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Investigations of Leucine-rich repeats and immunoglobulin-like domain-proteins 1 and 2 (LRIG1 and LRIG2) and their genes in cancer

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To my big family who never gives up to support

And

To all who serve the humanity

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Abstract

The mammalian leucine-rich repeats and immunoglobulin-like domains (LRIG) gene family consists of three different members, *LRIG1*, *LRIG2*, and *LRIG3*. These genes are expressed in all human and mouse tissues analyzed to date. All LRIG proteins share similar and evolutionary conserved structural domains including a leucine-rich repeat domain, three immunoglobulin-like domains, a transmembrane domain, and a cytosolic tail. Since the discovery of this family, around 20 years ago, various research groups have shown the importance of this family in cancer biology and prognosis. The aim of this thesis was to further investigate the role of LRIG1 and LRIG2 in cancer.

To investigate the roles of LRIG1 and LRIG2 in physiology and gliomagenesis, we generated *Lrig1*- and *Lrig2*-deficient mice and induced platelet-derived growth factor B (PDGFB)-driven gliomagenesis. We studied the effects of *Lrig2* ablation on mouse development and survival and investigated if the ablation of *Lrig1* or *Lrig2* affects the incidence or malignancy of induced gliomas. We also investigated if *Lrig2* ablation affects Pdgfr signaling in mouse embryonic fibroblasts (MEFs). Additionally, we analyzed the effects of ectopic LRIG1 expression in human primary glioblastoma cell lines TB101 and TB107, *in vivo* and *in vitro*. We reported no macroscopic anatomical defect but reduced growth and increased spontaneous mortality rate in *Lrig2*-deficient mice. However, the *Lrig2*-deficient mice were protected against the induced gliomagenesis. *Lrig2*-deficient MEFs showed faster kinetics of induction of immediate-early genes in response to PDGFB stimulation, whereas the phosphorylations of Pdgfra, Pdgfrb, Erk1/2, and Akt1 appeared unaltered. Lrig1-heterozygote mice showed a higher incidence of high-grade tumors (grade IV) compared to wildtype mice, demonstrating a haploinsufficient function of Lrig1. LRIG1 overexpression suppressed TB107 cell invasion *in vivo* and *in vitro*, which was partially mediated through the suppression of the MET receptor tyrosine kinase.

To identify LRIG1-interacting proteins, we used the yeast-two hybrid system and data-mined the Bio-Plex network of high throughput protein-protein

interaction database. To study the function of interactors, we used a triple co-transfection system to overexpress LRIG1 and PDGFRA and downregulate endogenous levels of interactors by short hairpin RNAs (shRNAs), simultaneously. This analysis demonstrated that CNPY3, CNPY4, GAL3ST1, GML, HLA-DRA, LRIG2, LRIG3, LRRC40, PON2, RAB4A, and ZBTB16 were important for the PDGFRA-downregulating function of LRIG1.

To investigate the clinical significance of *LRIG1* copy number alterations (CNAs) in breast cancer, we used droplet digital PCR (ddPCR) to analyze 423 breast cancer tumors. We found that *LRIG1* CNAs were significantly different in steroid-receptor-positive vs steroid-receptor-negative tumors and in ERBB2-amplified vs ERBB2-non-amplified tumors. In the whole cohort, patients with LRIG1 loss or gain had a worse metastasis-free survival than patients with normal *LRIG1* copy numbers, however, among the early-stage breast cancer subgroup, this difference was not significant.

In summary, *Lrig1* behaved like a haploinsufficient tumor suppressor gene in malignant glioma, whereas *Lrig2* appeared to promote malignant glioma. Our functional analysis of LRIG1 interactome uncovered several unanticipated and novel proteins that might be important for the regulation of receptor tyrosine kinases by LRIG1. *LRIG1* CNAs predicted metastasis-free survival time in breast cancer. Hopefully, our findings might lead to a better understanding of the regulation of growth factor signaling and its importance in cancer biology and prognosis.

