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Review

LRIG inhibitors of growth factor signalling – double-edged swords in human cancer?

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ABSTRACT

The leucine-rich repeats and immunoglobulin-like domains (LRIG) proteins are newly discovered negative regulators of growth factor signalling and proposed tumour suppressors. They antagonise signalling by interacting with growth factor receptors and by enhancing their ubiquitylation and degradation. Data on the expression of LRIG in human cancer have recently begun to accumulate; however, not all data appear consistent with the notion that the LRIG proteins always function as tumour suppressors. In the present review, we argue that the LRIG proteins could be double-edged swords, promoting or suppressing human cancer depending on cellular context.

1. Introduction

The leucine-rich and immunoglobulin-like domains (LRIG) proteins are newly discovered integral membrane proteins.[1–4] The founding member, LRIG1, antagonises growth factor signalling mediated by ErbB receptor tyrosine kinases.[5–7] Since deregulated ErbB signalling contributes to the development of many epithelial cancers, it was suggested that *LRIG1* was a human tumour suppressor gene.[2,8] Concordantly, LRIG1 was found to be downregulated in renal cell carcinoma[9] and cutaneous squamous cell carcinoma.[10] However, analyses of publicly available gene expression data sets revealed that *LRIG* genes were not always downregulated in human cancer; instead, they appeared to be upregulated in certain tumours, thus challenging the notion that LRIG proteins are tumour suppressors. In the present review, we summarise the available biochemical and clinical results, and argue that the LRIG proteins could be double-edged swords, promoting or suppressing human cancer depending on cellular context.

2. The LRIG gene family

The first *LRIG* transcript to be discovered, mouse *Lrig1* (also called *Lig-1*), was isolated in a screen for genes upregulated in retinoic acid-treated P19 cells committed to neural differentiation.[1] The human *LRIG* gene family comprises three paralogous genes, namely *LRIG1* (formerly *LIG1*),[2] *LRIG2*[3] and *LRIG3*.[4] The *LRIG* genes encode integral membrane proteins, with an extracellular or lumenal part consisting of a leucine-rich repeat (LRR) domain and three immunoglobulin-like domains, followed by a transmembrane region and a

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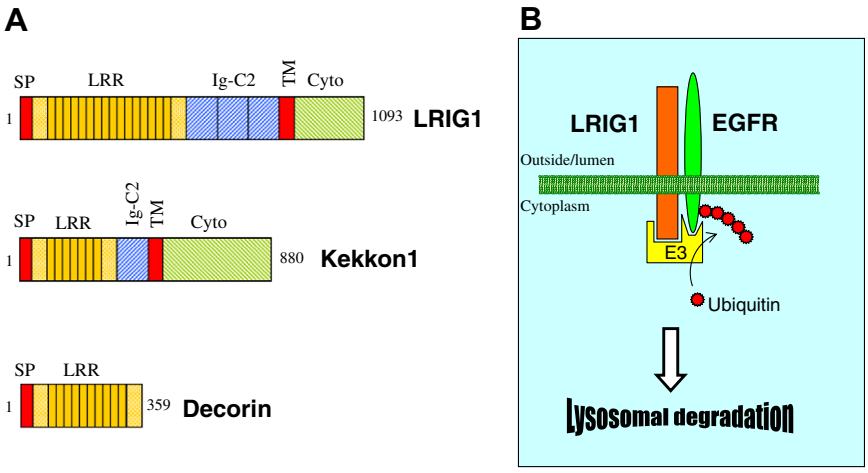

Fig. 1 – LRIG, kekkon and decorin domain organisation and molecular function of LRIG1. (A) Schematic drawing of the domain organisation of the LRIG, kekkon and decorin proteins. Indicated are the signal peptides (SP), the leucine-rich repeat domains, consisting of N- and C-terminal flanking regions and 6, 10, or 15 leucine-rich repeats (LRR), one or three immunoglobulin-like domains (Ig-C2), the transmembrane domains (TM) and the cytoplasmic tails (Cyto). Numbering refers to amino acids in *Drosophila* kekkon-1,[13] human decorin (PG40),[14] and human LRIG1,[2] respectively. (B) Schematic drawing of LRIG protein-induced ubiquitylation and degradation of ErbB receptors. LRIG1 interacts with ErbB receptors, including EGFR, via its extracellular domains, thereby recruiting cytoplasmic E3 ubiquitin ligases to the receptor complex; E3 ligase ubiquitylates the ErbB receptor and LRIG1 (not shown), resulting in receptor internalisation and lysosomal degradation.

