates with fIXa to form a proteolytic complex responsible for activating factor X to Xa. Factor Xa in complex with fVa are the final enzymes responsible for generating thrombin, the terminal protease in the blood coagulation cascade. In addition to regulating thrombosis by removing factors VIII and IXa, LRP1 also binds the plasminogen activators urokinase-type plasminogen activator (uPA) as well as tissue-type plasminogen activator.

Protease activity is tightly regulated by inhibitors. In the circulation, most protease inhibitors belong to the Serpin family of inhibitors. Early work by Ohlsson and colleagues [81] investigating the clearance of trypsin-inhibitor complexes from the circulation suggested the existence of an hepatic receptor responsible for the clearance of serpin-enzyme complexes. We now know that LRP1 is responsible for binding and mediating the removal of a number of serpin-enzyme complexes (Table 2). Most of these inhibitors only bind to LRP1 as a complex with a protease, but PAI-1 [82] and neuroserpin [83] appear capable of binding directly to LRP1 without a proteinase. Of interest, the tissue inhibitor of metalloproteinases 3 (TIMP3) also binds directly to LRP1 [84]. TIMP3 is an important inhibitor of several metalloproteinases.

8. SIGNALING PROPERTIES OF LRP1

Similar to the diversity of LRP1 ligands, LRP1 modulates a wide variety of signaling pathways, including the PDGF [85, 86], TGF β [21, 87, 88] and MAPK signaling pathways [89, 90], as well as the activation of Src family kinases [91], and the mediation of the bone morphogenic protein-binding endothelial regulator (BMPER) signaling pathway [92]. One mechanism by which LRP1 can precisely activate a wide array of signaling pathways is *via* interaction with co-receptors. In this paradigm, LRP1 ligands either recruit co-receptors *via* direct interaction with the co-receptor or induce conformational changes in LRP1 upon binding that enable LRP1 to interact with co-receptors. Co-receptor association with LRP1 results in the recruitment of additional scaffolding proteins [90], the phosphorylation of tyrosine residues within the LRP1 cytoplasmic domain [85, 86], and activation of specific signaling pathways [93]. Despite significant progress, mechanisms by which LRP1 regulates signaling pathways are still not fully understood.

8.1. Proteases and Protease Inhibitors as LRP1 Signaling Mediators

In addition to their role in degradation of the extracellular matrix, proteases and protease inhibitors compose a major class of LRP1 ligands that can initiate signaling events. In bone-marrow-derived macrophages, LRP1 has been demonstrated to regulate the inflammatory response by modulating NF κ B activation [66] (Table 3). Initial studies discovered that the response of macrophages to LPS was significantly enhanced when LRP1 was genetically deleted. Interestingly, both tissue-type plasminogen activator tPA (or an enzymatically inactive form of tPA) and activated forms of α_2 -macroglobulin (α_2 M*) inhibited the response of BMDM to lipopolysaccharide (LPS). This occurs *via* a process in which these two ligands prevent the LPS-mediated phosphorylation of I κ B α , a key regulator of the NF- κ B pathway. Late in the canonical NF- κ B pathway, I κ B α is phosphorylated and then tagged for proteasomal degradation. I κ B α degradation then allows translocation of the p50/p65 phosphorylated complex to the nucleus to induce transcription of NF- κ B regulated genes.

Studies have also revealed that another protease, matrix metalloproteinase-9 (MMP-9) also interacts with LRP1 to initiate a signaling cascade that may be significant in regulating Schwann cell migration upon peripheral nervous system injury [89]. This study observed that association of the MMP9 hemopexin domain with LRP1 activates a MAPK signaling cascade. Silencing of LRP1 or inhibition of MMP-9 hemopexin domain binding with receptor associated protein (RAP) which binds to LRP1 at multiple sites and inhibits its ability to bind ligands [94–96] prevented activation of Akt and ERK1/2 in Schwann cells. The MMP-9 signaling effect is not generalizable to other MMPs since MMP-2, which is not recognized by LRP1, did not induce activation of Akt and ERK1/2. Interestingly, in a crush injury model of peripheral nervous system injury, LRP1-dependent MMP-9 signaling was specific to only injured sciatic nerves with no noticeable pathway effects on undamaged nerves.

