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Review

Harnessing LRIG1-mediated inhibition of receptor tyrosine kinases for cancer therapy

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ABSTRACT

Leucine-rich repeats and immunoglobulin-like domains containing protein 1 (LRIG1) is an endogenous feedback regulator of receptor tyrosine kinases (RTKs) and was recently shown to inhibit growth of different types of malignancies. Additionally, this multifaceted RTK inhibitor was reported to be a tumor suppressor, a stem cell regulator, and a modulator of different cellular phenotypes. This mini-review provides a concise and up-to-date summary about the known functions of LRIG1 and its related family members, with a special emphasis on underlying molecular mechanisms and the opportunities for harnessing its therapeutic potential against cancer.

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1. Introduction

Since their discovery in the late 1970s, considerable interest has been dedicated to the study of receptor tyrosine kinases (RTKs), which strongly contribute to the regulation of many cellular processes in health and disease [1]. Dysregulation of RTK-related pathways are

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predominantly involved in the onset and the progression of a wide range of cancers [2,3], and the understanding of RTK signaling is therefore of great significance for the design of targeted therapies.

RTKs are endowed with an intrinsic kinase activity. After ligand-induced or spontaneous dimerization, auto- and transphosphorylation of tyrosine residues in the cytosolic kinase domain of the receptor allow signal transmission through activation of downstream pathways, leading to modulation of gene expression and cell phenotype and functions. This kinase activity currently represents a druggable target and lead to the development of specific tyrosine kinase inhibitors (TKIs), which typically block the ATP-binding sites of RTKs and therefore impair signal transmission inside the cell [4]. Nowadays, many of those TKIs are approved for clinical application and used to treat several types of malignancies (www.cancer.gov). Unfortunately, despite overall efficiency and high specificity, subsets of patients remain unresponsive and many develop resistance after long-term treatment [5]. This acquired resistance is thought to rely on diverse escape mechanisms associated with secondary mutations in the targeted RTK [6–8] or in the downstream pathways [9,10], or through the enrollment of other RTKs which take over the inhibited signaling [11–13].

Aside from this drug-based inhibition, RTKs are also controlled by cell-intrinsic mechanisms that ensure a finely-regulated signaling during homeostasis [14]. Endogenous inhibitors of RTK signaling include early attenuators, which directly impair RTK activity by interfering with phosphorylation or promoting degradation. Indeed, phosphatases (i.e. PTP1B or PTEN) can directly remove phosphate residues from RTKs or downstream kinase proteins and prompt inactivation [15], while ubiquitin ligases from the Cbl or Nedd families promote ubiquitination and degradation [16,17]. MiRNAs were also found to regulate RTK signaling through post-transcriptional mechanisms [18]. On the other hand, inducible late attenuators such as Mig6, Sef or Sprouty proteins are expressed downstream of RTK signaling and act as negative feedback regulators, also modulating phosphorylation or protein degradation [19–21].

Among the late attenuators is LRIG1, a member of the Leucine-rich Repeats and Immunoglobulin-like domains family of proteins. As such, LRIG1 plays a pivotal role in both tissue homeostasis and malignant transformation. Human LRIG1 was identified in the early 2000s [22] and initially characterized as a negative regulator of EGFR family proteins [23,24], promoting their ubiquitination and degradation, leading to impaired cell growth. Recent studies provided powerful *in vivo* evidence that ERBB family members are indeed targets of LRIG1. Loss of LRIG1 in genetically engineered mice leads to enhanced ERBB signaling in intestinal stem cells [25,26] (see later). The phenotype could be rescued pharmacologically by treatment with an EGFR inhibitor and genetically by crossing the mice with EGFR hypomorphic mice [25]. In addition to being an inhibitor of the EGFR family receptors, EGFR, ERBB2, ERBB3, and ERBB4, LRIG1 was further identified as an inhibitor of other RTKs, such as Ret [27], c-Met [28], PDGFRA [29] and TrkB [30]. Several studies also highlighted the significance of LRIG1 in cancer prognosis, as recently reviewed elsewhere [31].

