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## *Review*

# The low density lipoprotein receptor-related protein (LRP) 1 and its function in lung diseases

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**Summary.** The low density lipoprotein receptor-related protein (LRP) 1 is a ubiquitously expressed, versatile cell surface transmembrane receptor involved in embryonic development and adult tissue homeostasis. LRP1 binds and endocytoses a broad spectrum of over 40 ligands identified thus far, including lipoproteins, extracellular matrix proteins, proteases and protease/inhibitor complexes and growth factors. Interactions with other membrane receptors and intracellular adaptors/scaffolding proteins allow LRP1 to modulate cell migration, survival, proliferation and (trans) differentiation. Because LRP1 displays a wide-range of interactions and activities, its expression and function is temporally and spatially tightly controlled. It is not, therefore, surprising that deregulation of LRP1 production and/or activity is observed in several diseases. In this review, we will systematically examine the evidence for the role of LRP1 in human pathologies placing special emphasis on LRP1-mediated pathogenesis of the lung.

**Key words:** Low density lipoprotein receptor-related protein 1, Extracellular matrix, Proteases, Lung

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### Structure and function of LRP1

LRP1 is a large endocytic receptor widely expressed in several tissues and known to play a role in areas as diverse as lipid metabolism, protein degradation, cell migration, and entry of bacterial toxins and viruses (Lillis et al., 2008). LRP1, a member of the low-density lipoprotein (LDL)-receptor family, is a type I transmembrane receptor composed of two chains, a 515 kDa heavy chain and an 85 kDa light chain, both noncovalently associated on the cell surface. LRP1 consists of five structural subunits: (a) ligand binding type cysteine-rich repeats, (b) epidermal growth factor (EGF) receptor-like cysteine-rich repeats, (c) YWTD domains, (d) a single membrane-spanning segment, and (e) a cytoplasmic tail. Ligand binding-type cysteine-rich repeats are arranged in four clusters I-IV, whereby clusters II and IV are responsible for binding the majority of the currently known ligands of LRP1 (Neels et al., 1999). The cytoplasmic tail of LRP1 encompasses two di-leucine sequences, one YXXL motif, and two NPXY motifs (a proximal NPTY and a distal NPVY). The YXXL motif serves as the dominant endocytosis signal (Li et al., 2000), whereas the NPXY motifs, alongside their role in receptor endocytosis and recycling (Farfan et al., 2013), provide docking sites for the scaffolding/adaptor proteins involved in the signaling events.

LRP1 is a scavenger-type receptor that is constitutively endocytosed from the cell membrane and recycled back to the cell surface. It binds over 40 ligands including: lipoproteins, proteases, protease-inhibitor complexes, extracellular matrix proteins, growth factors,

bacterial toxins, and viruses (Lillis et al., 2008). Upon binding to LRP1, ligands are rapidly internalized *via* coated pit-mediated endocytosis and are successively degraded in lysosomes. The ability of LRP1 to bind and rapidly internalize different proteins with a wide range of functions suggests its important role in tissue homeostasis (Etique et al., 2013). Besides its role in endocytosis, LRP1 also functions as a signaling receptor. The phosphorylation of LRP1's cytoplasmic tail and subsequent binding of the scaffolding/adaptor proteins represents a mechanism to switch LRP1 function from endocytosis to signaling. Numerous cytosolic proteins including c-Jun-amino-terminal kinase-interacting protein (JIP) 1, Src homology 2 domain-containing transforming protein (Shc), FE65, disabled (DAB) 1, postsynaptic density protein (PSD)-95, and PTB domain-containing engulfment adapter protein (GULP) 1 were found to associate with LRP1 in order to deliver signals to the effector molecules (Lillis et al., 2008). For example, the binding of JIP1 to LRP1 was shown to hinder the activation of c-Jun and Elk-1, consequently reducing apoptosis of cerebellar neurons in response to DNA damage (Lutz et al., 2002). In addition, LRP1 was reported to control intracellular signaling pathways by regulating the activity and cell surface abundance of other receptors. In this respect, LRP1 was found to interact with the platelet-derived growth factor receptor (PDGFR)-β and urokinase-type plasminogen activator (u-PA)/u-PA receptor (u-PAR) complexes. Association of LRP1 with PDGFR-β was shown to modulate the access of PDGFR-β to the ubiquitination machinery and, thereby, the trafficking of PDGFR-β from the cell surface to intracellular compartments for degradation (Takayama et al., 2005). The binding of LRP1 to u-PA/u-PAR was reported to be crucial to restore wound repair capacity of lipid-loaded vascular smooth muscle cells (Lugano et al., 2013) and to promote cell survival, proliferation and migration. The multifunctional nature of LRP1 is underscored by the fact that the targeted disruption of the *Lrp1* gene in the mouse arrests the development of *LRP1*$^{-/-}$ embryos at the implantation stage (Herz et al., 1992).

In 1999 Quinn et al. described the existence of the truncated form of LRP1 referred to as soluble LRP1 (sLRP1) in human plasma (Quinn et al., 1999). sLRP1 comprises the complete ligand binding heavy chain and an $NH_2$-terminal portion of the light chain. Diverse proteases such as disintegrin and metalloproteinase (ADAM)-10, -17, -12, membrane type 1-matrix metalloproteinase (MT1-MMP), β-site of amyloid precursor protein cleaving enzyme (BACE), and tissue-type plasminogen activator (t-PA) were found to be involved in LRP1 processing and, thereby, in the regulation of sLRP1 abundance in the tissue (Etique et al., 2013). Although the physiological meaning of LRP1 extracellular cleavage remains elusive, it is believed that sLRP1, due to its ability to bind most of the ligands, quenches ligand-cell interactions and prevents ligand endocytosis or ligand-mediated activation of the signaling pathways. The amounts of sLRP1 were found to be increased in plasma of patients with liver abnormalities (Quinn et al., 1997), in endotracheal aspirates after cardiopulmonary bypass (Williams et al., 2005), and in cerebrospinal fluid of older individuals indicating that altered sLRP1 levels may be associated with pathologic conditions and aging (Liu et al., 2009).