Abbreviations

AKT	Ak strain transforming protein
Bio-Plex	Biophysical interactions of ORFeome-based complexes
CANT1	Calcium activated nucleotidase 1
CBL	Casitas b-cell lymphoma
CN	Copy number
CNAs	Copy number alterations
CNPY3	Canopy fibroblast growth factor signaling regulator 3
CNS	Central nervous system
ddPCR	Droplet digital PCR
DNA	Deoxyribonucleic acid
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EGFRvIII	EGFR mutant variant III
EMT	Epithelial-mesenchymal transition
ER	Estrogen receptor
ERBB	v-Erb-B erythroblastic leukemia viral oncogene homolog
ERK	Extracellular signal-regulated kinase
FACS	Fluorescence-activated cell sorting
FBS	Fetal bovine serum
FISH	Fluorescence in situ hybridization
FOS	FBJ murine osteosarcoma viral oncogene homolog
GAL3ST1	Galactosyl-3-sulfonyl-transferase-1
GBM	Glioblastoma
GLRX3	Glutaredoxin 3
GRB2	Growth factor receptor-bound protein 2
GTP	Guanosine triphosphate
HEK293	human embryonic kidney 293
HLA-DRA	Major histocompatibility complex, class II, DR
IDH	Isocitrate dehydrogenase
Ig	Immunoglobulin-like

JUN	v-Jun avian sarcoma virus 17 oncogene homolog
kDa	Kilodalton
LOH	Loss of heterozygosity
LRIG	Leucine-rich repeats and immunoglobulin-like domains
LRR	Leucine-rich repeats
LRRC40	Leucine-rich repeat containing 40
MAPK	Mitogen-activated protein kinase
MEF	Mouse embryonic fibroblast
MET	Mesenchymal-epithelial transition
MFS	Metastasis-free survival
MGMT	O6-methylguanine DNA methyltransferase
MIG-6	mitogen-inducible gene 6
miRNA	Micro-RNA
MTFR1L	Mitochondrial fission regulator 1 like
MYC	v-Myc avian myelocytomatosis viral oncogene homolog
NST	Nervous system tumor
OS	Overall survival
PBS	Phosphate-buffered saline
PCV	procarbazine, lomustine, and vincristine
PDGF	Platelet-derived growth factor
PDGFR	Platelet-derived growth factor receptor
PI3K	Phosphatidylinositol 3-kinase
PON2	Paraoxonase 2
PR	Progesterone receptor
PTEN	Phosphatase and tensin homolog
PTP	Protein tyrosine phosphatase
PTPRK	Protein tyrosine phosphatase, receptor type K
RAB4A	RAB4A, member RAS oncogene family
RAS	Rat sarcoma viral oncogene homolog
RAF	v-Raf murine leukemia viral oncogene homolog
RCAS	Replication competent ALV splice acceptor
RET	rearranged during transfection

RNA	Ribonucleic acid
RTK	Receptor tyrosine kinase
RT-PCR	Reverse transcriptase polymerase chain reaction
SCARA3	Scavenger receptor class A member 3
SCGB2A2	Secretoglobin family 2A member 2
SCRIB	Scribbled planar cell polarity protein
siRNA	Small interfering RNA
SRC	v-Src avian sarcoma viral oncogene homolog
TCGA	The cancer genome atlas
TERT	telomerase reverse transcriptase
TGF	Tumor growth factor
TP53	Tumor protein 53
Trk	tropomyosin-related kinase
TUBB8	Tubulin beta 8 class VIII
WHO	World health organization
Y2H	Yeast two-hybrid
ZBTB16	Zinc finger and BTB domain–containing 16

List of original papers

Paper I§

Rondahl, V., Holmlund, C., Karlsson, T., Wang, B., Faraz, M., Henriksson, R., and Hedman, H. (2013). *Lrig2*-deficient mice are protected against PDGFB-induced glioma. PLoS One 8, e73635.

Paper II§

Mao, F., Holmlund, C., Faraz, M., Wang, W., Bergenheim, T., Kvarnbrink, S., Johansson, M., Henriksson, R., and Hedman, H. (2018). *Lrig1* is a haploinsufficient tumor suppressor gene in malignant glioma. Oncogenesis, 7, 13.

Paper III§

Faraz, M., Herdenberg, C., Holmlund, C., Henriksson, R., and Hedman, H. (2018). A protein interaction network centered on leucine-rich repeats and immunoglobulin-like domains 1 (LRIG1) regulates growth factor receptors. Journal of Biological Chemistry, 293, 3421-3435.

Paper IV

Faraz, M., Tellström, A., Edwinsdotter, C., Grankvist, K., Huminiecki, L., Tavelin, B., Henriksson, R., Ingrid, L., and Hedman, H. (2018). *LRIG1* gene copy number analysis by ddPCR and correlation to clinical factors in breast cancer. Manuscript.

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Another published paper that was not included in this thesis: Hellstrom, M., Ericsson, M., Johansson, B., Faraz, M., Anderson, F., Henriksson, R., Nilsson, S.K., and Hedman, H. (2016). Cardiac hypertrophy and decreased high-density lipoprotein cholesterol in *Lrig3*-deficient mice. Am J Physiol Regul Integr Comp Physiol.