Prior studies have shown that the protease inhibitor plasminogen activator inhibitor-1 (PAI-1) can block SMC migration by regulating the accessibility of cell associated integrin attachment sites in the matrix [97]. This occurs when PAI-1 associates with vitronectin to block its interaction with integrin $\alpha_v\beta_3$. Interestingly, formation of a complex of PAI-1 with plasminogen activators results in loss of affinity of PAI-1 for vitronectin and restores cell migration. In addition to inhibiting SMC migration by blocking integrin binding sites in the matrix, PAI-1 can also stimulate cell migration *via* an LRP1-dependent mechanism [98]. Degryse *et al.* [98] demonstrated that PAI-1 stimulated rat SMC migration and this was attributed to the ability of either active or cleaved PAI-1 to mediate activation of the JAK/STAT signaling system. The effect of PAI-1 on LRP1-induced cell migration of rat SMC was independent of uPA, uPa receptor (uPAR), or vitronectin [98, 99]. More recent studies have suggested that PAI-1 also appears capable of modulating cell migration *via* an LRP1-dependent activation of β -catenin and ERK1/2 MAPK pathway [100]. Interestingly, PAI-1 induced transcription of beta-catenin-related genes in mouse embryonic fibroblasts expressing LRP1, but not in mouse embryonic fibroblasts deficient in LRP1.

8.2. Attenuation of TGF β Signaling by LRP1

Several studies support the notion that LRP1 is an important modulator of the TGF β signaling pathway. Initial studies by Huang *et al.* [101] demonstrated that ^{125}I -labeled TGF β 1 could be crosslinked to LRP1, and further demonstrated that murine fibroblasts in which LRP1 was genetically deleted were not sensitive to growth inhibition by TGF β [101] (Table 3). Further confirmation came from the studies of Tseng *et al.* [102] who demonstrated that TGF β 1 inhibits the growth of wild-type Chinese hamster ovary cells, but not the growth of LRP1-deficient Chinese hamster ovary cells. *In vivo* evidence that LRP1 modulates the TGF β signaling pathway is derived from vascular remodeling studies in mice in which LRP1 was selectively deleted from macrophages [21]. In these studies, macrophage LRP1 was found to protect the vasculature by limiting remodeling events associated with injury. This resulted from the ability of macrophage LRP1 to reduce TGF β 2 protein levels and to attenuate expression of the TGF- β 2 gene, resulting in suppression of the TGF β signaling pathway.

It is not clear how LRP1 attenuates the TGF β signaling pathway. One possibility is that LRP1 regulates levels of TGF β ligands by mediating their internalization. This notion is supported by surface plasmon resonance experiments revealing that TGF β 2 binds to LRP1 with affinity values close to those measured for the binding of TGF β 1 to soluble forms of TGFBR2 [21]. Furthermore, TGF β 2 is crosslinked to LRP1 in macrophages, and TGF β 2 antigen accumulates in conditioned media of LRP1-deficient macrophages [21]. In addition to its ability to reduce TGF β levels, LRP1 also appears to attenuate gene expression as well [21], and very likely modulates the TGF β signaling pathway by additional mechanisms.

Studies have revealed that the role of LRP1 in regulating TGF β signaling pathways often involve other molecules. For example, studies have shown that LRP1 functions as an endocytic receptor for decorin [103, 104], a small leucine-rich proteoglycan that is known to modulate TGF β signaling pathways [105]. Interestingly, Cabello-Verrugio and Brandan discovered a novel modulatory signaling pathway requiring both activation of the Smad pathway and phosphatidylinositol 3-kinase in which decorin and LRP1 cooperate to modulate TGF β signaling responses in myoblasts [106].