LRP1 is most prominently present in the brain, liver, lung, intestine and muscle (Herz et al., 1988). Tissue specific gene deletion studies as well as pharmacological approaches aimed at LRP1 inhibition revealed an important role of LRP1 in the development of atherosclerosis, neurodegenerative disorders, kidney fibrosis as well as in carcinogenesis (Lin and Hu, 2014). This review outlines the role of LRP1 in pathological conditions with the main focus being set on the importance of LRP1 in the development of lung diseases.

## LRP1 in vascular, neurodegenerative and kidney disorders and in carcinogenesis

The broad range of ligand diversity and the ubiquitous expression suggest that LRP1 is involved in diverse physiological and pathological processes. In the vascular wall, LRP1 maintains smooth muscle cell (SMC) homeostasis. Deletion of the *LRP1* gene in vascular SMC (VSMC) leads to VSMC proliferation, disruption of the elastic lamina, aortic aneurysm formation, and notably enhanced susceptibility to atherosclerotic lesion development (Boucher et al., 2003). The mechanism by which LRP1 protects against the formation of atherosclerotic lesions relies on its ability to dampen the activity of the PDGF-BB and the TGF-β signaling pathway in VSMC (Fig. 1A). LRP1 deficiency in VSMC is accompanied by increased expression and activation of PDGFR-β and enhanced phosphorylation of Smad2, a downstream component of the TGF-β signaling pathway (Boucher et al., 2007). Furthermore, the atheroprotective effects of LRP1 are displayed by its ability to decrease inflammatory responses (Overton et al., 2007), and to facilitate efferocytosis, a process by which apoptotic cells are removed by phagocytic cells, in macrophages (Yancey et al., 2011). However, LRP1 was also reported to mediate LDL accumulation in the vascular lesions (Llorente-Cortes and Badimon, 2005) and subsequent Wnt5a-dependent calcification (Woldt et al., 2012), thus stressing its multifaceted role in the development of vascular pathologies.

In the brain, LRP1 participates in the pathogenesis and the development of neurodegenerative disorders, such as Alzheimer's disease (Fig. 1B). Decreased LRP1 expression leads to diminished amyloid beta peptide (Aβ) catabolism and reduced transport across blood-brain-barrier resulting in an increased deposition of Aβ in senile plaques which are one of the major

histopathological hallmarks of Alzheimer's disease (Kanekiyo and Bu, 2014). In the central nervous system, LRP1 regulates the blood-brain-barrier integrity in response to t-PA under ischemic conditions, promotes Schwann cell survival and migration *via* ERK1/2, Akt and Rac1, and fosters regeneration after peripheral nerve injury (Lin and Hu, 2014). Mice lacking LRP1 in neurons develop behavioral and motor abnormalities, including hyperactivity, tremor and dystonia (May et al., 2004).

In kidney fibrosis, LRP1 controls t-PA-triggered fibrogenic activities *via* well-defined downstream mediators. Upon binding to LRP1, t-PA induces LRP1 tyrosine phosphorylation, which in turn promotes LRP1-mediated β1 integrin recruitment and the activation of integrin-linked kinase leading to fibroblast/myofibroblast proliferation and excessive matrix production in animal models of renal fibrosis (Hu et al., 2007). Accordingly, t-PA$^{-/-}$ mice show improved renal fibrosis resolution as a result of elevated apoptosis of fibroblasts/myofibroblasts (Hu et al., 2008). In addition, LRP1 promotes t-PA-triggered cell survival *via*