In order to update earlier review reports [32,33] and along with the growing knowledge concerning LRIG1, the present paper aims to provide a summary of the role of LRIG1 in molecular regulation of cell signaling and to shed light on its therapeutic potential.

2. LRIG1 regulates RTK signaling

2.1. Members of the LIG family are molecular regulators of membrane proteins

LRIG proteins include LRIG1, LRIG2 and LRIG3 isoforms. These proteins are composed of a cytosolic tail, a transmembrane domain and an extracellular, or vesicle-lumenal, domain including leucine-rich repeats (LRR) and immunoglobulin-like (IG) domains, thus resembling other proteins also carrying these extracellular domains (Fig. 1). This so-called LIG family includes several subfamilies (with respective members): LRIG (*LRIG1*, *LRIG2*, *LRIG3*), LINGO (*LINGO1*, *LINGO2*, *LINGO3*, *LINGO4*), AMIGO (*AMIGO1*, *AMIGO2*, *AMIGO3*), LINX/ISLR (LINX/ISLR2, ISLR), LRRC4/NGL (*LRRC4*, *LRRC4c*), and Trk receptors (*TrkA*, *TrkB*, *TrkC*), which all share a similar structure, possibly evolved from a common ancestral gene [34].

Many other proteins of the LIG family are mainly expressed in the developing nervous system. They interact with different types of membrane proteins, including the $p75^{NTR}$ receptor, the Nogo-66 receptor (NgR), or the GPI-anchored netrin-G molecules [35–37], thereby regulating neurite growth, synapse formation, neuronal survival and axonal guidance. These molecular interactions mostly depend on the LRR and Ig domains of the LIG family proteins, which are highly conserved and involved in many different protein-protein interactions [38]. This has been recently reviewed in the context of synaptic connections [39].

In addition to LRIG1, other members from the LIG family also interact with RTKs, such as Trk receptors [40,41]; Ret [34] or EGFR [42]. It is important to note that the extracellular domain of several RTKs contains itself variable numbers of either LRR (e.g. EGFR, InsR) or Ig domains (e.g. PDGFR, VEGFR), or both (Trk receptors) [3]. Based on the study of structural mutants, there is increasing evidence for a key role of ectodomains, and more specifically LRR, in the interaction of LIG proteins with RTKs and other targets, and their subsequent downregulation (Table 1). For example, it appears that without its ectodomain (LRR + Ig), LINGO1 is not able to inhibit Trk signaling [40]. The soluble LINGO1 ectodomain was also demonstrated to be sufficient for recognition of NgR and for repression of NgR-associated neurite outgrowth inhibition in cerebellar granule neurons [43], whereas the ectodomain of LRIG3 is involved in its interaction with FGFR1 [44]. LRRC4 also requires functional LRR domains (and more specifically LRR3) to reduce Akt and NF-κB signaling and proliferation in glioma cells, even if no information about a putative interaction with RTKs (or other proteins) was provided [45].

2.2. LRIG1 interacts with RTKs via its extracellular domain

The importance of the ectodomain, and more specifically LRR domain, is also progressively confirmed for LRIG1. The first investigation about LRIG1-mediated EGFR inhibition showed that the LRIG1 ectodomain was required to interact with the receptor [23], which was also recently confirmed for downregulating ErbB3 in breast cancer cells [46]. Other studies indicated that the LRR domain is mandatory for LRIG1 to interact with/inhibit the activity of TrkB in developing hippocampal neurons [30] or with LRIG3 and ERBB2 in breast cancer cells [47] (see Table 1).

Along with the observation that the LRIG1 ectodomain alone is able to exert RTK inhibition, several studies describe an anti-proliferative effect of the soluble LRIG1 ectodomain on glioma cells, either composed of LRR only [48] or both LRR and Ig domains [49,50], if added exogenously in the culture medium. Our team showed that the soluble LRIG1 ectodomain reduced glioblastoma (GBM) growth and increased survival in patient-derived xenografts [49]. The ectodomain of LRIG1 can be constitutively shed, and is therefore able to function as a soluble regulator of EGF signaling [50].