8.3. Co-receptor Involvement in LRP1 Signaling

LRP1 is capable of assembling unique co-receptor complexes that depend upon the specific ligand [90]. For example, studies found that treating PC12 and N2a neuron-like cells with tPA (or enzymatically inactive tPA) or $\alpha_2\text{M}^*$ resulted in rapid activation of ERK1/2 that was completely blocked by the LRP1 antagonist RAP [90]. tPA or $\alpha_2\text{M}^*$ treatment also resulted in phosphorylation of the tyrosine receptor kinase (Trk) receptors. All of these processes seemed to require the N-methyl-D-aspartate receptor (NMDAR) [90]. Together, these studies suggested that NMDARs cooperate with LRP1 in mediating the activation of the MAPK pathway. This cooperation may involve cytoplasmic adaptor proteins as studies have demonstrated that LRP1 can co-precipitate with NMDAR and postsynaptic density protein 95 [16, 107]. Interestingly, the tPA or $\alpha_2\text{M}^*$ mediated signaling events described above were ligand specific, as myelin-associated glycoprotein, another LRP1 ligand, failed to mediate the activation of ERK1/2 or induce phosphorylation of TrkA. In contrast, the association of

myelin-associated glycoprotein with LRP1 stimulated a completely different signaling pathway, which was accomplished by recruiting the p75 neurotrophin receptor into a complex with LRP1 resulting in the formation of activated RhoA [90].

A second example for a ligand-induced co-receptor function for LRP1 involves the PDGF signaling pathway. Initial studies [85] demonstrated that PDGF-BB directly binds to LRP1 and promotes transient tyrosine phosphorylation of the LRP1 cytoplasmic domain. This process requires activation of the PDGF receptor and activation of Src. Employing immunofluorescence and immunogold electron microscopy experiments, LRP1 and PDGF receptor- β were subsequently demonstrated to co-localize in coated-pits and endosomal compartments [86, 108]. Furthermore, phosphorylated forms of the PDGFR- β co-precipitated with LRP1 [108]. Interestingly, phosphorylation of LRP1 is blocked by treating the cells with dynamin inhibitors to prevent endocytosis [86]. Tyrosine phosphorylation of the LRP1 cytoplasmic domain generates a binding site on LRP1 for the adaptor proteins Shc [85, 109] and SHP-2 [110], a cytoplasmic SH2 domain containing protein tyrosine phosphatase, thereby modulating PDGF-mediated signaling events.

In addition to co-receptor complex formation, LRP1 can initiate signaling pathways by trans-activation of a second receptor. For example, ligand binding to LRP1 in embryonic sensory neurons results in the activation of Src family kinase members, which in turn mediated the transactivation of Trk receptors and lead to the activation of ERK1/2 and Akt [91]. Transfection of cells with a kinase-defective Src prevented phosphorylation of ERK1/2 and Akt. This signaling pathway is required for neurite sprouting in embryonic sensory neurons [111]. Finally, studies have also demonstrated that LRP1 interact with frizzled-1, a key co-receptor in the canonical Wnt signaling pathway [112]. This interact results in the formation of an inhibitory complex leading to suppression of the canonical Wnt signaling pathway.

8.4. CTGF-mediated LRP1 Signaling

Connective tissue growth factor (CTGF/CCN2) is a small cysteine-rich protein that is a member of the CCN family [113]. CTGF plays a variety of roles in the vasculature and contributes to angiogenesis, fibrogenesis, atherosclerosis and restenosis. Segarini *et al.* [114] were the first to identify LRP1 as a receptor for CTGF. CTGF protein levels, but not mRNA levels, were significantly increased in mice in which LRP1 was selectively deleted in SMC [8]. While not fully understood at present, LRP1 does influence signaling events mediated by CTGF. Thus, in renal myofibroblasts, CTGF mediates the tyrosine phosphorylation of the LRP1 cytoplasmic domain, an event that is required for the synergistic effect of CTGF and TGF- β to activate ERK1/2 and induce expression of α -SMA in these cells [115].

CONCLUSIONS

As a cellular receptor, LRP1 has the capacity to modulate an enormous number of biological processes, both by binding and removing molecules targeted for degradation and by initiating specific signaling pathways. The two major classes of ligands for this receptor, lipoproteins and proteases, contribute to important physiological processes. The diversity of LRP1 function arises from its capacity to interact with numerous ligands as well as other

transmembrane proteins to affect specific biological processes. Ligand-mediated assembly of co-receptors to participate along with LRP1 in signaling events provides remarkable specificity and diversity to this receptor's function. Evidence is beginning to accumulate revealing a critical function for this receptor in maintaining the integrity of the vasculature. This evidence includes genetic studies in humans as well as in mouse models in which the LRP1 gene has been selectively deleted in various cells. Defining the endocytic and signaling functions of LRP1 and how they integrate at the molecular level to affect cellular processes will require additional studies.