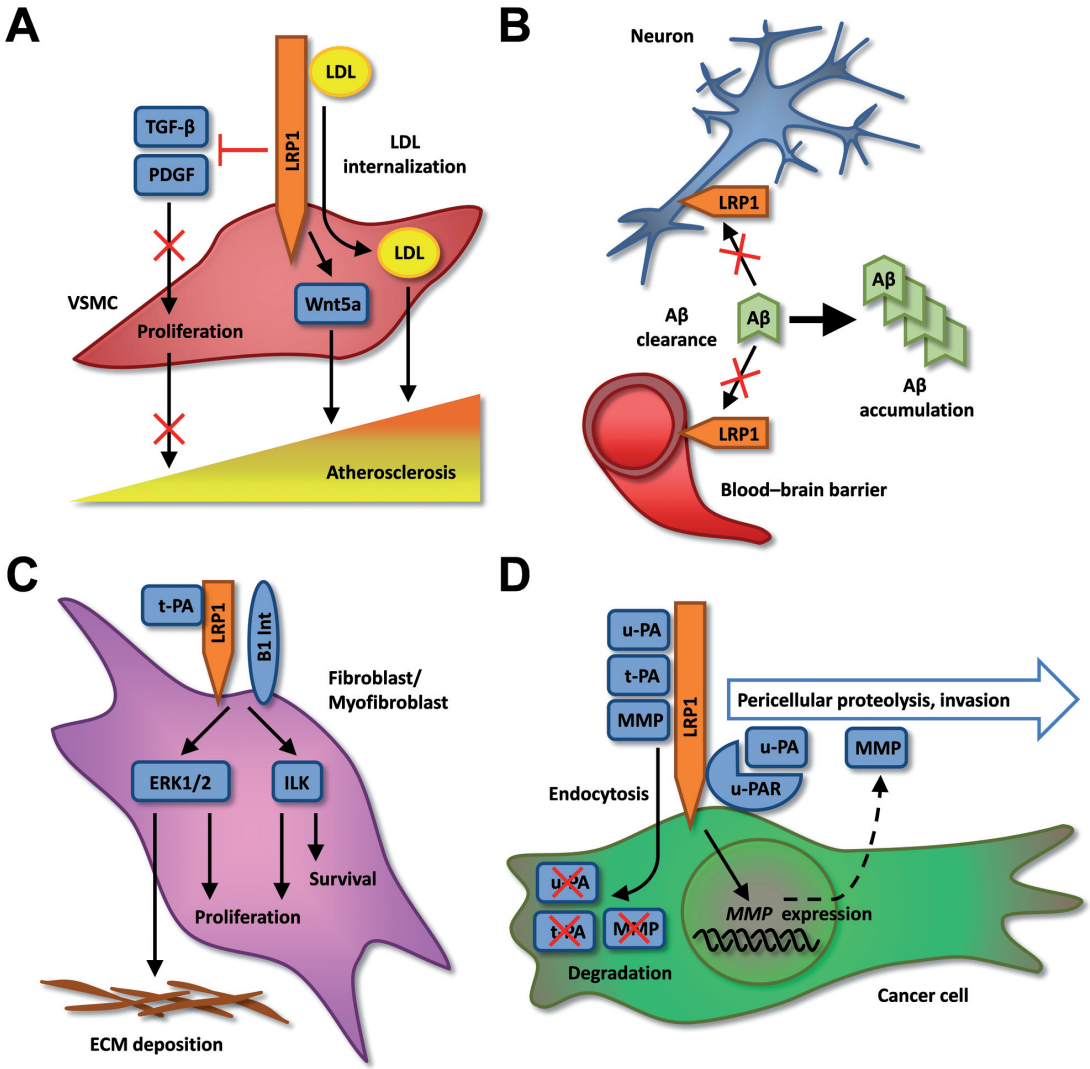


**Fig. 1.** Pathological processes mediated by LRP1 in non-pulmonary diseases. **A.** LRP1 plays a dual role in atherosclerosis. LRP1 inhibits TGF-β and PDGF-BB signaling pathways to limit vascular smooth muscle cell (VSMC) proliferation. This beneficial property is counteracted by LRP1-dependent low-density lipoprotein (LDL) accumulation and LRP1/Wnt5a-promoted calcification of atherosclerotic lesions. **B.** Reduced LRP1 expression in Alzheimer's disease perturbs amyloid beta peptide (Aβ) clearance and results in Aβ accumulation in the brain. **C.** LRP1 in concert with t-PA and β1 Integrin (β1 Int) drives kidney fibrosis by utilizing ERK1/2 and integrin-linked kinase (ILK) to induce proliferation and ECM deposition, and to support fibroblasts/myofibroblasts survival. **D.** Ambivalent role of LRP1 in cancer. LRP1-mediated u-PA, t-PA and MMP endocytosis and degradation may limit cancer cell motility. On the contrary, LRP1 supports cancer invasion by enhanced regeneration of free u-PAR and thus u-PA-dependent pericellular proteolytic activity and by increased MMP expression.

activation of an ERK1/2/p90RSK/Bad signaling pathway and cell proliferation through a mechanism involving ERK1/2, p90RSK, GSK3β and Cyclin D1 (Lin et al., 2010). Altogether, these findings signify the important role of LRP1 in the regulation of processes, which are detrimental to the development of renal fibrosis (Fig. 1C).

The role of LRP1 in cancer is determined by the tumor context. On the one hand, LRP1 may facilitate tumor progression but on the other it can also inhibit tumor growth and its expansion (Dedieu and Langlois, 2008). LRP1 levels were found to be reduced during progression of hepatocellular carcinoma, melanocytic tumors, Wilms tumor and lung adenocarcinomas. Often, lower LRP1 expression levels correlated with increased metastatic potential of tumor cells. On the contrary, abundant LRP1 expression was described in breast carcinoma, endometrial carcinoma, prostate cancer and glioblastoma (Li and Reynolds, 2012). This discrepancy may be explained by the plethora of processes coordinated by LRP1 (Fig. 1D). Since LRP1 internalizes u-PA, t-PA and matrix metalloproteinases (MMPs), LRP1 depletion, observed in some forms of cancer, leads to impaired protease endocytosis and thus, to the enhanced extracellular proteolytic activity and matrix remodeling, two processes which are advantageous for malignant tumors as they accelerate cell migration and invasion (Dedieu and Langlois, 2008). In contrast to this tumor-suppressive function, enrichment of LRP1 on the cell surface of other tumor cell types enhances regeneration of free u-PAR therefore increasing u-PA-dependent pericellular proteolytic activity and, in consequence, facilitating cancer invasion (Etique et al., 2013). The tumor-promoting properties of LRP1 are further underscored by reports showing that high LRP1 levels promote MMP-2 and MMP-9 expression, potentially *via* activation of an ERK1/2 signaling pathway (Song et al., 2009), and secure cell-matrix interactions, as LRP1 was found to control maturation of β1-integrin, endocytosis of αvβ3- and αvβ5-integrins, cytoskeleton reorganisation and turnover of adhesive complexes (Lillis et al., 2008).

## LRP1 in lung pathologies

Large genome-wide association with meta-analysis identified *LRP1* as a gene relevant for lung function (Soler Artigas et al., 2011). It is not therefore surprising that the involvement of LRP1 in the development of lung pathologies has been previously described. In this section we summarize reports highlighting the role of LRP1 in lung pathobiology and discuss new areas where LRP1 could contribute to the pathogenesis of pulmonary diseases.