Based on the structure of the LINGO1 ectodomain described previously [43], a study recently defined the three-dimensional crystal structures of the isolated LRR and Ig domains of LRIG1, produced in baculovirus-insect cells. This revealed a crescent-shaped and highly glycosylated LRR domain, whereas the three Ig domains formed a rod-shaped structure. Intriguingly, this study failed to detect a significant binding of LRIG1 ectodomain with EGFR, and the phosphorylation of EGFR remained unchanged [51].

In summary, while many targets of LIG proteins have been unequivocally identified, the exact protein-protein interaction motifs are still not fully understood. While the ectodomains of LIG proteins appear to be necessary for proper interaction with target proteins and for fine-tuning of their activity, it is not yet clear whether the LRR domains, Ig

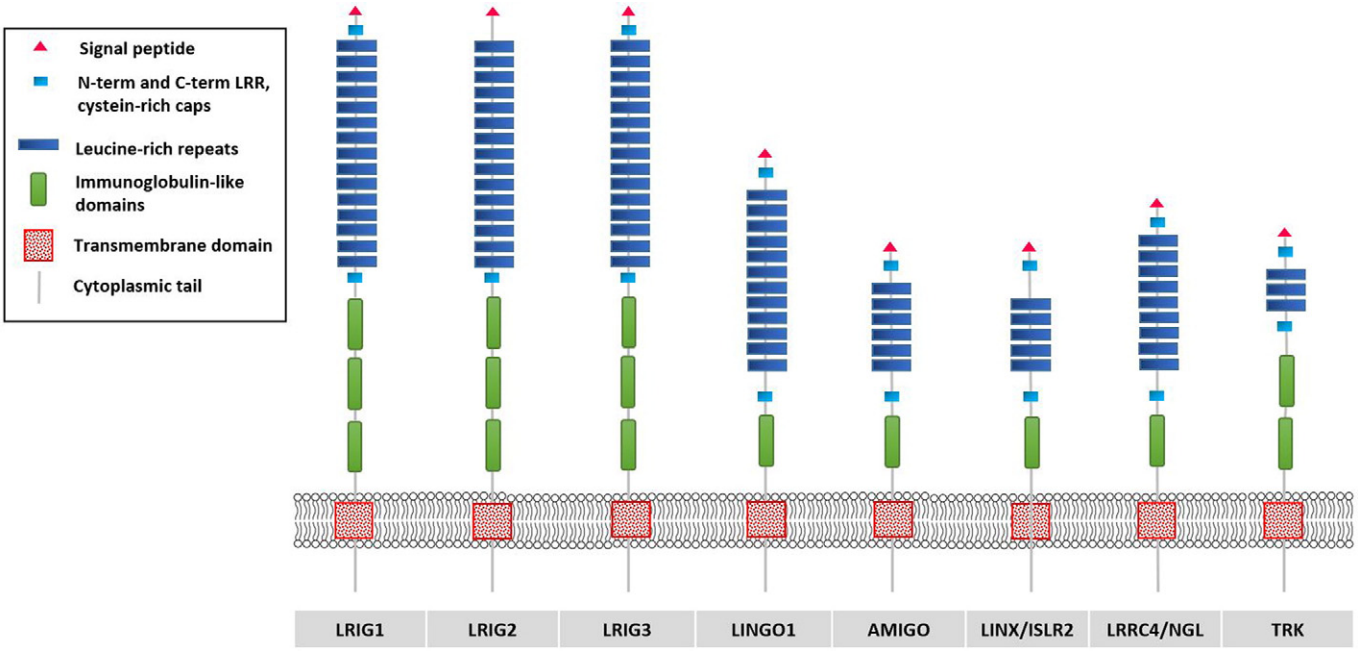

Fig. 1. Structural domains of human LIG proteins, showing respective differences in the number of leucine-rich repeats (LRR) or immunoglobulin-like domains (Ig) (NB: this representation does not reflect 3D protein structure).

domains, or both, are required for sustaining molecular interactions. As seen in Table 1, increasing evidence points to the importance of LRR domains, while the role of Ig domains remains controversial. It cannot also not be ruled out that the interaction of LRIG1 with its targets might be indirect and dependent on intermediate effectors. Further research should decipher the molecular mechanisms underlying LRIG1-RTK interaction, and for identifying the functional domains and minimal residues needed for this interaction.