Introduction

1. Receptor tyrosine kinases (RTKs)

The tyrosine kinase superfamily consists of cytoplasmic protein tyrosine kinases and receptor tyrosine kinases (RTKs). RTKs are transmembrane proteins with an extracellular domain that binds to ligands, a transmembrane domain, and an intracellular region that has a highly conserved kinase domain (Yarden and Ullrich, 1988). RTKs are found in metazoans with wide expression in different tissues (Grassot et al., 2003). They have very critical roles in cell biology. They regulate homeostasis by controlling various cellular processes like proliferation, differentiation, migration, metabolism, adhesion, communication, etc. There are 90 tyrosine kinase genes in the human genome of which 58 are RTKs, which are categorized into 20 subfamilies (Robinson et al., 2000). Figure 1 shows the RTK subfamilies with their members (Blume-Jensen and Hunter, 2001). In this thesis, three well-studied RTKs were of particular interest, namely platelet-derived growth factor (PDGF) receptor (PDGFR), epidermal growth factor (EGF) receptor (EGFR), and mesenchymal-epithelial transition receptor (MET).

1.1. PDGFRs

PDGFs were discovered as serum growth factors needed for the survival of fibroblasts, smooth muscle cells, and glia cells (Kohler and Lipton, 1974; Ross et al., 1974; Westermark and Wasteson, 1976). Some functions of PDGFs signaling pathway are highly conserved during evolution from worms to human with important roles in development. In mammals, PDGFs play critical roles in the development of oligodendrocytes, neural cells in the central nervous system (CNS), vascular smooth muscle cells, pericytes, kidney, and lung. Moreover, they are involved in the regulation of proliferation, migration, and tissue remodeling in neural crest cells (Hoch and Soriano, 2003; Noskovicova et al., 2015) and wound healing (Heldin and Westermark, 1999). The expression patterns of PDGFs and their receptors are regulated during development. However, deletion of either PDGFRA or PDGFRB receptor in mice is lethal

(Tallquist and Kazlauskas, 2004). PDGFRA is highly expressed in mesenchymal progenitors of lung, skin, and intestine and also progenitors of oligodendrocytes. PDGFRB is highly expressed in mesenchymal cells like vascular smooth muscle cells and pericytes (Andrae et al., 2008).

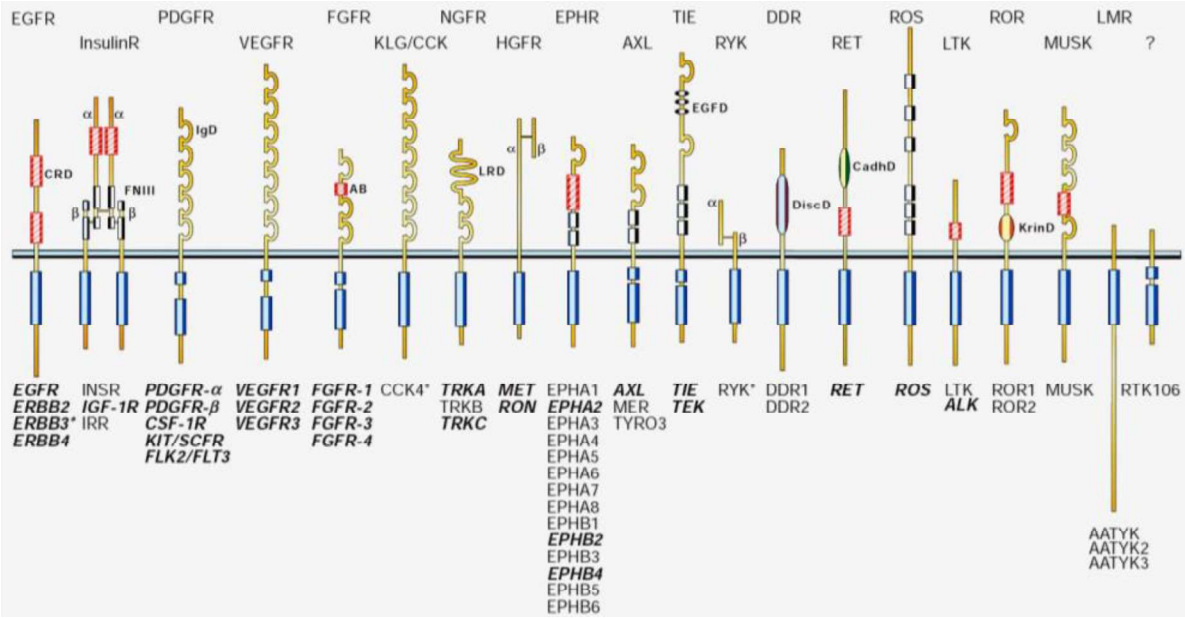

Figure 1. Receptor tyrosine kinase family (Blume-Jensen and Hunter, 2001; used with permission from Nature, Springer Nature).