### *LRP1 as a mediator of pleural injury and fibrosis*

Despite the fact that LRP1 regulates numerous processes that are critical for the development of lung pathologies, its role in lung diseases has hardly been explored. Tucker et al. demonstrated LRP1 immunoreactivity in pleural mesothelial cells (PMC) of the injured (pleuritis) and non-injured lungs (Tucker et al., 2012). PMC are the most abundant cells in the pleural space. They i) secrete glycosaminoglycans and other surfactant-like molecules to lubricate the pleural surface, ii) release pro- and anti-inflammatory mediators, iii) present antigens to lymphocytes, iv) regulate pleural permeability, v) promote both deposition and clearance of fibrin, and vi) participate in tissue repair processes by production of proteases, growth factors, and extracellular matrix proteins (for review, see (Batra and Antony, 2015)). Due to their broad spectrum of functions, PMC are believed to be essential for the maintenance of pleural homeostasis. Proinflammatory cytokines, such as TNF-α and IL-1β were found to decrease LRP1 expression in human PMC leading to the inhibition of u-PAR internalization and thus to increased u-PA enzymatic activity and consequently to augmented collagen I expression and PMC migration (Tucker et al., 2012). Although the molecular mechanism underlying u-PA-mediated induction of collagen I expression in PMC has not been fully deciphered, the involvement of TGF-β in this process cannot be excluded. LRP1 is identical to the TGF-β receptor type V (TβR-V) and mediates cell growth inhibition in response to TGF-β (Huang et al., 2003; Tseng et al., 2004). Suppression of LRP1 expression leads to the exaggerated stimulatory response of the cells to TGF-β. The enhanced activation of the mediators of TGF-β canonical (Smad2/Smad3) and non-canonical (ERK1/2) signaling pathways following LRP1 depletion was demonstrated in VSMC (Boucher et al., 2007) and macrophages (Muratoglu et al., 2011), respectively. In pleural fibrosis, TGF-β levels increase with disease progression (Sasse et al., 2003) and associate with the degree of fibrotic remodeling in experimental models (Decologne et al., 2007). Therefore, it is imaginable that suppressed LRP1 expression may amplify TGF-β signaling and thereby contribute to pathologic tissue remodeling. The ability of u-PA to favor PMC migration was attributed to the increased cell surface stability of u-PAR and thus to the activation of the plasmin-dependent proteolytic cascade (Tucker et al., 2012). Plasmin can potentiate matrix remodeling and thus cell migration, directly *via* its ability to degrade ECM proteins such as fibronectin and laminin or indirectly *via* its capability to activate MMPs (reviewed in (Smith and Marshall, 2010)). Increased collagen deposition and abnormal remodeling of extracellular matrix associated with augmented migratory capacity of PMC may culminate in progressive tissue scarring and the development of pleural fibrosis (Huggins and Sahn, 2004; Mutsaers et al., 2004). u-PA and its receptor were found to control PMC proliferation, their chemotactic activity, and, *via* generation of plasmin, mesothelial-to-mesenchymal transition, a process by which PMC acquire a profibrotic phenotype (Shetty et al., 1995; Tucker et al., 2012,

2014). Since activity and cell surface abundance of u-PA depend on the LRP1 level, downregulation of LRP1 expression may influence PMC profibrotic activities and consequently development of pleural fibrosis (Fig. 2A). Thus, the *in vivo* role of LRP1 in the pathogenesis of plural fibrosis is warranted to be investigated.

### *LRP1 as a modulator of pulmonary immune responses*

Surfactant proteins, SP-A and SP-D, belong to a family of mammalian C-type lectins, called collectins, which markedly contribute to surfactant homeostasis and pulmonary humoral and innate immunity (McCormack and Whitsett, 2002; van de Wetering et al., 2004; Kishore et al., 2005; Haczku, 2008). Their basic structure includes an amino-terminal collagen-like domain and a trimeric carboxy-terminal carbohydrate recognition domain (CRD). The globular CRD region binds, in a calcium-dependent manner, carbohydrate-based ligands on allergens, microbes and dying cells, while the collagen region docks with receptor molecules on immune cells, thus initiating the clearance mechanisms (Kishore et al., 2006). These well adaptable immune molecules are engaged in a range of immune functions, including viral neutralization (LeVine et al., 1999), inhibition of microbial growth (Wu et al., 2003), uptake of bacteria and fungi (Wright, 2005), clearance of apoptotic (Schagat et al., 2001) and necrotic cells (Vandivier et al., 2002), fine-tuning of allergic reactions (Hickling et al., 1998; Song and Phelps, 2000), polarization of helper T-cells (Singh et al., 2003), and resolution of inflammation (Schagat et al., 2001). Studies involving knock-out mice and murine models of lung hypersensitivity and infection have revealed the distinct roles of SP-A and SP-D in the modulation of lung inflammatory responses (reviewed in (Wright, 2005; Kishore et al., 2006)). These distinct roles depend on SP-A and SP-D orientation during their binding to the receptor on the alveolar macrophage (AM) surface (Gardai et al., 2003). In a resting, unstimulated lung, SP-A and SP-D interact with surface receptor signal inhibitory regulatory protein α (SIRPα) resulting in the activation of Src homology 2 domain-containing phosphatase-1 (SHP-1) and the suppression of the NF-ϰB system. However, upon exposure to LPS or apoptotic cells, SP-A and SP-D present their collagen-like domain to the calreticulin-LRP1 receptor complex to induce phagocytosis, proinflammatory cytokine production and innate and adaptive immune responses. Calreticulin is not a transmembrane protein, and similarly to heat shock proteins (hsp) gp96, hsp90, hsp70, it uses LRP1 as an adaptor molecule for phagocytosis and signal transduction (Basu et al., 2001). Besides its involvement in the SP-A- and SP-D-mediated inflammatory reactions, the calreticulin-LRP1 system also binds other members of the collectin family, including mannose-binding lectin, and it appears to operate not only in stress conditions but also in the naïve lung (Ogden et al., 2001; Vandivier et al., 2002). Thus, the calreticulin-LRP1 system, through its ability to recognize collectins, plays a crucial role in the initiation of inflammatory responses and may represent the mechanism to control a vast array of inflammatory processes in a diseased lung.

