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# 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.

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*<sup>+</sup> cells results in highly dysplastic adenomas in the intestine, supporting the idea that creating an initiating event in *Lrig1*<sup>+</sup> 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.

## LRIG1 IS A PAN-ERBB NEGATIVE REGULATOR

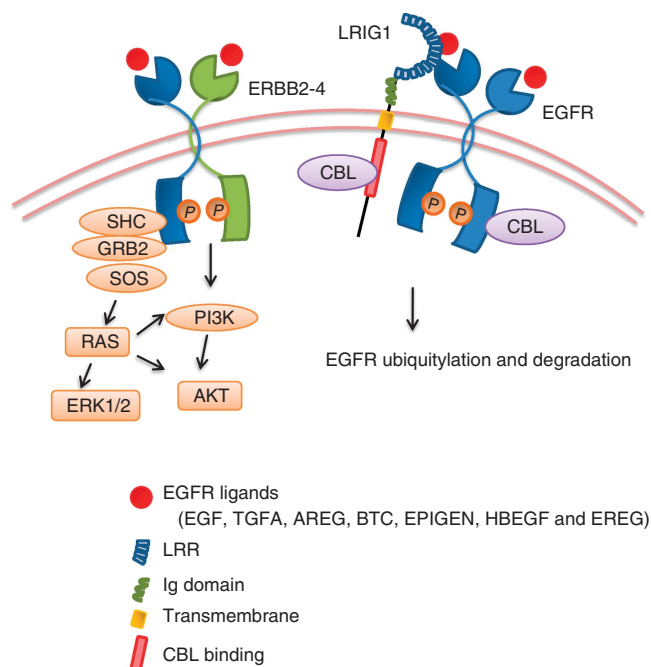
Since the cloning of mouse *Lrig1* (formerly called *LIG-1*) in 1996 (Suzuki *et al*, 1996), and human *LRIG1* in 2001 (Hedman *et al*, 2002), studies have focused on deciphering its molecular function. In two independent studies, LRIG1 co-immunoprecipitated with and downregulated all four members of the ERBB receptor family by heterologous expression; in the specific case of EGFR, LRIG1 can reduce receptor levels by promoting receptor ubiquitylation and lysosomal degradation, independent of EGFR ligands (see Figure 1) (Gur *et al*, 2004; Laederich *et al*, 2004). The interaction between EGFR and LRIG1 is thought to occur via their ectodomains; disruption of both the LRRs and Ig-like domains of LRIG1 is required to abrogate co-immunoprecipitation with EGFR (Gur *et al*, 2004). EGFR degradation by LRIG1 appears to be dependent on the E3 ligase, Cbl, as recruitment of Cbl to either EGFR or LRIG1 is sufficient to induce EGFR ubiquitylation and

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**Figure 1. LRIG1 negatively regulates EGFR signalling.** EGFR ligand binding results in phosphorylation of ERBBs and activation of downstream receptor tyrosine kinase signalling. In the current model, LRIG1 is postulated to associate with the EGFR ectodomain and accelerate recruitment of the E3 ligase c-CBL through a CBL-binding domain in the LRIG1 cytoplasmic tail. This effectively increases EGFR ubiquitylation and lysosomal degradation, resulting in decreased receptor levels at the cell surface. LRIG1 potentially downregulates other ERBBs through direct interaction or ERBB heterodimerisation with EGFR.

degradation (Gur *et al*, 2004). There is a marked reduction of EGFR degradation in cells expressing a mutant EGFR that lacks the putative Cbl-binding site (Y1045F) and a mutant LRIG1 lacking the Cbl-binding site. Interestingly, LRIG1 also efficiently downregulates EGFRvIII, an EGFR mutant lacking most of the ectodomain, in the presence of a dominant-negative form of Cbl, suggesting a possible Cbl-independent mechanism for mutant receptor downregulation in this instance (Stutz *et al*, 2008).

