LRIG1 is a triple threat: ERBB negative regulator, intestinal stem cell marker and tumour suppressor

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In baseball parlance, a triple threat is a person who can run, hit and throw with aplomb. Leucine-rich repeats and immunoglobulin-like domains 1 (LRIG1) is a cell surface protein that antagonises ERBB receptor signalling by downregulating receptor levels. Over 10 years ago, Hedman et al postulated that LRIG1 might be a tumour suppressor. Recently, Powell et al provided in vivo evidence substantiating that claim by demonstrating that Lrig1 loss in mice leads to spontaneously arising, highly penetrant intestinal adenomas. Interestingly, Lrig1 also marks stem cells in the gut, suggesting a potential role for Lrig1 in maintaining intestinal epithelial homeostasis. In this review, we will discuss the ability of LRIG1 to act as a triple threat: pan-ERBB negative regulator, intestinal stem cell marker and tumour suppressor. We will summarise studies of LRIG1 expression in human cancers and discuss possible related roles for LRIG2 and LRIG3.

LRIG1 IS A PAN-ERBB NEGATIVE REGULATOR

Since the cloning of mouse Lrig1 (formerly called LIG-1) in 1996 (Suzuki et al., 1996), and human LRIG1 in 2001 (Hedman et al., 2002), studies have focused on deciphering its molecular function. In two independent studies, LRIG1 co-immunoprecipitated with and downregulated all four members of the ERBB receptor family (see Figure 1). Emerging data have underscored the importance of LRIG1 in growth suppression and cancer. Loss of Lrig1 in mice leads to heightened Egfr signalling in keratinocytes (Suzuki et al., 2002), and multiple groups have reported that LRIG1 regulates ERBB receptor degradation (Gur et al., 2004; Laederich et al., 2004; Shattuck et al., 2007; Ledda et al., 2008). Based on immunofluorescent analysis, Lrig1 was proposed to mark a population of quiescent stem cells in the mammalian epidermis (Jensen et al., 2009). This proposal was strengthened by a genetic study demonstrating that Lrig1 marks intestinal stem cells using lineage mapping (Powell et al., 2012). This study also showed that Lrig1 loss results in spontaneous tumour formation, supporting a tumour suppressor role for Lrig1. Interestingly, disrupting one allele of the tumour suppressor gene, adenomatous polyposis coli (Apc), in Lrig1−/− cells results in highly dysplastic adenomas in the intestine, supporting the idea that creating an initiating event in Lrig1−/− stem cells gives rise to intestinal tumours (Powell et al., 2012). In this review, we discuss the current understanding of the roles of LRIG1 in growth factor signalling modulation, and the evidence that Lrig1 may act as a tumour suppressor.

Leucine-rich repeats and immunoglobulin-like domains 1 (LRIG1) is a type 1 transmembrane protein whose extracellular domain contains 15 leucine-rich repeats (LRRs) and three immunoglobulin (Ig)-like domains (see Figure 1). Emerging data have underscored the importance of LRIG1 in growth suppression and cancer. Loss of Lrig1 in mice leads to heightened Egfr signalling in keratinocytes (Suzuki et al., 2002), and multiple groups have reported that LRIG1 regulates ERBB receptor degradation (Gur et al., 2004; Laederich et al., 2004; Shattuck et al., 2007; Ledda et al., 2008). Based on immunofluorescent analysis, Lrig1 was proposed to mark a population of quiescent stem cells in the mammalian epidermis (Jensen et al., 2009). This proposal was strengthened by a genetic study demonstrating that Lrig1 marks intestinal stem cells using lineage mapping (Powell et al., 2012). This study also showed that Lrig1 loss results in spontaneous tumour formation, supporting a tumour suppressor role for Lrig1. Interestingly, disrupting one allele of the tumour suppressor gene, adenomatous polyposis coli (Apc), in Lrig1−/− cells results in highly dysplastic adenomas in the intestine, supporting the idea that creating an initiating event in Lrig1−/− stem cells gives rise to intestinal tumours (Powell et al., 2012). In this review, we discuss the current understanding of the roles of LRIG1 in growth factor signalling modulation, and the evidence that Lrig1 may act as a tumour suppressor.