Chronic obstructive pulmonary disease (COPD (Segura-Valdez et al., 2000)), emphysema (Imai et al., 2005), asthma (Jeffery et al., 1989), and cystic fibrosis (Vandivier et al., 2002) have been associated with a large number of apoptotic cells in the alveolar septae and airways. In parallel, impaired efferocytosis of AM and other cell types was described in these pathological conditions (Vandivier et al., 2006). Hodge and colleagues showed that cigarette smoke, a major risk factor for COPD, reduces the phagocytic ability of AM through downregulation of cell surface expression of molecules responsible for the removal of cellular debris, including LRP1 (Hodge et al., 2007). In fact, LRP1 inhibitory antibodies diminished clearance of apoptotic cells by AM. Furthermore, impaired efferocytosis of AM obtained from healthy smokers and COPD patients was partially restored by smoking cessation. This indicates that cigarette smoke controls LRP1 expression and thus the efficacy of apoptotic cell clearance by AM. Although the mechanism responsible for diminished LRP1 expression on AM cell surface in response to cigarette smoke still remains to be addressed, it appears that histone deacetylases (HDAC) may be involved. Pharmacological inhibition of HDAC in AM results in the downregulation of LRP1 expression leading to reduction of efferocytosis (Noda et al., 2013). Lung HDAC expression gradually decreases during COPD progression and is lowered in AM isolated from COPD patients (Ito et al., 2005). Interestingly, cigarette smoke may be one of the factors responsible for decreased HDAC levels in the lungs of COPD patients (Ito et al., 2001). Suppression of HDAC activity in COPD AM promotes the release of proinflammatory mediators and confers resistance to dexamethasone (Cosio et al., 2004; Ito et al., 2006), thereby explaining the infectivity of glucocorticoid therapy in that disease (Barnes, 2013). These studies provided the basis for clinical trials which confirmed the advantage of HDAC activation as an adjuvant therapy to glucocorticoids for COPD (Cosio et al., 2009; Ford et al., 2010). Therefore impaired HDAC activity in COPD may not only foster inflammation by release of proinflammatory mediators, but also repress LRP1-mediated apoptotic cell clearance by AM (Fig. 2B).

### *LRP1 as a regulator of basement membrane integrity in acute respiratory distress syndrome*

Acute respiratory distress syndrome (ARDS) is characterized by an acute onset of severe hypoxemia, bilateral chest opacities and non-hydrostatic pulmonary edema caused by a variety of direct and indirect insults (Ranieri et al., 2012). Typical features of the acute phase include loss of endothelial and epithelial barrier integrity, injury of alveolar basement membrane, and accumulation of protein-rich edema fluid in the alveolar

compartment. ARDS may resolve completely after the acute phase or progress to a prolonged fibroproliferative stage with pathologic tissue remodeling (Ware and Matthay, 2000).

Increased sLRP1 levels have been reported in bronchoalveolar lavage fluid (BALF) obtained from ARDS patients (Wygrecka et al., 2011). Incubation of human lung fibroblasts with TNF-α-containing ARDS BALF increased MT1-MMP expression and subsequently induced MT1-MMP-mediated LRP1 shedding leading to the impairment of MMP-2 and MMP-9 endocytosis and thus accumulation of these proteases in the extracellular milieu (Fig. 2C). Although the elevated levels of MMPs were found in the lungs of ARDS patients, their role in the pathology of ARDS is poorly understood. On the one hand MMPs might be