cytoplasmic tail (Fig. 1A). The extracellular/lumenal part, the transmembrane region and the cytoplasmic tail all contain amino acid stretches which are highly conserved between the three LRIG proteins,[4] implying that the LRIG proteins share some, if not all, of their molecular functions. All normal tissues and organs analysed to date express *LRIG* transcripts (Table 1) and proteins; however, not every cell expresses all LRIGs.[1–4,8,11,12]

Table 1 – LRIG mRNA expression in human tissues (transcripts/100 pg total RNA)

	LRIG1	*LRIG2*	*LRIG3*
Adrenal gland	6.8	50.8	0.9
Bladder	6.3	2.6	10.9
Blood	1.9	0.1	0.1
Brain	94.6	36.5	5.0
Cervix	21.2	12.8	10.4
Colon	18.0	6.8	12.1
Heart	6.1	1.0	0.6
Kidney	30.3	43.9	26.9
Liver	128.0	8.8	8.2
Lung	13.6	6.7	7.2
Mammary gland	16.2	0.4	14.3
Skeletal muscle	74.8	25.4	5.7
Ovary	40.6	123.8	10.0
Pancreas	33.6	2.5	13.2
Placenta	3.9	44.0	11.8
Prostate	51.9	19.1	12.9
Salivary glands	13.2	3.2	9.3
Skin	25.6	83.5	87.6
Small intestine	105.2	33.3	30.7
Spleen	6.5	8.4	7.2
Stomach	103.0	32.6	56.9
Testicle	22.8	7.5	4.8
Thyroid	51.8	13.7	44.2
Trachea	39.4	9.5	14.8
Uterus	43.5	128.0	8.6

Data are from Guo *et al.*[4]

3. LRIG1 suppresses cell proliferation and ErbB receptor tyrosine kinases

LRIG proteins show structural similarities to two previously identified inhibitors of epidermal growth factor (EGF) signalling: the insect cell surface protein, kekkon-1,[13] and the mammalian extracellular matrix proteoglycan, decorin (PG40) [14] (Fig. 1A). Strikingly, the LRIG proteins share with kekkon-1 and decorin a structurally related N-terminal LRR domain. In addition, the LRIG proteins and kekkon-1 have their overall domain organisation in common; *i.e.* the LRR domain is followed by one or three immunoglobulin-like domains, a transmembrane domain and a cytoplasmic tail. In the fruit fly, *Drosophila melanogaster*, kekkon-1 is expressed in response to EGF signalling and is part of an EGF-driven negative feedback loop.[15] Kekkon-1 physically interacts through its LRR domain with the *Drosophila* EGF receptor (EGFR) and inhibits its binding of EGF and receptor signalling.[15,16] Decorin is a secreted extracellular matrix proteoglycan whose polypeptide comprises almost entirely its LRR domain.[14] Decorin is a ligand of EGFR and attenuates EGFR signalling by inducing receptor internalisation and persistent protein downregulation by an ubiquitin-independent pathway involving caveolae.[17–19] Because of its structural similarity to insect kekkon-1, it was suggested that human LRIG1 might be an inhibitor of EGFR-mediated signalling and a tumour suppressor in man.[2,8] Indeed, human LRIG1

was recently shown to be a negative regulator of EGFR signalling.[5,6] Concordantly, LRIG1 inhibits proliferation of human embryonic kidney-293 cells,[5] NIH3T3 fibroblasts,[6] keratinocytes,[20] bladder carcinoma cells,[21] and glioma cells (Wei *et al.*, our unpublished observations). Its mode of EGFR-inhibition, however, appears to be different from the mechanisms exploited by kekkon-1 and decorin (Fig. 1B). Analogous to kekkon-1, LRIG1 interacts through its extracellular LRR domain (and immunoglobulin-like domains) with the extracellular parts of the human ErbB family members, EGFR (ErbB1), ErbB2, ErbB3, and ErbB4. In contrast to decorin and kekkon-1, however, LRIG1 recruits cytoplasmic E3 ubiquitin ligases including c-Cbl,[5,22] resulting in ErbB receptor ubiquitylation, internalisation and lysosomal degradation.[5,6] Human LRIG1, thus, negatively regulates growth factor receptors belonging to the ErbB family by enhancing receptor ubiquitylation and degradation. Notably, it was recently shown that a recombinant LRIG1 ectodomain fragment, including only the LRR domain, by itself could suppress cell proliferation and EGFR signalling.[7] Whether LRIG1 fragments are naturally released and play a physiological role *in vivo* is currently unknown; however, the results, nevertheless, suggest that LRIG1 might suppress growth factor signalling by multiple mechanisms. Whether LRIG2 and LRIG3 have similar functions as LRIG1, and whether LRIG proteins affect other growth factor receptors than ErbB family members are important and still unresolved questions.

