Commentary & View

LRP-1

A new modulator of cytoskeleton dynamics and adhesive complex turnover in cancer cells

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The low-density lipoprotein receptor-related protein-1 (LRP-1) is a large scavenger receptor mediating the internalization and catabolism of various biological components from the extracellular matrix. In the past decade, LRP-1 appeared as an attractive receptor for targeting the invasive behavior of cancer cells since this protein is able to reduce the accumulation of extracellular proteases by endocytosis. However, recent data suggest that LRP-1 could support carcinoma cell invasion depending on the cellular environment. Indeed, in addition to its well-determined role in ligand binding and endocytosis, LRP-1 emerges as a central cellular environment. Indeed, in addition to its well-determined role in ligand binding and endocytosis, LRP-1 emerges as a central molecular regulator of cytoskeleton organization and adhesive complex turnover in malignant cells. This commentary reviews the functions played by LRP-1 in cancer-related events and discusses the potential mechanisms whereby LRP-1 is able to control the cellular phenotype of cancer cells.

The low-density lipoprotein (LDL) receptor-related protein-1 (LRP-1) is a large endocytic receptor belonging to the LDL receptor gene family. It consists of a 515-kDa heavy chain containing the extracellular ligand-binding domains and a non-covalently associated 85-kDa light chain including the transmembrane domain and a short cytoplasmic tail. LRP-1 functions as a clearance receptor mediating the uptake and catabolism of various ligands from the pericellular environment, including extracellular matrix macromolecules, active proteases and proteinase/inhibitor complexes. For instance, LRP-1 allows internalization and subsequent lysosome-mediated degradation of metalloproteases (MMP-2, -9 and -13) and serine proteinases such as tissue-type and urokinase-type plasminogen activators (tPA and uPA, respectively). Through this mechanism, LRP-1-dependent endocytosis prevents excessive extracellular matrix remodeling and basement membrane degradation, two key elements of cancer progression and metastasis. Neutralizing the endocytic function of LRP-1 commonly increases extracellular proteolysis and stimulates the capacity of cancer cells to invade the surrounding tissues. Consistent with this, a decreased expression of membrane-anchored LRP-1 is frequently associated with tumor growth and metastasis development. This concerns malignant cells derived from various tissues such as prostate, kidney, colon, breast, endometrial and thyroid. LRP-1-mediated endocytosis therefore emerges as an innovative approach for targeting the proteolytic cascade associated with invasive cancers. However, its accurate role in the context of cancers remains highly controversial and finally poorly understood.

Recent results from our group provide new insights into LRP-1 roles in multiple cancer-related events. Indeed, we highlighted that LRP-1 silencing prevented thyroid carcinoma cell invasiveness. Interestingly, this inhibition occurred despite a major increase in pericellular proteolytic activities. We were therefore convinced that the altered invasive capacity of LRP-1-silenced cells was not due to change in pericellular proteolysis and could be directly associated to migration defects. Thus, we postulated that LRP-1 could control critical events influencing cell migration and especially the balance between traction forces and cell adhesiveness. We therefore established that LRP-1 expression is necessary for two- and three-dimensional cell migration and identified LRP-1 as a pivotal mediator of cancer cell deadhesion. Indeed, accelerated rate of cell attachment and inhibited cell-matrix detachment were observed when LRP-1 was silenced. Consistent with these observations, LRP-1-silenced cancer cells exhibited atypical overspread morphology, abnormal stress fibers distribution and failed to extend membrane projections, thereby indicating that LRP-1 somehow controls the malignant cell phenotype. Altogether, we demonstrated that LRP-1 promotes carcinoma cell invasion by subtly controlling the adhesive properties and the actin network structure. Looking ahead, the development of genic therapy strategies based on LRP-1 silencing could constitute new alternative approaches in oncology.

Nevertheless, the mechanisms by which LRP-1 controls the cellular phenotype of malignant cells, i.e., the cytoskeleton organization and adhesive complex turnover, remain largely unclear. How to explain the effects of LRP-1-silencing on cell spreading and motility?

The first hypothesis consists in explaining them by a defect in LRP-1-mediated ligand binding and endocytosis (Fig. 1A). Indeed, several membrane-associated proteins capable of interacting with LRP-1 are involved in cell adhesion and migration. It is the case for the uPA receptor (uPAR), which is intimately linked to cancer-related
New functions for LRP-1 in cancer