2.3. Role of ubiquitin and protein degradation in LRIG1-mediated RTK inhibition

Receptor degradation allows to regulate the duration of signaling, by irreversibly eliminating activated RTKs. The classical mechanism underlying RTK degradation includes ubiquitination of the receptor, followed by internalization and degradation by the proteasome or after transport to the lysosome. This process is tightly dependent on the level of ubiquitination, which is regulated by enzymes with some level of specificity for target proteins. Among these, Cbl enzymes are E3 ubiquitin-ligases specifically recognizing phosphorylated tyrosine residues on RTKs and promoting poly-ubiquitination of the receptors [52].

The first reports relating the inhibition of EGFR signaling by LRIG1 proposed that LRIG1 expression increased ligand-induced ubiquitination of EGFR [23,24], and that EGFR degradation was maximal upon co-expression of LRIG1 and Cbl [23]. LRIG1-mediated receptor degradation was diminished upon expression of a dominant-negative Cbl protein [53] and functional Cbl enzyme was required for the degradation of LRIG1-EGFR complexes by the proteasome [23]. A Cbl recognition site that was necessary for inhibition of EGFR signaling was identified on the cytosolic tail of LRIG1 [23].

More recent literature however has questioned the role of receptor ubiquitination/degradation in LRIG1-induced signaling. In this context, several recent studies reported that the reduction of receptor activity was unrelated to ubiquitination and degradation (see Table 2). For example, LRIG1 was found to effectively induce destabilization of the glioma-associated mutant EGFRvIII [54] or Met [28] in a Cbl-independent fashion (i.e. upon Cbl knockdown, or in presence of a dominant-negative Cbl). Other studies showed that decreased activity of EGFR, ErbB2

Table 1
Current knowledge of the specific contribution of functional domains for LIG protein interaction with target proteins and inhibition.

LIG Protein	Target	Target downregulation by **full-length** protein?	Domain involved in interaction (INT) and/or downregulation (DR) of the target?								Downstream effects of Target downregulation	Ref
			Ecto		*LRR only*		*Ig only*		*TM/Cyto*			
			INT	*DR*	*INT*	*DR*	*INT*	*DR*	*INT*	*DR*		
LINGO1	TrkA	YES	+	+	ns	ns	ns	ns	−	−	Reduction of neuronal differentiation in PC12	[38]
LINGO1	NgR	ns	+	+	ns	ns	ns	ns	ns	ns	Reversion of neurite outgrowth inhibition by myelin in CGN	[41]
LRIG3	FGFR1	YES	+	ns	+	ns	+	ns	−	ns	Reduction of neural crest induction in *Xenopus*	[42]
LRRC4	ns	YES	ns	+	ns	+	ns	−	ns	−	Reduction of proliferation in U251	[43]
LRIG1	EGFR	YES	+	ns	+	ns	+	ns	−	+	Reduction of *c-fos* expression in HEK293	[23]
LRIG1	ErbB3	YES	ns	+	ns	ns	ns	ns	ns	−	Reduction of proliferation in MCF7	[44]
LRIG1	TrkB	YES	+	+	+	+	−	−	−	−	Reduction of dendritic growth in hippocampal primary neurons	[28]
LRIG1	LRIG3	YES	+	+	+	+	+	−	−	−	ns	[45]
	ErbB2	YES	+	+	+	+	+	−	−	−	ns	
LRIG1	EGFR	YES	ns	+	ns	ns	ns	ns	ns	−	Reduction of glioma and COS-7 cell proliferation. Inhibition of EGF-induced *c-fos* transcription.	[48]

Legend: ns = not specified in the indicated publication; − = no, + = yes.