4. The role of LRIG1 in stem cell quiescence and skin homeostasis

The *LRIG* genes are highly expressed in normal skin (Table 1).[3,4,11,12] LRIG1 expression is associated with patches of basal and hair follicle cells, corresponding to the foci of epidermal stem cells.[11,20] LRIG1 seems to regulate epidermal stem cell quiescence by downregulating EGFR and suppressing stem cell proliferation.[20] Intriguingly, ablation of the *Lrig1* gene in mice results in the development of psoriatic lesions, including hyperproliferation of epidermal keratinocytes.[11] Since psoriasis is associated with deregulated EGFR signalling,[23,24] these results are consistent with a role for LRIG1 in restricting cell proliferation and EGFR signalling in normal skin. Furthermore, in cutaneous squamous cell carcinoma, which is also associated with deregulated EGFR signalling,[25,26] LRIG1 is downregulated in high grade tumours.[10] Taken together, these findings suggest that LRIG1 is important for the homeostasis of epidermis and its loss is associated with the development of psoriasis and high grade skin tumours.

5. LRIG expression in non-cutaneous cancers

Overexpression and hyperactivation of ErbB receptors is not only found in psoriasis and skin cancer, rather it is a feature of many epithelial cancers, including carcinomas of the kidney, bladder, cervix, breast, colon and lung.[27,28] This prompts the question of whether deregulation of LRIG protein expression contributes to the dysregulation of ErbB signalling in these malignancies. In the majority of renal cell carcinoma, LRIG1 is downregulated (Table 2),[9] and strikingly, the *EGFR*/*LRIG1* mRNA ratio was increased in nine of nine analysed kidney tumours compared to matched uninvolved kidney cortex. In transitional cell carcinoma of the bladder, LRIG1 protein levels were downregulated compared to clinically normal bladder tissue, and significant inverse correlations were observed between LRIG1 levels and tumour grade and clinical stage (Table 2). Similarly, LRIG1 protein levels were downregulated in high grade cervical carcinomas compared to low grade tumours (Table 2). A meta-analysis of publicly available gene expression data sets revealed downregulation of *LRIG1* in lung carcinoma (Table 2). Thus, in addition to its possible

Table 2 – Tumours showing underexpression (LRIG1-down) or overexpression (LRIG1/2-up) of LRIG genes or proteins compared to the corresponding normal tissues

Cancer	Expression	Detection method	Reference
Skin	LRIG1-down	IHC[a]	10
Renal	LRIG1-down	QRT-PCR[b]; IHC[a]	9
Bladder	LRIG1-down	IHC[a]	Yang Wei-Minc[c]
Cervix	LRIG1-down	IHC[a]	Hellberg *et al.*[c]
Lung	*LRIG1*-down	Meta-analysis[d]	Kilpinen and Kallioniemi[c]
Prostate	*LRIG1*-up	Microarray[e]	43,44
		Meta-analysis[d]	Kilpinen and Kallioniemi[c]
Muscle	*LRIG2*-up	Meta-analysis[d]	Kilpinen and Kallioniemi[c]
Lung carcinoid	*LRIG2*-up	Microarray[e]	45
Astrocytoma	*LRIG1*-up	SAGE[f]	NCBI[g]
Leukaemia	*LRIG1* and *LRIG2* – some very high[h]	Meta-analysis[d]	Kilpinen and Kallioniemi[c]

a Immunohistochemistry.
b Quantitative real-time reverse-transcription PCR.
c Unpublished observation.
d Meta-analysis of publicly available gene expression data sets, provided by Kilpinen and Kallioniemi (VTT Medical Biotechnology, Finland).
e Microarray gene expression; data extracted from the Oncomine 3.0 database (www.oncomine.org).[46]
f Serial analysis of gene expression.
g National Center for Biotechnology Information GEO data sets (www.ncbi.nlm.nih.gov).
h Very high expression in some "outlier" cases; however, the median expression levels were not increased as compared to normal bone marrow.

role in suppressing psoriasis and cutaneous squamous cell carcinoma, these findings are consistent with a role for LRIG1 in restraining EGFR signalling in the normal kidney, bladder, cervix and lung epithelia. In other words, downregulation of LRIG1 in these tissues could unleash EGFR signalling which might contribute to the development of various carcinomas.