Events such as cellular attachment, contraction and migration \(^8\) LRP-1 mediates the removal of uPAR from the membrane surface. \(^9\) When LRP-1-dependent endocytosis is abrogated, the amount of uPAR expressed at the cell surface may increase, which in turn may affect the integrin-mediated adhesion processes. Indeed, uPAR/α5β1 integrin complex was evidenced to promote fibronectin-induced adhesion and to inhibit cell migration. \(^10,11\) Besides, uPAR was previously reported to interact with the α3β1 integrin triggering adhesion to vitronectin and regulating uPA-stimulated cell spreading. \(^12\) Other studies revealed that the disruption of the uPA/uPAR/αvβ3 complex prevented the localization of αvβ3 to focal contacts and triggered cell detachment from vitronectin. \(^13,14\) Thus, in absence of LRP-1, inhibition of uPAR internalization could increase the association and stabilize uPAR/integrin complexes. This could regulate the conformation of integrins and subsequently affect their ability to transduce signals. \(^11\) For example, activation of extracellular signal-regulated kinases (ERKs) in cancer cells is highly dependent on the stability of the uPA/uPAR/β-integrin complexes and could be modulated by LRP-1. Furthermore, consistent with our findings, accumulation of uPA and/or integrins at the cell membrane has already been correlated to changes in cell size and to cytoskeleton reorganization. \(^14\) By internalizing diverse membrane-linked proteins, LRP-1-mediated endocytosis therefore modifies the membrane composition, affects cell/matrix interactions along with the associated signaling pathways and then, may control the adhesive properties of tumor cells. Interestingly, Cao and colleagues \(^15\) have recently proposed an attractive model in macrophages in which LRP-1 could facilitate the cell detachment at the rear of the cell by mediating the internalization of adhesion complexes containing integrins. Such a molecular model is in agreement with our recent results and could probably occur during tumorigenesis. Overall, LRP-1 appears as a scavenger receptor regulating cellular matrix attachment sites and modulating a subtle balance between adhesion and deadhesion events in cells, especially in a tumoral context.

Nevertheless, in contrast to our recent observations, blocking the endocytic activity of LRP-1 by using the LRP-1-associated protein RAP commonly led to increased tumor cell invasion and migration. \(^3,4\) This indicates that the mechanisms by which LRP-1 promotes tumor cell migration do not only rely on its endocytic function. This is undeniably much more complex.

Recently, LRP-1 was reported to traffic β1 integrin at the cell surface independently of its endocytic function \(^16\). The capacity of LRP-1 to regulate protein secretory pathways represents a novel mechanism by which LRP-1 could influence the membrane topography and subsequently cellular adhesiveness, signaling and migration (Fig. 1B). \(^17\) Briefly, three spatially and temporally coordinated events are required for cell migration. This consists of cell attachment at the leading edge, cell contraction and cell rear detachment. \(^18\) We propose a model in which LRP-1 would represent a main molecular relay triggering a range of signaling pathways controlling these sequential events that contribute to tumor cell motility (Fig. 1B). The cytoplasmic domain of LRP-1 possesses two NPxY signaling motifs that serve as docking sites for signaling adaptors and scaffold proteins. This mainly concerns c-Jun N-terminal kinase (JNK)-interacting protein-1/2, FE65, Disabled-1, PSD-95 and Shc proteins that may

![Figure 1. Schematic illustration of the multiple functions played by LRP-1 in cancer-related events. LRP-1 could control the cellular phenotype of malignant cells and contribute to cancer cell motility through endocytosis (A) and/or signaling functions (B).](Image)
modulate cell spreading and adhesiveness in part through mitogen-activated protein kinases. For example, tyrosine phosphorylation of the second NPXY motif in LRP-1 cytoplasmic tail may generate a docking site for the Shc adaptor protein, which is well known for transducing signals through Ras and ERK in normal and pathological contexts. Furthermore, a recent study demonstrated that LRP-1-dependent intracellular signaling pathways regulated the expression of target genes directly involved in the extracellular matrix composition and stiffness. This constitutes a new way to modulate the local landscape of the extracellular matrix and thus cell-matrix adhesion. It is likely that the cytoplasmic domain of LRP-1 could be released from the membrane after proteolytic cleavage and translocated to the nucleus to recruit transcriptional activators. Nevertheless, very few genes transcriptionally controlled by LRP-1 have been identified. Other target genes associated to the actin network or focal adhesion complexes are probably regulated by LRP-1 through transactivation or transrepression processes. Identifying these target genes will be an exciting challenge for scientists interested in targeting the behavior of malignant cells through alternative strategies. By interacting with other signaling receptors at the cell surface, LRP-1 may modulate transduction pathways widely involved in the regulation of cell spreading, actin filament structuration and detachment of the rear of the cell. This includes integrin-mediated signaling and involves Src, ERK, JNK, Ras and/or Rac1 kinases. For example, LRP-1 is able to mediate the β1-integrin recruitment and the subsequent stimulation of the β1-integrin-linked kinase (ILK). ILK is a focal adhesion protein kinase known to strengthen the integrin/cytoskeleton connections. By regulating ILK activity, LRP-1 could play a pivotal role in controlling the actin polymerization and cytoskeleton dynamics during retraction of migrating cells. LRP-1 is besides found associated with the cell surface form of calreticulin in endothelial cells. Such interaction stimulates the GTPase signaling pathway and its downstream effectors, phosphoinositide 3 kinase (PI3K) and ERK, that allow the intermediate level of adhesion required for optimal cell migration. Although LRP-1 was first located in clathrin-coated pits, more recent data identified LRP-1 as being transiently associated with lipid rafts/caveolae. LRP-1 clustering in lipid rafts helps to understand how LRP-1 can be bridged to uPAR or platelet-derived growth factor receptor (PDGFR) and to modulate the downstream signaling pathways leading to cell migration and actin network reorganization. Indeed, a PDGFR/LRP-1 complex was detected in caveolae. Such a signaling complex promotes smooth muscle cell migration through Src and/or PI3K-dependent tyrosine phosphorylation of the LRP-1 cytoplasmic domain. The capacity of PDGFR to directly phosphorylate LRP-1 has recently been evoked but never demonstrated at this day. Moreover, Spijkers and colleagues elegantly demonstrated that LRP-1 is involved in β2 integrin-mediated adhesion of leukocytes. This probably occurs independently of the endocytic function of LRP-1 and requires LRP-1/β2 integrin association in lipid rafts. As a result, it would appear that the cellular distribution of LRP-1 at the plasma membrane could play a central role in mediating specific intracellular signaling responses. It is now well established that raft-associated signaling proteins are connected by means of structural proteins to the dynamic filamentous actin network. The presence of LRP-1 in such plasma membrane microdomains may explain, at least in part, its capacity to influence the cytoskeleton architecture and dynamics. However, up to now, little is known about the LRP-1 clustering at the plasma membrane. This phenomenon seems widely dependent of the cellular context. To improve our understanding about the LRP-1-dependent signaling pathways in tumor cells, it will be necessary to establish how LRP-1 trafficking between membrane microdomains is regulated. It is now becoming clear that the nature of the intracellular and extracellular ligands bound to LRP-1 influences its distribution at the plasma membrane.