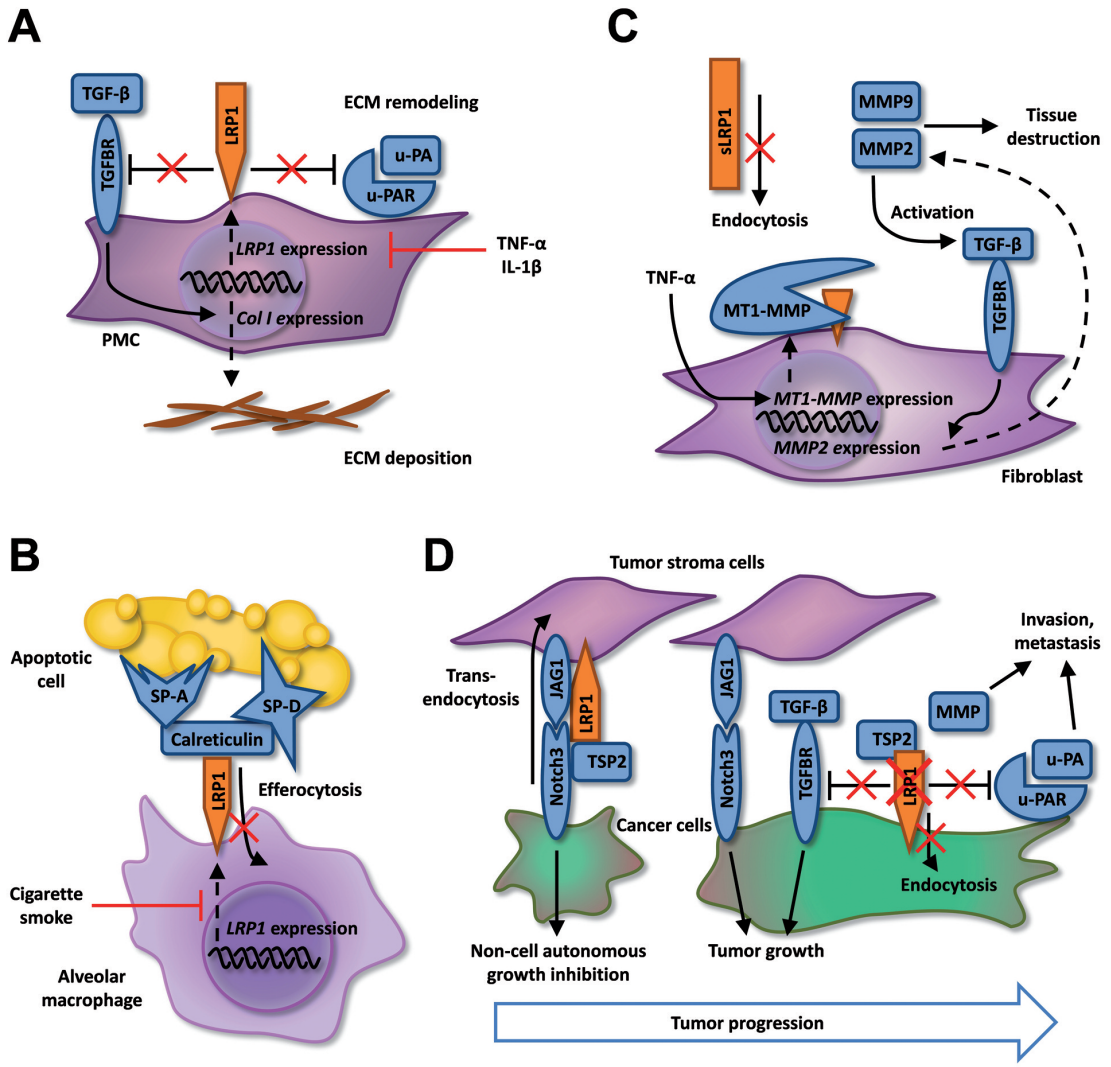


**Fig. 2.** LRP1 in lung diseases. **A.** Inflammatory mediators (TNF-α and IL-1β) may decrease LRP1 expression in pleural mesothelial cells (PMC) during pleuritis. Diminished LRP1 levels may insufficiently block u-PA/u-PAR-mediated EMC remodeling and promote TGF-β/TGF-β receptor (TGFBR) activity resulting in collagen I (Col I) expression and ECM deposition. Ultimately, these processes drive tissue remodeling and may result in pleural fibrosis. **B.** In COPD, impaired efferocytosis in alveolar macrophages may result from reduced LRP1 expression, and subsequently perturbed calreticulin/surfactant protein (SP)-A/SP-D-mediated apoptotic cell clearance, in response to cigarette smoke. **C.** LRP1 shedding leads to MMP2 and MMP9 accumulation and tissue destruction in ARDS. TNF-α induces MT1-MMP expression which in turn cleaves LRP1, thus impairing endocytosis of MMP and enhancing extracellular proteolysis. In addition, MMP2 may activate TGF-β signaling to induce its own expression in a positive feedback loop manner. **D.** A putative model of lung tumor suppression by LRP1. Expression of LRP1 on the tumor stroma cell surface mediates trans-endocytosis of Jagged 1 (JAG1)/TSP2/Notch3 signaling complex and controls non-cell autonomous growth inhibition of cancer cells at early stages of carcinogenesis. In a more advanced tumor, loss of LRP1 expression in stromal and cancer cells triggers pro-proliferative function of Notch3 and TGF-β signaling pathways, respectively. In addition to growth induction, LRP1 deficiency allows extracellular MMP accumulation and activation of u-PA/u-PAR system thus promoting cancer invasion and subsequently metastasis.

beneficial, as they can contribute to the clearance of the deposited ECM components and thus to the reconstitution of the normal lung structure, but on the other, they may also have deleterious effects and drive destruction of basement membrane, facilitate abnormal matrix remodeling and promote cell migration/invasion (Davey et al., 2011). These considerations are supported by the findings which demonstrated increased levels of laminin and type IV collagen degradation products in ARDS BALF (Torii et al., 1997; Wygrecka et al., 2011). As sLRP1 levels in ARDS BALF positively correlated not only with MMP-2 and MMP-9, but also with laminin (Wygrecka et al., 2011), it is tempting to speculate that enhanced cleavage of LRP1 ectodomain augmented pericellular concentration of MMPs and subsequently accelerated basement membrane destruction leading to increased permeability of the alveolar-capillary barrier. Interestingly, elevated ARDS BALF levels of sLRP1, MMP-2, MMP-9, and laminin have also been observed in the later course of the disease, suggesting the importance of LRP1 shedding in the progression of ARDS towards fibrosis (Wygrecka et al., 2011). Potentiated extracellular proteolytic activity due to impaired MMP clearance may result in disorganized and insufficient epithelial repair, fibroblast proliferation and migration, and abnormal ECM remodeling, ultimately leading to the formation of scar tissue (Davey et al., 2011). However, LRP1, as a scavenger receptor mediating the uptake of many other proteases including u-PA and t-PA (in free form or in complex with the plasminogen activator inhibitor-1) as well as ECM constituent, thrombospondin 1 (TSP1), may play a more complex role in ARDS.