Although LRIG1 is downregulated in carcinomas of the skin, kidney, bladder, cervix and lung, a survey of publicly available gene expression data sets does not reveal a general downregulation of *LRIG* gene expression in human tumours. For example, mining the Oncomine database reveals overexpression of *LRIG* genes in prostate cancer and lung carcinoids compared to the corresponding normal tissues (Table 2). Similarly, analysis of NCBI's serial analysis of gene expression (SAGE) data sets suggests high expression of *LRIG1* in astrocytoma (Table 2). The previously mentioned meta-analysis also revealed upregulation of *LRIG1* in prostate cancer and *LRIG2* in sarcoma, and a very high expression (*outlier expression*) of *LRIG1* and *LRIG2* in some leukaemia (Table 2). The situation in breast and colon carcinoma appears complex, with underexpression in some tumours and overexpression in others[29] (Ljuslinder *et al.*, submitted manuscript). It should be noted that the *LRIG* genes are relatively newly discovered and, hence, reports on their expression were scarce until recently. Especially for *LRIG3*, expression data are still very poor. Nevertheless, although the data are incomplete, published reports, the herein discussed unpublished results and publicly available data sets convincingly show that *LRIG* genes are both under- and overexpressed in human cancers.

6. LRIG proteins: double-edged swords in human cancer?

As discussed above, there is considerable experimental and clinical evidence that LRIG1 functions as a tumour suppressor in certain human tissues. Several receptor tyrosine kinases, including the LRIG1 targets ErbB1 and ErbB2, are well established proto-oncogenes, whose overexpression and activation are associated with tumour progression and poor patient outcome.[27,28,30] However, since the *LRIG* genes are not universally downregulated in human cancers, and in fact upregulated in some, it might be asked whether LRIG proteins always function as tumour suppressors, or even whether LRIG proteins under certain circumstances function as tumour promoters. Although this would appear contradictory to the role of LRIG proteins as suppressors of growth factor signalling, effects on certain tumour promoting growth factor receptors, differential subcellular protein localisation, compensatory feedback mechanisms or bystander effects might account for LRIG protein overexpression in some human tumours.

6.1. *Effects on tumour suppressing growth factor receptors*

Excessive receptor tyrosine kinase signalling is not stimulatory to cancer cell growth in all settings. The EGFR overexpressing A431 epidermoid cancer cell line, for instance, responds to EGF stimulation by undergoing cell growth arrest and apoptosis.[31–33] Thus, in situations of "growth factor susceptible" cancer cells thriving in growth factor-rich milieus,

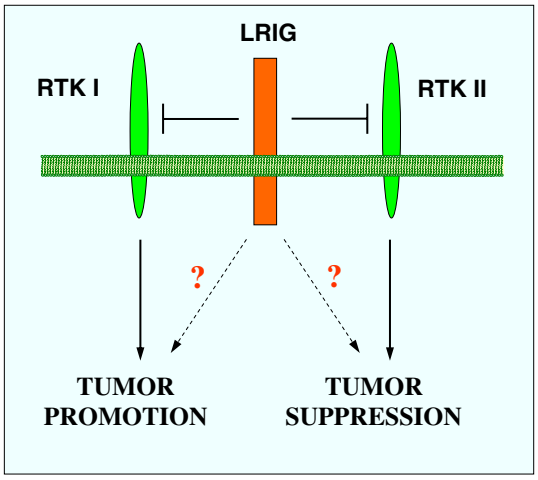

Fig. 2 – Schematic model of proposed functions of LRIG proteins in human cancer. LRIG proteins inhibit receptor tyrosine kinases (RTK), whose activities might be either tumour promoting (left) or tumour suppressing (right). Specific receptor tyrosine kinases with tumour promoting and tumour suppressing activities, respectively, are exemplified in the text. The LRIG proteins might have additional, yet unknown, functions which may in turn be either tumour promoting or tumour suppressing (dashed arrows).