Table 2
Role of ubiquitination/degradation in LRIG1-mediated RTK inhibition.

Protein	Receptor	Receptor ubiquitination?	Mechanism	Downstream effects of target downregulation	Domain involved	Ref
LRIG1	EGFR	+	Cbl	EGFR degradation	LRIG1-Cyto	[23]
LRIG1	Met	−	Cbl-independent	Met degradation by the lysosome and not by the proteasome	ns	[26]
LRIG1	EGFRvIII	ns	Cbl-independent	ns	ns	[54]
LRIG1 ecto	EGFR	−	ns	No internalization, but inhibition of EGFR activity	ns	[46]
LRIG1	EGFR, ErbB2	−	Cbl-independent	Internalization of EGFR, degradation of EGFR and ErbB2	ns	[45]
LRIG1	RET	−	ns	No downregulation, but inhibition of RET signaling	ns	[25]
LRIG1	TrkB	+ *(but dependent on the level of TrkB activation)*	ns	TrkB degradation by the proteasome	ns	[28]

Legend: ns = not specified in the indicated publication.

or TrkB was not associated with LRIG1-mediated ubiquitination of RTKs [30,47,48]. Moreover, under certain conditions, LRIG1 or its ectodomain were able to negatively regulate Ret and EGFR signaling even in the apparent absence of receptor downregulation [27,50].

Thus the role of ubiquitin-dependent RTK inhibition by LRIG1 is still a question of debate and may be context-dependent. Although the level of receptor ubiquitination is systematically verified in most studies, other aspects such as receptor levels at the membrane, receptor internalization, or involvement of lysosome/proteasome machineries, are only partially covered. As mentioned above, RTK degradation is a negative feedback mechanism that regulates and is dependent on RTK activation. As discussed in [30], increased levels of ubiquitination may therefore be a consequence of reduced activation of RTK by LRIG1, rather than a cause. Similarly, the key role of the LRIG1 ectodomain, highlighted in the previous section, may also suggest an alternative model of LRIG1 activity. Importantly, LRIG1 ectodomain is active when applied as a soluble protein, indicating that the mechanism underlying RTK inhibition may exclusively depend on extracellular interactions. For instance, the impact of LRIG1-RTK interaction on the efficacy of ligand binding is not known. As it is the case for Kekkon-1, a *Drosophila* paralog of LRIG1 [55], LRIG1 may also dimerize with RTKs, hence limiting dimerization between RTK members, and reducing the amount of active receptors at the membrane. Further investigations are required to precisely decipher how LRIG1 - and LRIG1 ectodomain only - inhibit RTK signaling, and to shed light on the role of ubiquitin and the protein degradation machinery in this process.

2.4. Contrasting functions of LRIG2 and LRIG3 isoforms

The role of LRIG2 and LRIG3 in regulation of cell signaling remains unclear. Mutations in *LRIG2* were associated with urofacial syndrome [56]. *LRIG2*-deficient mice were shown to be protected against PDGF-induced gliomagenesis [29], implying that LRIG1 and LRIG2 could display opposite activities. Similar to the LRIG1 ectodomain [50], LRIG2 ectodomain is also shed from the membrane of glioma cells, however soluble LRIG2 increased cell proliferation by interacting with/enhancing the activity of EGFR [57]. In view of these data, it seems that even if LRIG1 and LRIG2 ectodomains share a similar structure (Fig. 1), they support different molecular interactions and activities. Recently, LRIG2 was described to negatively regulate the ADAM17-mediated shedding of the axon guidance receptor Neogenin at the membrane of cortical neurons [58]. It was therefore suggested that regulation of ectodomain shedding could constitute a new putative mechanism underlying LRIG-dependent regulation of membrane proteins, including RTKs. LRIG3 was described to interact with FGFR1 in the Xenopus, subsequently downregulating downstream signaling during neural crest formation [44]. In GBM cell lines, LRIG3 overexpression inhibited EGFR signaling and cell growth [59]. Conversely, LRIG3 apparently displays opposite activity to LRIG1 in breast cancer cells, contributing to the stabilization of ErbB receptors at the membrane [47]. Further studies are needed to understand LRIG2 and LRIG3 activities, which will also shed light on the mechanisms underlying LRIG1 activity as well.