Regulation of focal adhesions turnover is crucial for normal and cancer cell migration. We recently established that LRP-1 is capable of regulating the amount, localization and composition of focal adhesion sites in cancer cells. Focal adhesions transmit both mechanical and biochemical signals that regulate cell shape, actin cytoskeleton organization and finally cell motility. The LRP-1-mediated stimulation of focal adhesion turnover could support the malignant cell migration especially by facilitating the cell rear detachment from the matrix. The amounts of focal adhesion kinase (FAK) and paxillin that control focal adhesion dynamics and cytoskeleton arrangement were found drastically reduced in LRP-1-silenced thyroid carcinomas. LRP-1 appears also able to control the intracellular amount of talin and the cellular distribution of α-actinin. Moreover, we evidenced that both paxillin and FAK association to focal contacts occurred in a LRP-1-dependent manner. It is interesting to underline that such a regulation mediated by LRP-1 seems highly specific. Indeed, the amounts of most focal adhesion or actin-binding proteins that we assessed do not change according to LRP-1 expression. All these new findings contribute to explain why LRP-1-deficient malignant cells exhibit abnormal filamentous actin architecture and altered adhesive properties. The LRP-1-mediated regulation of FAK, paxillin and talin amounts and/or distribution probably results in the control of integrin activation and adhesion complex turnover. Nevertheless, how LRP-1 regulates the amounts and/or the distribution of specific focal adhesion components remains poorly understood. Given the important role of FAK during tumorigenesis, many scientists, interested in targeting the behavior of cancers, try to develop efficient FAK inhibitors. In this context, in association with discriminating cytotoxic agents, strategies aiming at FAK downregulation through LRP-1 targeting could be of great interest to develop new antitumor therapies. Furthermore, we imagine that LRP-1 may directly contribute to generate traction forces required for cell migration. Arguments exist suggesting that LRP-1 could act as a regulator of the actinomysin network through the stimulation of the myosin light chain phosphorylation. This could be mediated by Rho family GTPases and myosin light chain kinase. By this mean, LRP-1 could contribute to polarize the cytoskeleton structure, to enhance the cell contractility and to improve the cell-matrix detachment at the rear of the cell, thereby leading to efficient directed cell migration.

In the light of recently discovered results, LRP-1 may promote the development of cancer metastases in vitro and in vivo, depending on the cellular context. The expression of LRP-1 at the plasma membrane may contribute to maintain an optimal balance between cell attachment at the leading edge and cell detachment at the trailing edge, thereby favoring cancer cell invasion. Finally, we consider the endocytic receptor LRP-1 as a mechano-sensor receptor that plays important roles in controlling several structures that mediate cell-matrix interactions in cancer cells. As summarized in Figure 1, this concerns the control of (1) matrix stiffness and proteolysis, (2) actin cytoskeleton...
architecture and dynamics and (3) focal adhesion composition and distribution. Beyond its endocytic function, LRP-1 is able to control such molecular events by transmitting diverse signaling pathways that may lead to transregulation of target genes. Further in-depth studies will be necessary in the future years to decrypt the multiple molecular mechanisms regulated by LRP-1. This will improve our understanding of how LRP-1 contributes to cancers and will permit to develop alternative strategies against malignancies.

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