LRIG protein overexpression could enable rather than restrict tumour growth (Fig. 2). In fact, ectopic expression of LRIG1 protects A431 cells from EGF-induced cell growth arrest and apoptosis.[5] Furthermore, some targets of LRIG protein-induced downregulation might be predominantly tumour suppressive receptors. Although the role of the LRIG1 target ErbB4 in human cancer is still not entirely clear,[34] there is emerging evidence that ErbB4 might function as a tumour suppressor in breast,[35–37] prostate,[38] and kidney[39] epithelia. If so, suppression of ErbB4 by LRIG1 could promote rather than suppress tumour growth. It is, thus, possible that downregulating certain growth factor receptors could promote rather than suppress tumour growth, and hence, might account for the overexpression of *LRIG* genes observed in some cancers.

6.2. *The role of subcellular protein localisation*

When normal or malignant tissues are stained with anti-LRIG antibodies, or when cell lines are transformed with green fluorescent protein (GFP)-tagged LRIG proteins, the LRIG proteins exhibit differential subcellular distribution in a cell type-specific manner. Thus, when LRIG-GFP fusion proteins are ectopically expressed in Vero fibroblasts or U-105 MG glioma cells, the LRIG proteins localise to the plasma membrane, whereas when the same LRIG proteins are expressed in human embryonic kidney-293 cells or GL15 glioma cells, the LRIG proteins predominantly localise to the perinuclear region.[3,40] At least a part of the intracellular LRIG pool co-localises with early endosomes and the trans-Golgi network[5] (Nilsson *et al.*, our unpublished observations). Similarly, *in vivo*, nuclear, perinuclear, cytoplasmic and plasma membrane localisation is observed in a cell type-specific manner

(Fig. 3). Moreover, in astrocytic tumours, perinuclear localisation of LRIG proteins predicts better patient survival,[40] and in oligodendroglial tumours, cytoplasmic expression of LRIG2 predicts worse patient outcome (Holmlund *et al.*, our unpublished observations). It could, thus, be speculated that the LRIG proteins exert their tumour suppressive functions in the perinuclear region, whereas cytoplasmic localisation would represent "mislocalisation" of the LRIG proteins. In any case, the subcellular distribution of LRIG proteins seems to have important clinical implications.

6.3. *Feedback regulation and possible linkage between LRIG1 and neighbouring oncogene(s)*

LRIG1 suppresses cell proliferation and is transcriptionally upregulated by certain growth signals; *e.g.* by EGF in HeLa cells[5] and by androgens in prostate carcinoma cells.[41] Consequently, overexpression of LRIG proteins could represent compensatory feedback mechanisms rather than being causative in tumourigenesis. Thus, even if the LRIG proteins function as tumour suppressors, growth signal feedback regulation might result in the overexpression of LRIG-proteins in some tumours compared to the corresponding normal tissues.

Over-expression of *LRIG* genes in human tumours could also be secondary to other oncogenetic events. The *LRIG1* locus at chromosome 3p14 shows increased copy number in a subset of breast carcinomas.[29] This increase in *LRIG1* gene copy number could possibly be attributed to linkage of the *LRIG1* gene with nearby located oncogene(s), which could result in coupled co-amplification of *LRIG1*. It has, for example, been shown that at least twelve neighbouring genes at chromosome 17q12 are co-amplified and overexpressed in conjunction with amplification of the proto-oncogene *ErbB2* in breast carcinoma.[42] Even accidental coupled co-amplification of *LRIG1* would be predicted to result in increased LRIG1 levels, which, in turn, could theoretically drive the selection of breast cancer cells with compensatory upregulation of LRIG1 targets such as ErbB2. In fact, there is a strong association between amplification of the *ErbB2* gene and increased gene copy number of *LRIG1* [29] (Ljuslinder *et al.*, our unpublished observations). However, the presence of oncogenes in the vicinity of the *LRIG1* locus is an unproven hypothesis which awaits experimental verification.