Abundance of u-PA in sepsis, a major risk factor for developing ARDS, is increased and higher in non-survivors than survivors (Philippe et al., 1991). u-PA enhances release of proinflammatory cytokines by neutrophils, and u-PA$^{-/-}$ and u-PAR$^{-/-}$ mice show reduced lung injury in response to insults (Abraham et al., 2003; van Zoelen et al., 2009). However, the u-PA/u-PAR system is also required for the induction of proper inflammatory responses as it promotes recruitment of neutrophils to the sites of injury (Gyetko et al., 1996, 2000; May et al., 1998). Considering the fact that LRP1 controls neutrophil trafficking during acute inflammation by maintaining neutrophil adhesion (Weckbach et al., 2014), it is plausible to postulate that LRP1 alone or in concert with u-PA/u-PAR complexes may facilitate neutrophil extravasation into tissue and thus play a dual role during inflammation. Following this concept, shedding of LRP1 could disturb the delicate balance between pro- and anti-inflammatory u-PA/u-PAR functions in the lung.

TGF-β is a critical mediator of ARDS (Fahy et al., 2003; Budinger et al., 2005) and several LRP1 ligands, including MMP-2, MMP-9 and TSP1 (Mikhailenko et al., 1995; Chen et al., 1996), appear to regulate cell proliferation, adhesion, and migration as well as ECM deposition *via* their ability to activate TGF-β (Schultz-Cherry et al., 1995). Thus, augmented extracellular cleavage of LRP1 in ARDS lungs could contribute to overactivation of the TGF-β signaling pathway *via* extracellular MMPs and TSP1 retention. This process seems to operate in a positive feedback loop manner as TGF-β signaling was shown to promote MMP-2 expression (Kasai et al., 2005). The causative link between sLRP1 and ARDS pathogenesis was underscored by the positive correlation between the BALF levels of sLRP1 and disease severity (Wygrecka et al., 2011). Therefore, inhibition of LRP1 shedding emerges as a challenging therapeutic concept in ARDS.

### *LRP1 as a lung cancer suppressor*

Although levels of LRP1 expression in cancer are tumor cell type-dependent, LRP1 is clearly down-regulated in lung cancer (Meng et al., 2011). Yamamoto and colleagues reported that LRP1 expression is hardly detectable in primary lung adenocarcinoma (Yamamoto et al., 1997), a surprising finding concerning the evidence of high LRP1 levels in alveolar epithelium and pulmonary fibroblasts (Wygrecka et al., 2011). Interestingly, reduced LRP1 expression was also observed in brain metastases originating from lung adenocarcinoma, indicating that LRP1 suppression may be preserved throughout the metastatic process (Yamamoto et al., 1997). As gene expression profiles of primary lung adenocarcinoma and lung adenocarcinoma-derived brain secondary tumors significantly differ, LRP1 depletion in both types of neoplastic lesions demonstrates that LRP1 is a notable exception from this rule (Kikuchi et al., 2006). More recently, molecular analysis of a large cohort of patients suffering from various types of tumors not only confirmed LRP1 downregulation in lung cancer but, in addition, revealed that this type of malignancy displays the lowest LRP1 expression amongst all lesions characterized (Meng et al., 2011). Moreover, the authors reported that sustained low LRP1 mRNA expression in lung adenocarcinoma correlates with reduced survival independently of the tumor differentiation stage. Detailed immunohistochemical analysis of tumor specimens revealed low LRP1 levels in cancer cells and high LRP1 expression in stroma cells. Remarkably, high LRP1 levels in stroma fibroblasts are associated with a better clinical outcome, suggesting that LRP1 may display anti-tumor properties in this type of lung cancer. In fact, co-culture of primary or metastatic adenocarcinoma cells with LRP1-depleted fibroblasts enhanced tumor cell proliferation (Meng et al., 2011). Although the lung tumor growth-suppressive mechanism relying on the stromal LRP1 expression has not yet been investigated *in vivo*, existing data suggest that it may involve the Notch3 signaling. Notch3 is over-expressed in lung cancer cells and the presence and processing of this receptor is necessary for cell autonomous growth and survival (Haruki et al., 2005; Konishi et al., 2007; Osanyingbemi-Obidi et al., 2011). On the contrary, the non-cell autonomous growth of

Notch3-positive lung cancer cells co-cultured with fibroblasts expressing a Notch3 ligand Jagged1 is greatly reduced. Furthermore, this proliferation-restricting, transcellular receptor-ligand interaction is enhanced by Thrombospondin 2 (TSP2), a common partner for Notch3 and Jagged1. Interestingly, LRP1 binds TSP2, Notch3 and Jagged1 and drives their trans-endocytosis, while LRP1 inhibition abrogates TSP2 impact on the Notch3-dependent signaling and cell proliferation (Meng et al., 2010), suggesting that LRP1 in Jagged1-positive signal-sending stroma cells controls the proliferation of signal-receiving, Notch3-expressing tumor cells. Whether this mechanism operates in a native tumor environment remains to be elucidated. Furthermore, it would be interesting to test whether depletion of LRP1 drives the conversion of the cell autonomous Notch3 function from antiproliferative to growth promoting mode.

Similarly, lung cancer cells may escape growth inhibitory and apoptotic signals by suppressing the LRP1 expression in order to modulate their responsiveness to TGF-β. TSP2 not only modulates Notch3 signaling but also prevents activation of latent TGF-β (Schultz-Cherry et al., 1995). Expression levels of TGF-β are elevated in several cancers, and in the lung they correlate with advanced tumor stage and decreased survival (Hasegawa et al., 2001). A current concept describes a dual role of TGF-β in carcinogenesis in which it serves as a tumor-suppressor during the initial steps of malignancy but then progressively converts to the promoter of cancer growth, invasion and metastasis (Lebrun, 2012). *In vivo*, TGF-β restricts tumor growth at the primary site and, depending on the operational signaling, modulates the behavior of secondary tumors (Vazquez et al., 2013). Strikingly, over-expression of LRP1 in the human adenocarcinoma cell line derived from lymph node metastasis (characterized by low endogenous LRP1 production) rescued cell sensitivity to TGF-β as evident by the inhibition of proliferation (Huang et al., 2003). Given the modulatory role of LRP1 in the regulation of TGF-β signaling, the gradual loss of LRP1 could switch the TGF-β function from growth-inhibitory at early cancer stages to growth-promoting at the more advanced cancer stages. Interestingly, this concept is supported by the findings showing enhanced survival of lung cancer patients displaying LRP1 expression, suggesting that the presence of this receptor, in concert with TGF-β, may indeed restrict tumor development (Meng et al., 2011).