1.1.1. Role of PDGFRs in cancer

Most RTKs behave like proto-oncogenes that are necessary for normal cell behavior but when deregulated may convert to dominant oncogenes (Holbro et al., 2003). The mechanisms of deregulation are different and include mechanisms that occur at the deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein levels. Somatic gain-of-function mutations frequently happen in human cancer. Chromosomal rearrangements can generate fusion genes that juxtapose RTKs catalytic domain next to another protein segment that has a dimerization function. Another mechanism that causes RTK signaliing deregulation is the upregulation of RTKs expression. RTKs may become overexpressed by gene amplifications, increased transcription by other

means, or a reduced protein degradation rate (Blume-Jensen and Hunter, 2001).

Mutations in PDGFs or the PDGFRs are relatively common in different cancer types, including lung cancer (10-20%), bladder cancer (15-20%), malignant melanoma (10-30%), glioblastoma (GBM) (15-20%), prostate cancer (20%), ovarian cancer (10-20%), and colorectal cancer (10-15%). In GBM and lung cancer, most mutations are detected in PDGFRA (Farooqi and Siddik, 2015). Activating point mutations in the kinase domain of PDGFRA are reported in some of gastrointestinal stromal tumors. In addition to mutations, PDGFRA gene is amplified in some cancers such as GBM, anaplastic oligodendroglioma, esophageal squamous cell carcinoma, etc. Autocrine PDGF stimulation is common in human GBM, osteosarcoma, and other tumor types. In addition to the autocrine mechanism, PDGF can induce neighboring cells by the paracrine mechanism. Tumor-produced PDGF stimulates endothelial cells to produce more vessels. PDGF signaling stimulates neural stem cells to divide and trigger carcinogenesis (Heldin, 2012). Intriguingly, epithelial cells do not express PDGFs and PDGFRs normally, however, during the epithelial-mesenchymal transition, expression of PDGF and PDGFR is induced. Ectopic expression of PDGFB in neural progenitor cells or astrocytes in the mouse brain results in the development of glioma (Dai et al., 2001). PDGFRA and PDGFRB are associated with aggressiveness in different tumors. Thus, elevated PDGFB expression levels in experimental glioma yields more aggressive tumors (Shih et al., 2004). Several studies have demonstrated that highly active PDGF signaling promotes proliferation, survival, and invasion of tumor cells and remodeling of the stroma to worsen the carcinogenesis (Pietras et al., 2003).

1.1.2. PDGFR signaling pathways

All PDGFs are expressed as dimers composed of two polypeptide chains that are attached to each other by disulfide bonds. In mammals, there are four PDGF chains (A, B, C, and D) and different dimers have been shown to be expressed (AA, BB, CC, DD, and AB) (Fredriksson and Eriksson, 2004). These ligands

bind to two different receptors, PDGFRA and PDGFRB, which structurally are similar (Claesson-Welsh, 1988). After ligand binding, the receptors dimerize and autophosphorylate each other in trans on specific tyrosine residues, which results in a conformational change (Kazlauskas and Cooper, 1989). The ligand-receptor complex is internalized via clathrin-coated pits into the endosomes (Wang et al., 2004). The catalytically active receptors phosphorylate different intracellular substrates. Moreover, phosphorylated tyrosines provide docking sites for signal transduction proteins with enzymatic activities as well as various adaptor proteins. Many of the docking proteins have a Src homology-2 domain that binds to specific phosphorylated tyrosines (Heldin and Westermark, 1999). Signaling proteins like v-Src avian sarcoma viral oncogene homolog (SRC), growth factor receptor-bound protein-2 (GRB-2), phospholipase C-γ, and non-receptor type-11 protein tyrosine phosphatase are activated downstream of PDGFR (Valgeirsdottir et al., 1995). PDGFRA, PDGFRB, and PDGFRA/B activate many shared signaling pathways. However, it was shown that there are some pathways that are differentially regulated by PDGFRA and PDGFRB, for example, nuclear factor kappa B and interleukin-6 pathways by PDGFRA/B, steroid hormone synthesis by PDGFRA, and angiogenesis or EGFR signaling pathways by PDGFRB (Wu et al., 2008). Figure 2 shows a published summary of PDGFRA signaling pathways (Corless et al., 2011).