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LIST OF ABBREVIATIONS

LDL	Low density lipoprotein
LRP	LDL receptor-related protein
SMC	Smooth muscle cells
RAP	Receptor associated protein
TGFβ	Transforming growth factor β
EGF	Epidermal growth factor
TAA	Thoracic aortic aneurysms
AAA	Abdominal aortic aneurysms
GWAS	Genome-wide association study
SNP	Single nucleotide polymorphism
MMP	Matrix metalloprotease
ERK	Extracellular signal-regulated kinases
JNK	c-Jun N-terminal kinase
ADAMTS	A disintegrin and metalloproteinase with a thrombospondin type 1 motif
HtrA1	High temperature requirement A1
PDGF	Platelet derived growth factor
PAI-1	Plasminogen activator inhibitor-1
TIMP3	Tissue inhibitor of metalloproteinase 3

Trk	Tyrosine receptor kinase
NMDAR	N-methyl-D-aspartate receptor

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Fig. 1. Core members of the LDL receptor family.

Core members of this receptor family include similar domain organization consisting of ligand binding repeats, epidermal growth factor (EGF) repeats, β-propeller domains, a transmembrane domain and cytoplasmic domains containing one or more NPxY motifs.

Fig. 2. LRP1 and the LDL receptor family are highly conserved.

(A) The percent identity of human DNA and protein sequences for the LDL receptor family members against their predicted homologs in 12 species were retrieved from the NCBI HomoloGene database. Tiles with a black circle indicate that there is currently no annotation for a receptor homolog in the indicated species. The high levels of sequence identity (black) indicate that the family is particularly well conserved in vertebrate species. For example, human LRP1 protein is 92%, 99%, 98%, 98%, 98%, 87%, 83%, 77%, 40% and 41% identical to *P.troglodytes*, *C.lupus*, *B.taurus*, *M.musculus*, *R.norvegicus*, *G.gallus*, *X.tropicalis*, *D.rerio*, *D.melanogaster* and *A.gambiae* LRP1 homologs. (B) The sequence identity of prominent LRP1 ligands in these species indicate that they are generally less conserved than LRP1 (open circles). This suggests that the biological role of LRP1 extends beyond the interaction with any single ligand.

Fig. 3. LRP1 gene structure and mapped SNPs.

The human LRP1 gene contains 89 exons and is located on chromosome 12q13.3. Genetic studies have revealed the association of LRP1 SNPs with abdominal aortic aneurysms (black), aortic dissections (open), and Marfan Syndrome (dotted). LRP1 SNPs associated with abdominal aortic aneurysms or aortic dissections are located within the first two introns while the LRP1 SNP associated with Marfan Syndrome, a missense mutation (G > A), is located in exon 15. (Ensembl Release 89 – May 2017; Gene ENSG00000123384; Transcript ID ENST00000243077.7).

Table 1.

Genetic studies of LRP1 associated with aortic aneurysms, aortic dissections, and disease.

	SNP	Functional Class	Risk Allele	OR (95% CI)	p-value	Method	References
Aortic Aneurysms							
Abdominal	rs1466535	Intron	C	1.15 (1.10–1.21)	4.52×10^{-10}	GWAS	[40]
	rs4759044	Intron	G	0.85 (0.79–0.92)	5.40×10^{-5}	GWAS	[41]
	rs1466535	Intron	A	0.84 (0.77–0.91)	2.12×10^{-5}	GWAS	[41]
	rs1466535	Intron	T	1.85 (1.2–2.84)	0.01	TaqMan	[42]
	rs1385526	Intron	C	0.91 (0.877–0.944)	6.4×10^{-7}	GWAS	[35]
Aortic Dissections							
Cervical Artery	rs11172113	Intron	C	0.82 (0.76–0.89)	3.03×10^{-7}	GWAS	[43]
	rs1466535	Intron	A	0.83 (0.77–0.90)	4.94×10^{-6}	GWAS	[43]
Sporadic Thoracic Type A + B	rs11172113	Intron	C [†]	0.82 (0.76–0.89)	2.74×10^{-8}	Exome	[44]
Sporadic Thoracic Type A	rs11172113	Intron	C [†]	0.81 (0.75–0.89)	3.11×10^{-6}	Exome	[44]
Other Diseases							
Marfan Syndrome	n/a	Exon	A	n/a	n/a	Exome	[45]