Apart from having an influence on cell signaling processes, the absence of LRP1 can lead to the accumulation of MMP-2 and MMP-9 in the extracellular milieu (Hahn-Dantona et al., 2001; Emonard et al., 2004; Wygrecka et al., 2011). The importance of MMP-mediated degradation of extracellular matrix and activation of growth factors, including TGF-β, for promotion of cancer cell proliferation, invasiveness and metastasis formation is well established (reviewed in (Kessenbrock et al., 2010)). High levels of MMP-2 and MMP-9 in lung cancer cells and surrounding stroma correlate with a more advanced tumor stage, presence of metastases and a poorer survival outcome (Ishikawa et al., 2004; Guo et al., 2007; Leinonen et al., 2008; Schveigert et al., 2013). Moreover, lymph node metastases originating from lung tumors preserve a high MMP-9 expression signature (Zheng et al., 2010). In accordance with these observations, specific inhibition of MMP-9 reduces proliferation, migration and invasion of a lung cancer cell line (Yang et al., 2009). Furthermore, blockage of MMP-2 expression decreases migration and invasion of lung cancer cells *in vitro*, reduces tumor growth and VEGF-dependent angiogenesis (Itoh et al., 1998; Chetty et al., 2006, 2010), while simultaneous MMP-2 and MMP-9 inhibition prevents lung cancer metastasis in mice (Nakamura et al., 2004). The influence of reduced LRP1 expression on MMP-dependent tumor growth and metastasis has not yet been investigated in the context of lung malignancies. Liver cancer bears a resemblance to lung cancer in the form of abundant MMP-2 and MMP-9 expression (Arii et al., 1996; Ogata et al., 1999) and reduced LRP1 levels that are associated with decreased survival (Huang et al., 2012). Huang and coworkers reported an inverse relationship between LRP1 expression and invasiveness of several hepatocarcinoma cell lines. Moreover, LRP1 silencing increased MMP-9 abundance and activity *in vitro*, and enhanced tumor growth and metastasis *in vivo* (Huang et al., 2012). Given the similar LRP1 and MMP-9 expression profile in lung and liver cancer, it is tempting to speculate that lung cancer cells could exploit low LRP1 expression to promote metastasis through elevated levels of MMP-9.

The MMPs activity can also be induced by the u-PA/u-PAR system. The expression of u-PA and its receptor was found to be increased in various types of cancer, including lung cancer (Pedersen et al., 1994; Salden et al., 2000). By converting plasminogen to plasmin, u-PA/u-PAR facilitates degradation of ECM and stimulates MMP activity to promote invasion and metastasis of lung cancer cells (Lakka et al., 2001; Rao et al., 2005). An inhibitory function of LRP1 in the u-PA/u-PAR system resulting in limited ECM remodeling and cell migration functions under normal conditions (Weaver et al., 1997; Gaultier et al., 2010; Noh et al., 2013) and has been proposed to be disturbed in rat prostate cancer characterized by low LRP1 expression and high u-PAR abundance (Gilardoni et al., 2003). Whether an analogical mechanism operates in lung cancer remains to be elucidated.

As outlined above, it appears that LRP1 exerts anti-cancer properties in the lung by transducing the proliferation-limiting signals and restricting the extracellular matrix remodeling (Fig. 2D). Therefore, approaches aimed at the restoration of LRP1 expression in malignant cells or enhancing LRP1 production in stromal fibroblasts could offer promising therapeutic options for the treatment of lung cancer. This indication is appealing since tumor stroma, as opposed to

malignant cells, is genetically stable and rarely develops resistance to anti-cancer therapy (Quail and Joyce, 2013).

## Conclusions

Extracellular proteases play an important role in normal cellular and tissue function, but inadequate and excessive protease activities are likely to be highly detrimental and lead to pathological tissue remodeling. Thus, not only the regulation of proteases expression and activation but also the control of mechanisms involving protease inhibitors as well as scavenger receptors (which help to clear protease-inhibitor complexes) may balance extracellular protease functions. In this regard, LRP1 mediates the cellular catabolism of many proteases from sites of expression and from the circulation. In addition to the contribution to the extracellular matrix balance, LRP1-regulated proteolysis directs cell migration and fine-tunes inflammatory responses. Furthermore, LRP1 mediates the uptake of lipoproteins and apoptotic cell debris, further signifying its role in tissue homeostasis. However, LRP1 also displays other functions not directly related to scavenger activities. Its broad spectrum of interactions allows modulation of cell signaling pathways involved in controlling gene expression and cell behavior. In this case, LRP1 provides an interface for growth factor-receptor interaction to regulate cell proliferation and motility. Moreover, LRP1-mediated signal transduction is not limited to a single cell, but may also operate in a juxtacrine signal mode between adjacent cells. Thus, the characterization of LRP1-mediated multi-component cellular processes that contribute to matrix remodeling, efferocytosis or cell migration and proliferation in the development of lung diseases await precise analysis. The possible anti-remodeling, anti-inflammatory, or anti-tumor strategies which arise from these investigations may provide a basis for future therapeutic approaches.

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