Keywords: LRIG1; tumour suppressor; EGFR signalling; stem cell

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A tumour suppressor is a gene in which loss-of-function results in transformation. Classically, both alleles are affected; however, there are examples of haplo-insufficient tumour suppressors, such as p27 (Fero et al, 1998). It is also increasingly appreciated that a tumour suppressor may act at distinct steps in the neoplastic process, such as initiation, invasion or metastasis. Although LRIG1 has been proposed to be a tumour suppressor for over a decade, only recently has genetic evidence demonstrated that Lrig1 ablation in mice leads to spontaneous tumour formation (Powell et al, 2012). Powell et al (2012) engineered a mouse model in which a CreERT2 cassette was inserted into the translational start site of the endogenous Lrig1 locus; mice were generated on a 129S7/SvEv and C57BL/6 mixed background. Mice homozygous for Lrig1-CreERT2 are functionally null for Lrig1, which we refer to as Lrig1Cre/Cre (see Table 1). Of note, we have observed embryonic lethality in Lrig1Cre/Cre mice backcrossed into a pure C57BL/6 background (unpublished results), indicating that in this inbred background, Lrig1 is essential for development. Consistent with the known function of Lrig1 in negatively regulating ErbBs and downstream signalling, the intestines of Lrig1Cre/Cre mice exhibit significantly increased ErbB1-3 protein levels and phosphorylated Erk1/2 (p-Erk1/2), as measured by immunoblot and/or immunohistochemistry. Over 88% of Lrig1Cre/Cre mice develop low-grade duodenal tumours overlying significantly expanded Brunner’s glands; levels of ErbB1-3 and p-Erk1/2 in these tumours are higher than in matched grossly normal small intestinal tissue. Interestingly, these tumours do not exhibit nuclear β-catenin, suggesting that tumours are not due to increased canonical Wnt signalling but more likely from enhanced ErbB signalling. This is consistent with the notion that proper calibration of ErbB signalling by Lrig1, especially in intestinal stem cells, is critical for intestinal cell and tissue homeostasis.

Genetic ablation of Lrig1 was first reported in 2002, when Suzuki et al (2002) engineered an Lrig1 null allele through insertion of a neomycin cassette after the first half of exon 1, resulting in a premature translational stop. These Lrig1 null mice, referred to as Lrig1neo/neo, also generated on a 129S7/SvEv and C57BL/6 mixed background, developed skin lesions resembling psoriasis on their tail, face and ears, but remained tumour free (see Table 1). Interestingly, as mentioned above, genetic background seems to affect phenotypes in Lrig1 mutant mice. Recently, when Wong et al (2012) crossed Lrig1neo/neo mice into an FVB/N background, they observed increased intestinal size and crypt expansion throughout the small intestine, resulting from increased epithelial proliferation at postnatal day 6. The mice appeared to be extremely malnourished and had to be killed, eliminating the possibility of intestinal tumorigenesis studies. Of note, the neomycin cassette is retained in this mutant; constitutive expression of neomycin in the homozygous state has been reported to contribute to various phenotypes, such as embryonic lethality, depending on the gene that it affects (Scacheri et al, 2001). Thus, it cannot be ruled out that neomycin expression in Lrig1neo/neo

Table 1. Genetic models for Lrig family members

| Genetic mutants | Phenotype |
|-----------------|-----------|
| Lrig1neo/neo    | Skin psoriasis (129-BL6) (Suzuki et al, 2002) | Severely distended abdomens (FVB/N) (Wong et al, 2012) |
| Lrig1Cre/Cre    | Duodenal adenomas (129-BL6) (Powell et al, 2012) | Embryonic lethality (BL6) (unpublished) |
| Lrig3−/−        | Defects in inner ear morphogenesis (Abraira et al, 2008) |

Abbreviation: Lrig1 = leucine-rich repeats and immunoglobulin-like domains 1. 129-BL6 indicates 129S7/SvEv and C57BL/6 mix background; FVB/N signifies FVB/N background; and BL6 indicates C57BL/6 background.
mice potentially contributes to the phenotypes observed. Despite the different phenotypes in these two Lrig1-null mouse models, it is clear that ablation of Lrig1 in mice leads to enhanced ErbB activity and increased growth, supporting a role for Lrig1 in intestinal homeostasis.