Germline ablation of *Lrig1* in two independent mouse models resulted in elevated levels of ErbB1-3 in the intestine (Powell *et al*, 2012; Wong *et al*, 2012), providing *in vivo* evidence to support its role in degrading ErbBs. LRIG1 can also associate with the receptor tyrosine kinase MET, independent of ligand stimulation and receptor activation, and is thought to enhance its degradation without affecting receptor ubiquitylation, independent of Cbl (Shattuck *et al*, 2007; Lee *et al*, 2012). Distinct from its mechanism of downregulating ERBB and MET, LRIG1 associates with the RET receptor tyrosine kinase and antagonises glial-derived neurotrophic factor ligand binding to and activating RET (Ledda *et al*, 2008). LRIG1 silencing in human glioma cells leads to increased proliferation and invasion (Mao *et al*, 2012). Thus, it appears that LRIG1 may control cell growth through antagonising multiple receptor tyrosine kinases. However, much remains to be learned about how endogenous LRIG1 is regulated, and the structural details through which it interacts with RTKs.

## LRIG1 IS A TUMOUR SUPPRESSOR IN MOUSE

A tumour suppressor is a gene in which loss-of-function results in transformation. Classically, both alleles are affected; however, there

**Table 1. Genetic models for Lrig family members**

Genetic mutants	Phenotype
<i>Lrig1<sup>neo/neo</sup></i>	Skin psoriasis (129-BL6) (Suzuki <i>et al</i> , 2002)
	Severely distended abdomens (FVBN) (Wong <i>et al</i> , 2012)
<i>Lrig1<sup>Cre/Cre</sup></i>	Duodenal adenomas (129-BL6) (Powell <i>et al</i> , 2012)
	Embryonic lethality (BL6) (unpublished)
<i>Lrig3<sup>-/-</sup></i>	Defects in inner ear morphogenesis (Abraira <i>et al</i> , 2008)

Abbreviation: Lrig1 = leucine-rich repeats and immunoglobulin-like domains 1. 129-BL6 indicates 129S7/SvEv and C57BL/6 mix background; FVBN signifies FVBN background; and BL6 indicates C57BL/6 background.

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 *Lrig1<sup>Cre/Cre</sup>* (see Table 1). Of note, we have observed embryonic lethality in *Lrig1<sup>Cre/Cre</sup>* 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 *Lrig1<sup>Cre/Cre</sup>* 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 *Lrig1<sup>Cre/Cre</sup>* 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  $\beta$ -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 *Lrig1<sup>neo/neo</sup>*, 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 *Lrig1<sup>neo/neo</sup>* mice into an FVBN 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 *Lrig1<sup>neo/neo</sup>*

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).

Table 2. Expression of *LRIG1* in human cancers

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

Abbreviation: *LRIG1* = leucine-rich repeats and immunoglobulin-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.

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<sup>+</sup> status, 76% of ERBB2<sup>+</sup> breast cancer tumours displayed decreased *LRIG1* transcript or protein expression, compared with patient-matched normal tissue. In contrast to ERBB2<sup>+</sup> breast tumours, ERα<sup>+</sup> 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 ERα<sup>+</sup> breast cancer patients (Krig *et al*, 2011).

These two studies provide *in vitro* mechanisms to reconcile the different *LRIG1* expression patterns observed in ERBB2<sup>+</sup> and ERα<sup>+</sup> 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<sup>+</sup> breast cancers. In addition, Krig *et al* (2011) demonstrated that *LRIG1* is a direct transcriptional target of ERα, thus suggesting higher *LRIG1* expression in ERα<sup>+</sup> tumours may be due to ERα activity. Further, they also showed that ERBB2 activation decreases ERα 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<sup>+</sup> 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