3. LRIG1-mediated RTK signaling contributes to stem cell maintenance

Based on single cell gene expression profiling, LRIG1 was identified a few years ago as a marker and important regulator of cell quiescence in human epidermal stem cells. It was shown to downregulate EGFR, downstream MAPK pathways and c-Myc trancription, leading to reduced cell proliferation [60,61]. These multipotent LRIG1 + epidermal stem cells are virtually able to give rise to any cell type of the human skin and contribute to the formation of both sebaceous gland and interfollicular epidermis *in vivo* [62]. Nevertheless different stem cell populations exist in the mouse skin and it was recently shown that compared to other stem cell subtypes (Lgr5 + or Lgr6 + cells), LRIG1 expressing cells give rise to different types of tumors upon oncogenic β-catenin activation [63]. Similar LRIG1 + stem cell niches were identified in the intestinal crypts [25,26], wherein LRIG1 regulates stem cell maintenance through the balance of EGFR/ErbB signaling. These LRIG1-positive stem cells can give rise to colonic tumors, when lacking one allele of the *adenomatous polyposis coli (APC)* gene, then recapitulating features of patients with familial adenomatous polyposis [64]. Using an LRIG1 reporter mouse, two subpopulations of LRIG1 expressing stem cells were subsequently identified in the gut: one subset was Lgr5 + and expressed glycosylated LRIG1, whereas a 2nd subset was Lgr5 − and expressed a non-glycosytated form of LRIG1 [65]. More recently, LRIG1 was also shown to be expressed in smooth muscle progenitor cells in the small intestine, driving their fate towards two distinct subtypes of interstitial cells of Cajal (ICC), respectively associated with the deep muscular plexus (ICC-DMP) and the submucosal plexus (ICC-SMP) [66]. In the cornea, LRIG1 is expressed in epithelial stem cells, and loss of LRIG1 impairs stem cell recruitment and turnover after injury. These authors further described that LRIG1 regulates STAT3-dependent pathway and subsequent inflammation [67]. Based on these observations, LRIG1 is used as a marker for tissue-specific stem cells in both physiological and pathological conditions, i.e. in the skin [68,69], the gut [70,71], the ocular epithelium [72] and in cervical cancer [73].

4. LRIG1-mediated RTK regulation in health and disease

LRIG1 negatively regulates RTKs, both in healthy cells and in disease. As mentioned in the previous section, LRIG1 interferes with EGFR and ErbB receptors in tissue-specific stem cells [25,60,61]. In neuronal cells, LRIG1 specifically impairs neurite outgrowth through the inhibition of Ret or TrkB [27,30] both *in vitro* and in the developing brain.

In various models of cancer, LRIG1 negatively regulates EGFR [23,54], ErbB2,3,4 [24, 46, 74], Met [28,75], but also Ron [75,76], or PDGFRα [29], which results in reduced proliferation of tumor cells *in vitro* and *in vivo*. Furthermore, several papers also demonstrated that LRIG1 impairs the migration and invasion abilities of glioma [54], breast cancer [28] or bladder cancer cell lines [77]. This inhibitory effect on invasion was correlated with the downregulation of matrix metalloproteases (MMPs) upon LRIG1 overexpression [78,79]. Interestingly, the LRIG1-EGFR interaction was proposed to mediate cell-cell contact inhibition and the

acquisition of an invasive phenotype, by regulating EGFR/E-cadherin complexes in lung epithelium [80].