7. Conclusions and future perspectives

The LRIG proteins appear to be *bona fide* tumour suppressors in certain epithelia. However, in some tumours, LRIG protein expression is upregulated. Thus, it is intriguing that not only the LRIG protein expression levels are important determinants, but that the subcellular protein localisation also appears biologically significant. However, at present, neither the mechanisms regulating the subcellular distribution of LRIG proteins, nor the biochemical consequences thereof are known. Clues to these questions are likely to come from the identification of proteins interacting with the LRIG proteins in the various subcellular compartments. It will also

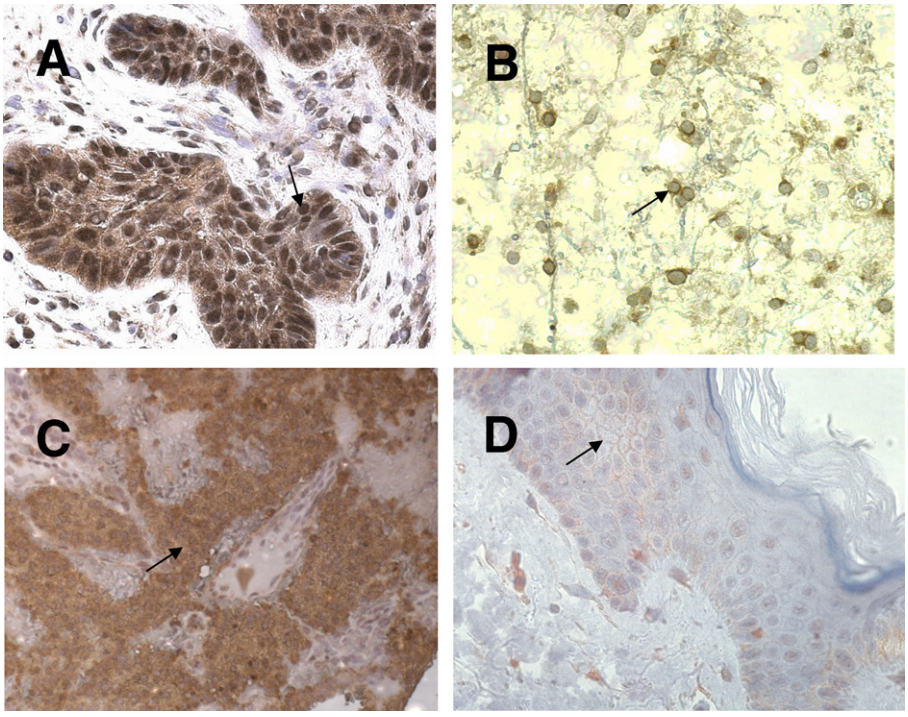

Fig. 3 – Clinical examples of differential subcellular localisation of LRIG proteins. (A) Nuclear (and cytoplasmic) staining of LRIG1 in basal cell carcinoma. (B) Perinuclear staining of LRIG3 in anaplastic astrocytoma (From Ref. [4], Fig. 4d, ©Springer-Verlag 2006, with kind permission of Springer Science and Business Media). (C) Cytoplasmic staining of LRIG2 in oligodendroglioma. (D) Plasma membrane staining of LRIG3 in normal human skin.

be important to extend the LRIG studies from epidermal stem cells to cover other stem cells, including cancer stem cells. Whether restricting growth factor signalling, or other yet unknown functions of the LRIG proteins sometimes can promote rather than suppress human cancer are interesting and still open questions. Future studies will further delineate the molecular functions of the LRIG proteins, the mechanisms and role of differential LRIG subcellular distribution, and the role of dysregulated LRIG proteins in the genesis and progression of human cancers.

Conflict of interest statement

The authors have no conflict of interest to declare.