**STATUS OF LRIG1 IN HUMAN CANCERS**

The LRIG1 locus, 3p14.3, is deleted in some human cancers, including nasopharyngeal (Sheu et al., 2009), renal (Willers et al., 1996) and breast cancers (Maitra et al., 2001). However, according to the TCGA cancer data sets (colorectal, lung, glioblastoma and ovarian), the LRIG1 locus is rarely lost or mutated. Here, we discuss multiple studies of LRIG1 expression status in human cancers (also summarised in Table 2).

In a recent bioinformatics gene expression analysis of five cancers (breast, lung, bladder, glioma and melanoma) from eight independent studies (Rouam et al., 2010), LRIG1 was one of four genes whose decreased expression best correlated with poor survival. Low LRIG1 expression also correlated with worse outcome in squamous epithelial uterine cervical cancer (Lindstrom et al., 2008), lymphocytic leukaemia (Hanlon et al., 2009) and colorectal cancer (Figure 2). In a genetic screen, using Villin-CreERT2 to drive an activating Kras mutation on a Cre-activatable Sleeping Beauty transposon background, Lrig1 was the second most frequent gene to be disrupted in the subset of adenomas that advanced to cancer (see discussion in Powell et al., 2012).

In other cancers, LRIG1 expression inversely correlates with tumour stage and can also differ by cancer subtype. For example, in squamous cell carcinoma (SCC) of the skin, LRIG1 was expressed at greater levels in well-differentiated SCC than in poorly differentiated SCC, and SCCs expressing lower LRIG1 correlated with metastasis and decreased survival (Tanemura et al., 2005). In addition, Thomasson et al. (2012) reported that LRIG1 transcript and/or protein expression was decreased in clear cell renal cell carcinoma, but not in other histological subtypes.

LRIG1 expression in human cancer must be examined carefully, with attention to tissue context, cancer stage and cancer subtype. This is best exemplified in breast and prostate cancers, where oestrogen and androgen regulation of LRIG1 expression becomes a confounding factor (Miller et al., 2008; Thomasson et al., 2010; Krig et al., 2011). Miller et al. (2008) reported decreased LRIG1 transcript and protein levels in 63% of breast cancers examined that inversely correlated with tumour grade, as determined by Oncomine database and immunoblot analyses, respectively. When these data were further scored based on ERBB2 + status, 76% of ERBB2 + breast cancer tumours displayed decreased LRIG1 transcript or protein expression, compared with patient-matched normal tissue. In contrast to ERBB2 + breast tumours, ERx - breast tumours displayed increased levels of LRIG1 transcript by Oncomine database and immunoblot analysis, and intermediate-to-high LRIG1 gene expression correlated with longer relapse-free survival in ERx + breast cancer patients (Krig et al., 2011).

These two studies provide in vitro mechanisms to reconcile the different LRIG1 expression patterns observed in ERBB2 + and ERx - breast cancers. Miller et al. (2008) showed that constitutively active ERBB2 had a negative effect on LRIG1 transcript and protein, suggesting that oncogenic ERBB2 may employ a mechanism to decrease the tumour suppressive benefits of LRIG1, thereby imparting an advantage to ERBB2 + breast cancers. In addition, Krig et al. (2011) demonstrated that LRIG1 is a direct transcriptional target of ERx, thus suggesting higher LRIG1 expression in ERx + tumours may be due to ERx activity. Further, they also showed that ERBB2 activation decreases ERx levels, indirectly antagonising LRIG1 expression. This provides a mechanism for disparate LRIG1 expression observed in these subtypes of breast cancer and illustrates the importance of context-specific analysis of LRIG1 expression in human cancer. In a separate analysis, based on intrinsic subtypes of breast cancer, low LRIG1 expression was confirmed in the ERBB2 + subset; LRIG1 expression was highest in the luminal A subtype, the subtype with the best clinical outcome, and high LRIG1 expression correlated with a greater probability of