Along these lines, LRIG1 was recently identified as an inhibitor of epithelial-to-mesenchymal transition (EMT) in cancer cells [76]. This process classically takes place during normal embryonic development, but is also thought to contribute to cancer initiation, progression and metastasis [81,82]. EMT is characterized by specific changes in membrane composition, cell-cell interaction and adhesion, coupled to a defined transcriptional program. It was shown that LRIG1 silencing in mammary epithelial cells leads to stem cell enrichment and induction of the EMT process through upregulation of i.e. vimentin and CD44. The opposite was observed when overexpressing LRIG1 in invasive breast cancer cells: signaling downstream of EGFR, Met and Ron receptors was inhibited and invasion was reduced [76]. Another study highlighted the ability of LRIG1 to attenuate alterations in E-cadherin and vimentin and reverse hypoxia-induced EMT in glioma cells, through EGFR inhibition. Vasculogenic mimicry by tumor cells under hypoxic conditions was also affected by LRIG1 overexpression [83].

In summary, it appears that LRIG1 affects a wide array of phenotypes which are not limited to cell proliferation, and which may be cell context-dependent. In light of the complexity of tumor development and progression, the effects of LRIG1 on RTK-regulated cell processes including proliferation, quiescence, adhesion and cell-cell contact, migration, invasion, angiogenesis, etc. warrant future investigations.

5. Regulation of *LRIG1* expression in cancer

Along with its role in stem cell maintenance, LRIG1 was identified as a genuine tumor suppressor gene. Indeed, genetic ablation of the *LRIG1* gene in mouse skin and intestine led to aberrant proliferation of stem cells in the interfollicular epidermis [61], and to crypt expansion and duodenal adenomas in the gut [25,26], respectively. LRIG1 depletion was recently confirmed to first promote expansion of Brunner's submucosal glands in the intestine, further developing into duodenal adenomas with gastric metaplasia. Of note, the authors also showed that a subset of human duodenal adenomas lack LRIG1 expression, display gastric metaplasia and increased EGFR activity [84].

In view of these findings, understanding how the expression of this tumor suppressor gene is regulated is of high significance in cancer research. As an endogenous feedback inhibitor of RTKs, the expression of LRIG1 mRNA is induced downstream of EGFR [23] or TrkB signaling [30]. On the other hand, ErbB2 can downregulate LRIG1 levels, thereby enhancing its own expression levels [74]. More recently, it was suggested that LRIG1 gene expression is silenced by methylation in colorectal cancer cells, therefore unable to inhibit EGFR signaling [85]. Hypermethylation of the 3p14 locus, containing the LRIG1 gene, was also frequently observed in patients with cervical carcinoma. The outcome of those patients was worse if promoter hypermethylation was combined to loss of LRIG1 gene [86].

LRIG1 was also shown to be regulated by androgens [87] and estrogens, the latter through estrogen receptor alpha (ERα). LRIG1 is overexpressed in ERα-positive breast cancer, and ER-dependent induction of LRIG1 contributes to reduced tumor growth and increased relapse-free survival [46,88]. Other recent work indicates that loss of LRIG1 in intestinal stem cells is mediated by Mushashi RNA binding proteins (Msi1/2), which include RNA recognition motifs and therefore play a role in post-transcriptional gene regulation [89]. Msi1/2 bind LRIG1 transcripts, induce cell transformation by upregulation of the ErbB pathway [90] and lead to colorectal carcinomas.

6. Therapeutic potential of LRIG1 in cancer

Altogether, the aforementioned studies highlight the strong potential of LRIG1 in RTK modulation, which could be harnessed as a therapeutic anti-cancer strategy. A key advantage of LRIG1 may be its efficacy against multiple RTKs as well as its potential to circumvent resistance mechanisms generally activated by small molecule inhibitors. In this line, the emerging role of the LRIG1 ectodomain is promising, especially since the first experimental attempts to apply it as a soluble compound in patient-derived glioma models *in vivo* provided very encouraging results in different GBMs with variable RTK expression [49]. As mentioned in the previous sections, increased knowledge on the mechanistic aspects of LRIG1-RTK interaction and downstream signaling will facilitate its application as a therapeutic agent. Currently this application is hampered by the fact that LRIG1 is a large protein with multiple protein-protein interaction domains. An important step will therefore be the identification of the minimal active part of the protein. The synthesis of recombinant proteins and the development of a purified active LRIG1 domain will be essential in a perspective of anti-cancer treatment.