[†]Minor allele associated with a decreased risk. n/a: Not applicable

Table 2. Proteases, co-factors, protease inhibitors and protease-inhibitor complexes that bind to LRP1.

	Function	References
Proteases that degrade the extracellular matrix		
ADAMTS-4	Degrades cartilage proteoglycans	[116]
ADAMTS-5	Degrades cartilage proteoglycans	[117]
MMP-13	Degrades extracellular matrix	[118]
MMP-9	Local proteolysis of extracellular matrix	[119]
TSP-2:MMP-2 complexes	Degrades extracellular matrix	[79]
HtrA1	Degrades extracellular matrix	[8]
Proteases and cofactors involved in blood coagulation		
Factor IXa	Blood coagulation	[120]
Factor VIII	Blood coagulation	[121, 122]
Factor V	Blood coagulation	[123]
Proteases involved in fibrinolysis		
tPA	Fibrinolysis	[79, 124]
Pro-uPA and uPA	Fibrinolysis	[125]
Protease inhibitors that regulate blood coagulation		
Heparin cofactor II:thrombin complexes	Regulation of blood coagulation	[126]
ATIII-thrombin complexes	Regulation of blood coagulation	[126]
Protein C inhibitor:protease complexes	Regulation of blood coagulation	[127]
TFPI and TFPI:VIIa complexes	Regulation of blood coagulation	[128]
Protease nexin 1:protease complexes	Regulation of blood coagulation, development	[129]
Protease inhibitors that regulate fibrinolysis		
PAI-1 and PAI-1:protease complexes	Regulation of fibrinolysis	[130]
Neuroserpin and Neuroserpin:protease complexes	Plasminogen activator inhibitor in brain, synaptic plasticity	[131]
Pan-protease inhibitors and inhibitors that regulate inflammation and complement activation		
Activated α_2 M and α_2 M:protease complexes	Inflammation, pan-protease inhibitor	[10, 11]
PZP protease complexes	Pan-protease inhibitor	[132]

References	
[133]	Regulation of complement activation
[126]	Neutrophil elastase inhibitor, protects against inflammation and matrix degradation
Protease inhibitors that regulate matrix degradation	
	Timp3
[134]	
Regulation of matrix degradation	

Table 3.

LRP1-mediated signaling pathways.

Primary Ligands	Co-receptors	Function	Pathway Interactions	References
tPA or α_2M	Trk NMDAR	Fibrosis, inflammation, cell survival	ERK1/2, SFK, RhoA, PI3K, NF- κ B, PSD-95,	[16, 66, 90, 91, 107, 111]
MMP-9		Cell migration, Peripheral nervous system injury	ERK1/2, PI3K	[89]
PAI-1		Cell survival, cell migration	ERK1/2, β -catenin, PI3K, JAK1/STAT1, vitronectin, integrin $\alpha_v\beta_3$	[97, 98, 100]
uPA	EGFR	Cell migration, fibrosis	ERK	[99]
TGF- β	TGF- β RI TGF- β RII Decorin	Cell growth, matrix remodeling	Smad2/3, ERK1/2	[21, 87, 88, 101, 102, 106]
PDGF	PDGFR- β	Aneurysm formation	ERK1/2, Shc, SFKs	[85, 86]
CTGF		Fibrosis, fibroblast differentiation	ERK1/2, Akt	[115]
BMPIR		Angiogenesis, endothelial cell proliferation, cell cycle progression	PARP-1, CDK2, Rb, NFATc1, ERK, INF- γ , NF- κ B, NF45	[24, 92]
Frizzled	Frizzled-1	Tissue development	Wnt3A, LRP6	[112]