![Figure 2. LRIG1 expression in colorectal cancers. Box plot of the LRIG1 gene signature in the TCGA colorectal adenocarcinoma data set. LRIG1 expression is significantly downregulated in tumours compared with normal tissues. P<0.001.](Image 350x104 to 530x246)

| Cancer | Expression | Prognosis |
|--------|------------|-----------|
| Breast | ↓(ERBB2 -), ↑(ERBB2 +) (Miller et al., 2008) | ↑ ≈ Better (ERs -) (Krig et al., 2011) |
|        |            | ↑ ≈ Better (UNC) and (Rouam et al., 2010) |
| Lung   |            | ↑ ≈ Better (Rouam et al., 2010) |
| Colorectal | ↓(Ljuslinder et al., 2007) and TCGA | ↓ ≈ Better (Thomasson et al., 2012) |
| Renal cell | ↓ (Clear cell) (Thomasson et al., 2012) | ↓ ≈ Better (Lindstrom et al., 2008) |
|        |            | ↓ ≈ Better (early stage) (Hellberg et al., 2009) |
| Cervix |            | ↓ ≈ Better (Lindstrom et al., 2008) |
|        |            | ↓ ≈ Better (Ljuslinder et al., 2007) |
| Prostate | ↓ (Thomasson et al., 2010) | ↓ ≈ Better (glioma) (Rouam et al., 2010) |
| Brain  |            | ↓ ≈ Better (glioma) (Rouam et al., 2010) |
| Lymphocytic leukemia | ↓ (Hanlon et al., 2009) | ↓ ≈ Better (Rouam et al., 2010) |
| Melanoma |        | ↓ ≈ Worse (squamous cell) (Tanemura et al., 2005) |
| Skin   |            |        |

Abbreviation: LRIG1 = leucine-rich repeats and immunglobulin-like domains 1. ≈ Indicates correlated to; ↓ indicates upregulated in cancer compared with normal/control group; ↓ indicates downregulated in cancer compared with normal/control group; Worse denotes worse clinical outcome (poor/decreased survival); Better denotes better clinical outcome/survival; TCGA signifies statistical analysis using TCGA colorectal adenocarcinoma data set by Yan Guo; UNC indicates statistical analysis using UNC337 breast cancer data set by Charles M. Perou.
Given that LRIG1 is dysregulated in a number of human cancers, restoring its tumour suppressor function may attenuate growth factor signalling and reduce tumour growth. Interestingly, LRIG1 can associate with and destabilise EGFrVIII, a constitutively active mutant EGFR variant, suggesting there may be a possible therapeutic potential of LRIG1 in cancer, especially in glioblastoma where EGFRvIII is most commonly observed (Stutz et al, 2008). In addition, the soluble ectodomain of LRIG1, containing only the LRRs, associates with and inhibits EGFR activation, regardless of ligand stimulation, demonstrating a potentially novel mechanism of EGFR signalling modulation by LRIG1 (Goldoni et al, 2007). In this context, it has been shown recently that human glioblastomas expressing wild type or VIII mutant EGFR when placed within mouse brain are growth inhibited by nearby encapsulated cells secreting soluble LRIG1 ectodomain (Johansson et al, 2013). An in vitro study also showed that shedding of the LRIG1 ectodomain occurs endogenously and this ectodomain suppresses EGFR activation in a paracrine manner, without downregulating receptor levels (Yi et al, 2010).