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REFERENCES

1. Suzuki Y, Sato N, Tohyama M, et al. cDNA cloning of a novel membrane glycoprotein that is expressed specifically in glial cells in the mouse brain. LIG-1, a protein with leucine-rich repeats and immunoglobulin-like domains. *J Biol Chem* 1996;**271**:22522–7.
2. Nilsson J, Vallbo C, Guo D, et al. Cloning, characterization, and expression of human LIG1. *Biochem Biophys Res Commun* 2001;**284**:1155–61.
3. Holmlund C, Nilsson J, Guo D, et al. Characterization and tissue-specific expression of human LRIG2. *Gene* 2004;**332**:35–43.
4. Guo D, Holmlund C, Henriksson R, et al. The LRIG gene family has three vertebrate paralogs widely expressed in human and mouse tissues, and a homolog in Ascidiacea. *Genomics* 2004;**84**:157–65.
5. Gur G, Rubin C, Katz M, et al. LRIG1 restricts growth factor signaling by enhancing receptor ubiquitylation and degradation. *EMBO J* 2004;**23**:3270–81.
6. Laederich MB, Funes-Duran M, Yen L, et al. The leucine-rich repeat protein LRIG1 is a negative regulator of ErbB family receptor tyrosine kinases. *J Biol Chem* 2004;**279**:47050–6.
7. Goldoni S, Iozzo RA, Kay P, et al. A soluble ectodomain of LRIG1 inhibits cancer cell growth by attenuating basal and ligand-dependent EGFR activity. *Oncogene* 2006; doi:10.1038/sj.onc.1209803.
8. Hedman H, Nilsson J, Guo D, et al. Is LRIG1 a tumour suppressor gene at chromosome 3p14.3? *Acta Oncol* 2002;**41**:352–4.
9. Thomasson M, Hedman H, Guo D, et al. LRIG1 and epidermal growth factor receptor in renal cell carcinoma: a quantitative RT–PCR and immunohistochemical analysis. *Br J Cancer* 2003;**89**:1285–9.
10. Tanemura A, Nagasawa T, Inui S, et al. LRIG-1 provides a novel prognostic predictor in squamous cell carcinoma of the skin: immunohistochemical analysis for 38 cases. *Dermatol Surg* 2005;**31**:423–30.
11. Suzuki Y, Miura H, Tanemura A, et al. Targeted disruption of LIG-1 gene results in psoriasiform epidermal hyperplasia. *FEBS Lett* 2002;**521**:67–71.
12. Nilsson J, Starefeldt A, Henriksson R, et al. LRIG1 protein in human cells and tissues. *Cell Tissue Res* 2003;**312**:65–71.
13. Musacchio M, Perrimon N. The Drosophila kekkon genes: novel members of both the leucine-rich repeat and immunoglobulin superfamilies expressed in the CNS. *Dev Biol* 1996;**178**:63–76.
14. Krusius T, Ruoslahti E. Primary structure of an extracellular matrix proteoglycan core protein deduced from cloned cDNA. *Proc Natl Acad Sci USA* 1986;**83**:7683–7.
15. Ghiglione C, Carraway III KL, Amundadottir LT, et al. The transmembrane molecule kekkon 1 acts in a feedback loop to negatively regulate the activity of the Drosophila EGF receptor during oogenesis. *Cell* 1999;**96**:847–56.
16. Ghiglione C, Amundadottir L, Andresdottir M, et al. Mechanism of inhibition of the Drosophila and mammalian EGF receptors by the transmembrane protein Kekkon 1. *Development* 2003;**130**:4483–93.
17. Moscatello DK, Santra M, Mann DM, et al. Decorin suppresses tumor cell growth by activating the epidermal growth factor receptor. *J Clin Invest* 1998;**101**:406–12.
18. Iozzo RV, Moscatello DK, McQuillan DJ, et al. Decorin is a biological ligand for the epidermal growth factor receptor. *J Biol Chem* 1999;**274**:4489–92.
19. Zhu JX, Goldoni S, Bix G, et al. Decorin evokes protracted internalization and degradation of the epidermal growth factor receptor via caveolar endocytosis. *J Biol Chem* 2005;**280**:32468–79.
20. Jensen KB, Watt FM. Single-cell expression profiling of human epidermal stem and transit-amplifying cells: Lrig1 is a regulator of stem cell quiescence. *Proc Natl Acad Sci USA* 2006;**103**:11958–63.
21. Yang WM, Yan ZJ, Ye ZQ, Guo DS. LRIG1, a candidate tumour-suppressor gene in human bladder cancer cell line BIU87. *BJU Int* 2006;**98**:898–902.
22. Rubin C, Gur G, Yarden Y. Negative regulation of receptor tyrosine kinases: unexpected links to c-Cbl and receptor ubiquitylation. *Cell Res* 2005;**15**:66–71.
23. Vassar R, Fuchs E. Transgenic mice provide new insights into the role of TGF-alpha during epidermal development and differentiation. *Genes Dev* 1991;**5**:714–27.
24. Cook PW, Piepkorn M, Clegg CH, et al. Transgenic expression of the human amphiregulin gene induces a psoriasis-like phenotype. *J Clin Invest* 1997;**100**:2286–94.
25. Shimizu T, Izumi H, Oga A, et al. Epidermal growth factor receptor overexpression and genetic aberrations in metastatic squamous-cell carcinoma of the skin. *Dermatology* 2001;**202**:203–6.
26. Budiyanto A, Bito T, Kunisada M, et al. Inhibition of the epidermal growth factor receptor suppresses telomerase activity in HSC-1 human cutaneous squamous cell carcinoma cells. *J Invest Dermatol* 2003;**121**:1088–94.
27. Salomon DS, Brandt R, Ciardiello F, et al. Epidermal growth factor-related peptides and their receptors in human malignancies. *Crit Rev Oncol Hematol* 1995;**19**:183–232.
28. Citri A, Yarden Y. EGF-ERBB signalling: towards the systems level. *Nat Rev Mol Cell Biol* 2006;**7**:505–16.
29. Ljuslinder I, Malmer B, Golovleva I, et al. Increased copy number at 3p14 in breast cancer. *Breast Cancer Res* 2005;**7**:R719–27.