LRIG1 could also be applied in combination strategies. Downregulation of LRIG1 was shown to increase the activity of RTKs, thereby contributing to resistance to SMAC mimetics (antagonists of inhibitor of apoptosis (IAP) proteins) in breast cancer cells [75,91]. This resistance might be overcome upon TKI treatment, and therefore possibly by increased LRIG1 expression as well. In bladder cancer cells, adenovirus-based LRIG1 delivery was shown to inhibit EGFR expression and tumor growth [92]. LRIG1 also counteracted the side effects of cisplatin, through reducing cisplatin-induced nuclear accumulation of phosphorylated EGFR and related DNA damage response/repair [93]. Recent paper on breast cancer cells reported that fulvestrant (inhibitor of ERα) decreased LRIG1 expression, which subsequently induced upregulation of ErbB3 and tumor growth [46]. Inducing LRIG1 activation together with fulvestrant treatment could therefore be considered as an adequate therapeutic approach. To combat the acquired resistance of cancer cells to TKI treatment, coupling LRIG1 activation with TKIs could also be an interesting strategy to improve the response to RTK inhibition. It remains to be seen how the beneficial effect of LRIG1 can be best harvested in a therapeutic setting.

The design of a LRIG1-based therapeutic strategy must take into consideration the general purpose of RTK modulation. In the case of neuronal pathologies, RTK inhibition often reduces neuronal growth, hence the focus may be to activate RTK and block inhibitors. For example, recent approaches developed anti-LINGO-1 blocking antibodies to promote neuronal growth and/or remyelination in acute optic neuritis [94]. Conversely, in the context of cancer, therapeutic strategies focus on RTK inhibition in order to reduce tumor growth.

Importantly, the expression level of LRIG1 is significantly associated with tumor progression, and has prognostic implication in various cancer types, as has been reviewed in detail in [29]. In the same line, LRIG1 gene was recently identified as a key gene in colorectal cancer progression based on a transposon mutagenesis screen [95].

7. Conclusion

Since the identification of the human *LRIG1* gene in the early 2000s, a series of important studies have validated LRIG1 as a potent inhibitor of RTK signaling, a tumor suppressor and identified its therapeutic potential in various models of cancers. As mentioned previously, LRIG1 negatively regulates a wide panel of RTKs and related cell phenotypes, and has therefore great perspective as a therapeutic inhibitor of pan-RTK signaling. Additional research is needed to elucidate the precise mechanism of action of these proteins and pave the way for the establishment of specific and targeted therapies for regulating RTK signaling, in a range of pathological conditions.

Abbreviations

ADAM17	a disintegrin and metalloproteinase 17
AMIGO	Adhesion molecule with Ig like domain 1
ATP	Adenosine triphosphate
EGF	Epidermal growth factor

EGFR	Epidermal growth factor receptor
EMT	Epithelial-to-mesenchymal transition
ERα	Estrogen receptor α
FGFR	Fibroblast growth factor receptor
GBM	Glioblastoma
GDNF	Glial cell-derived neurotrophic factor
GPI	Glycophosphatidylinositol
IAP	Inhibitor of apoptosis
Ig	Immunoglobulin
InsR	Insulin receptor
LIG	Leucine-rich repeats and immunoglobulin-like domains
	LRIG/LINGO Leucine-rich repeats and immunoglobulin-like domains containing protein
LRR	Leucine-rich repeats
LRRC	Leucine-rich repeats containing
MAPK	Mitogen-activated protein kinase
MMP	Matrix metalloprotease
Msi	Mushashi
NgR	Nogo-66 receptor
PC12	Pheochromocytoma 12
PDGFR	Platelet-derived growth factor receptor
PTEN	Phosphatase and tensin homolog
PTP1B	Protein-tyrosine phosphatase 1B
RTK	Receptor tyrosine kinase
TKI	Tyrosine kinase inhibitor
Trk	Tropomyosin receptor kinase
VEGFR	Vascular endothelial growth factor receptor

Conflict of interest statement

The authors declare no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found, in online version.

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