The three LRIG protein family members have homologous functional domains. The extracellular regions of each contain 15 LRRs and three Ig-like extracellular domains and have 57–67% amino-acid sequence identity. Much less is known about the functions of LRIG2 and LRIG3 compared with LRIG1.

Overexpression of LRIG3 in HEK293T cells resulted in upregulation of ERBB receptors, in contrast to LRIG1, which is known to downregulate ERBB receptor levels (Aabraira et al, 2010). However, when LRIG3 was knocked down in a human glioblastoma cell line, GL15, both total EGFR and phospho-EGFR were moderately upregulated, consistent with observed increases in proliferation, adhesion and invasion (Cai et al, 2009). Therefore, the precise function of LRIG3 in regard to ERBB receptor signalling modulation may be context-dependent.

The only genetic study regarding Lrig3 in mouse focused on inner ear morphogenesis. Aabraira et al (2008) observed that Lrig3 expression was restricted to the lateral canal during embryogenesis; Lrig3 loss led to defects in inner ear morphogenesis and it was shown that during inner ear development, Lrig3 acts to repress Netrin transcription (see Table 1). Although LRIG3 can associate with EGFR, ERBB2 and ERBB4 in vivo, this inner ear phenotype is unlikely associated with ErbB signalling (Aabraira et al, 2010). A separate study in Xenopus laevis reported Lrig3 expression in the neural plate and neural crest, and that loss-of-function prevented neural crest marker expression (Zhao et al, 2008). In contrast,
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LRIG3 gain-of-function induced neural crest marker expression and attenuated Fgf signalling in animal caps, similar to Wnt3a gain-of-function, suggesting that LRIG3 negatively modulates Fgf signalling during X. laevis development (Zhao et al. 2008).

Even less is known about the role of LRIG2 and LRIG3 in human cancers. Mutations in LRIG2 and LRIG3 are rare, according to the TCGA data set. High LRIG2 expression is a poor prognostic marker in early stages of cervical cancers by immunohistochemistry (Hedman et al., 2010). In brain tumours, by semi-quantitative immunohistochemistry, cytoplasmic expression of LRIG2 correlated with decreased survival in oligodendrogliomas (Holmlund et al. 2009) and higher tumour grade in meningiomas (Ghasimi et al., 2012). In addition, LRIG2 protein is highly expressed in invasive pituitary adenomas, but not expressed in non-invasive cases by qPCR (Zhang et al., 2011), suggesting LRIG2 may be differentially regulated during tumour progression. LRIG3 downregulation was identified as one of 12 promising serum biomarkers for early stage non-small-cell lung cancer (Ostroff et al., 2010). Although the three LRIG family members share structural similarities, it is unclear whether they exhibit functional redundancy. The Lrig1 and Lrig3 knockout mice display distinct phenotypes. However, the expression pattern of the Lrig family members in the affected tissues in these knockout mice is unknown. Future studies will need to address this issue of functional redundancy.

CONCLUSION

In a recent review by Hanahan and Weinberg, perturbation of negative-feedback mechanisms that attenuate proliferative signalling was noted as an emerging hallmark of cancer (Hanahan and Weinberg, 2011). These negative-feedback programs are often regulated by tumour suppressor genes, directly or indirectly. LRIG1 qualifies as a negative-feedback inhibitor of ERBBs and other RTKs, and there is now in vivo evidence that it acts as a tumour suppressor. However, it remains to be demonstrated conclusively that its tumour suppressor activity is due directly to its ability to negatively regulate ErbBs and other RTKs. Going forward, it will be important to determine how LRIG1 physically interacts with EGFR and other RTKs, and how LRIG1 is regulated at the transcriptional and post-transcriptional level. It will also be of interest to determine by lineage labelling if Lrig1 marks stem cell populations in other organs than the intestine. Additional questions include whether Lrig1 maintains stem cell quiescence and whether this activity contributes to its tumour suppressor function. The triple threat features of LRIG1 – ERBB negative regulator, stem cell marker and tumour suppressor – clearly underscore the importance of understanding the function of LRIG1 in health and disease.

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