30. Slamon DJ, Clark GM, Wong SG, et al. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 1987;**235**:177–82.
31. Gill GN, Lazar CS. Increased phosphotyrosine content and inhibition of proliferation in EGF-treated A431 cells. *Nature* 1981;**293**:305–7.
32. Barnes DW. Epidermal growth factor inhibits growth of A431 human epidermoid carcinoma in serum-free cell culture. *J Cell Biol* 1982;**93**:1–4.
33. Gulli LF, Palmer KC, Chen YQ, et al. Epidermal growth factor-induced apoptosis in A431 cells can be reversed by reducing the tyrosine kinase activity. *Cell Growth Differ* 1996;**7**:173–8.
34. Gullick WJ. c-erbB-4/HER4: friend or foe? *J Pathol* 2003;**200**:279–81.
35. Jones FE, Welte T, Fu XY, et al. ErbB4 signaling in the mammary gland is required for lobuloalveolar development and Stat5 activation during lactation. *J Cell Biol* 1999;**147**:77–88.
36. Sartor CI, Zhou H, Kozlowska E, et al. Her4 mediates ligand-dependent antiproliferative and differentiation responses in human breast cancer cells. *Mol Cell Biol* 2001;**21**:4265–75.
37. Witton CJ, Reeves JR, Going JJ, et al. Expression of the HER1-4 family of receptor tyrosine kinases in breast cancer. *J Pathol* 2003;**200**:290–7.
38. Williams EE, Trout LJ, Gallo RM, et al. A constitutively active ErbB4 mutant inhibits drug-resistant colony formation by the DU-145 and PC-3 human prostate tumor cell lines. *Cancer Lett* 2003;**192**:67–74.
39. Thomasson M, Hedman H, Junttila TT, et al. ErbB4 is downregulated in renal cell carcinoma: a quantitative RT-PCR and immunohistochemical analysis of the epidermal growth factor receptor family. *Acta Oncol* 2004;**43**:453–9.
40. Guo D, Nilsson J, Haapasalo H, et al. Perinuclear leucine-rich repeats and immunoglobulin-like domain proteins (LRIG1-3) as prognostic indicators in astrocytic tumors. *Acta Neuropathol* 2006;**111**:238–46.
41. Febbo PG, Lowenberg M, Thorner AR, et al. Androgen mediated regulation and functional implications of fkbp51 expression in prostate cancer. *J Urol* 2005;**173**:1772–7.
42. Kauraniemi P, Bärlund M, Monni O, et al. New amplified and highly expressed genes discovered in the ERBB2 amplicon in breast cancer by cDNA microarrays. *Cancer Res* 2001;**61**:8235–40.
43. Welsh JB, Sapinoso LM, Su AI, et al. Analysis of gene expression identifies candidate markers and pharmacological targets in prostate cancer. *Cancer Res* 2001;**61**:5974–8.
44. Lapointe J, Li C, Higgins JP, et al. Gene expression profiling identifies clinically relevant subtypes of prostate cancer. *Proc Natl Acad Sci USA* 2004;**101**:811–6.
45. Bhattacharjee A, Richards WG, Staunton J, et al. Classification of human lung carcinomas by mRNA expression profiling reveals distinct adenocarcinoma subclasses. *Proc Natl Acad Sci USA* 2001;**98**:13790–5.
46. Rhodes DR, Yu J, Shanker K, et al. ONCOMINE: a cancer microarray database and integrated data-mining platform. *Neoplasia* 2004;**6**:1–6.