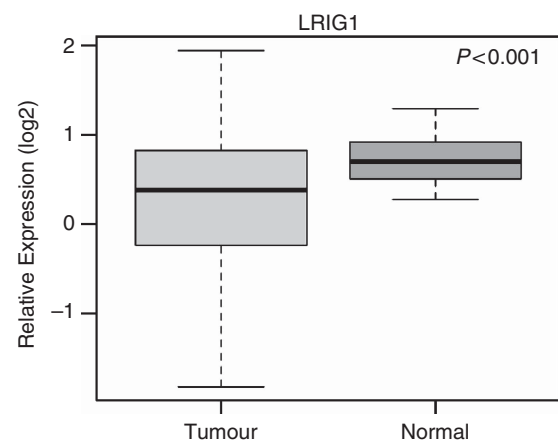
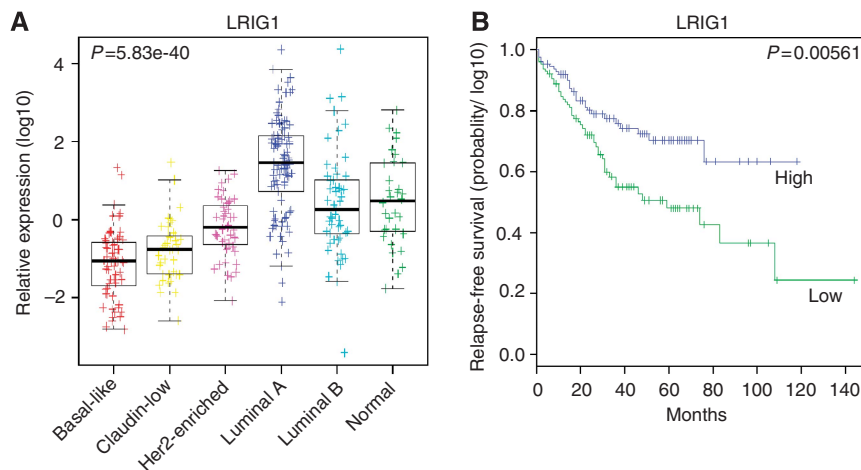


Figure 2. *LRIG1* expression in colorectal cancers. Box plot of the *LRIG1* gene signature in the TCGA colorectal adenocarcinoma data set. *LRIG1* expression is significantly downregulated in tumours compared with normal tissues.  $P < 0.001$ .



**Figure 3.** LRIG1 expression in breast cancers. **(A)** Box plot of the *LRIG1* gene signature in the UNC337 human breast tumour data set based on intrinsic subtypes. LRIG1 expression appears to be significantly upregulated only in the Luminal A intrinsic subtype compared with normal ( $P = 5.83 \times 10^{-40}$ ). **(B)** When divided into high (50/128) and low (29/128) LRIG1-expression groups, patients with high LRIG1 expression exhibit longer relapse-free survival. *P*-values reflect statistical significance of analysis of variances (ANOVAs;  $P = 0.00561$ ).

relapse-free survival (Figure 3, personal communication with Dr Charles M Perou).

Prostate cancer has emerged as an important example of how LRIG1 expression may not directly reflect a tumour suppressor effect. Thomasson *et al* (2010) examined two independent groups of prostate cancer patients; LRIG1 protein expression correlated with better survival of one group, in which patients had undergone prostatectomy, but worse survival of the second group, in which patients had not received treatment. To address this paradox, the authors showed that LRIG1 transcript and protein are upregulated by androgen signalling. In fact, LRIG1 expression may act as a surrogate marker for androgen activity, which is most often the main driver for prostate carcinogenesis. In this context, the authors suggest that although LRIG1 overexpression decreases PC3 prostate cancer cell growth, tumour-promoting androgen signalling prevails over the tumour suppressive impact of LRIG1 in patients with increased androgen activity and increased LRIG1 (Thomasson *et al*, 2010). Thus, in a high androgen environment, LRIG1 may reflect androgen signalling and may not exert enough tumour suppressive activity to overcome the threshold of oncogenic events, and thus expression should be carefully evaluated as its role may be masked by other factors.

Finally, a study by Hellberg *et al* (2009) exemplifies the significance of considering cancer biomarker expression in the context of other factors. This study demonstrated that the prognostic significance of LRIG1 protein expression in cervical cancer was dependent on how the data were correlated with other factors, such as cancer subtype, stage and other known tumour suppressors and oncogenes (Hellberg *et al*, 2009). Moving forward, it will be important to evaluate LRIG1 expression in human cancer with consideration of cancer stage, subtype and additional factors (such as estrogens) that may influence its expression.

#### THERAPEUTIC POTENTIAL OF LRIG1

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).

#### OTHER LRIG FAMILY MEMBERS: LRIG2 AND LRIG3

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 (Abraira *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. Abraira *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 (Abraira *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,



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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