1.1.3. Negative regulation of PDGFRs

Negative regulation of RTK signaling can be achieved through different mechanisms, including: (1) prevention of ligand binding to the receptor, (2) inhibition of RTK autophosphorylation, and (3) activation of negative regulators that reduce the signaling output (Ledda and Paratcha, 2007).

The PDGFR signaling pathways, like other RTK pathways, have to be tightly regulated to avoid oncogenic signaling leading to carcinogenesis and abnormalities in development (Andrae et al., 2008). Receptor endocytosis is a

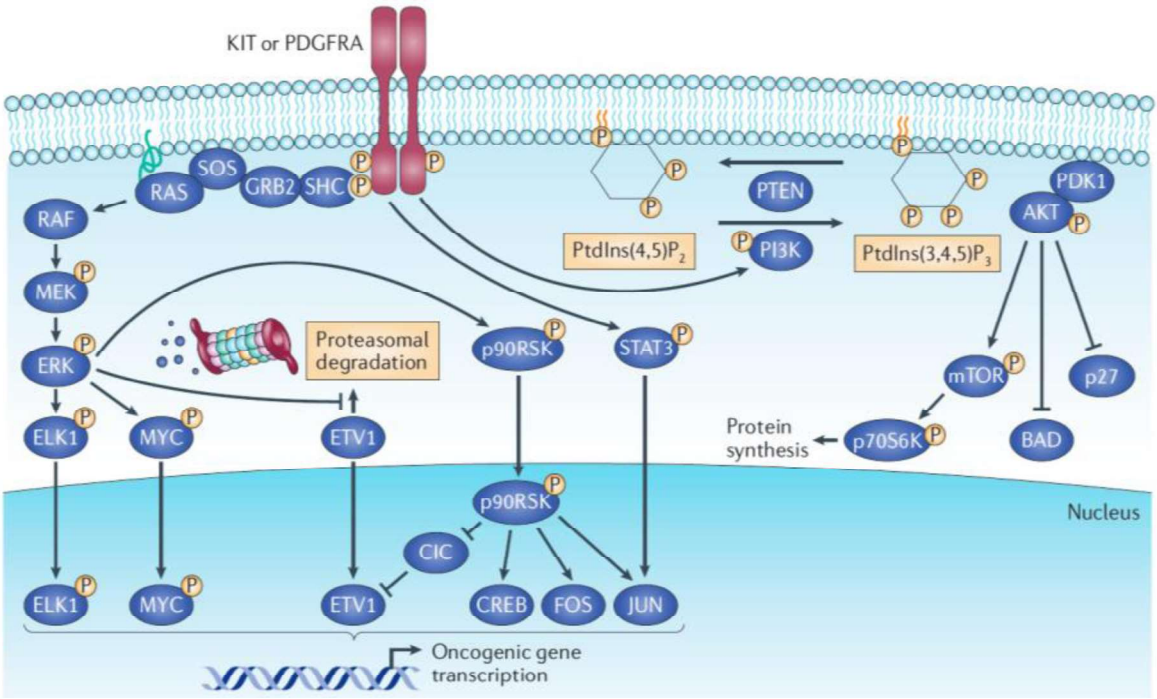

Figure 2. PDGFRA oncogenic signaling pathways (Corless et al., 2011; used with permission from Nature Reviews Cancer, Springer Nature).

major negative regulatory feedback mechanism that can terminate signaling. Casitas b-cell lymphoma (CBL) family of E3 ubiquitin ligases ubiquitinates the receptor-ligand-adaptor complex, thereby labeling them for degradation in lysosomes or proteasomes (Miyake et al., 1999). Non-receptor type-11 protein tyrosine phosphatase (Lechleider et al., 1993), and non-receptor type-2 protein tyrosine phosphatase (Karlsson et al., 2006) negatively regulates PDGFRs by de-phosphorylating receptors and substrates. PDGFRB but not PDGFRA was shown to be negatively regulated by v-Myc avian myelocytomatosis viral oncogene homolog (MYC) in a negative feedback loop (Oster et al., 2000). The exact mechanism of this regulation is not clear. However, MYC might have a direct effect on the PDGFRB promoter through enhancer elements. The PDGFRB promoter has different binding sites for various transcription factors like GATA binding protein-1, serum response factor, v-Jun avian sarcoma virus 17 oncogene homolog (JUN), and neurofibromin 1. Thus, the differential regulation of PDGFRB in contrast to PDGFRA, might be related to these transcription factors that specifically target *PDGFRB* gene. Rat sarcoma viral oncogene homolog (RAS)- GTPase activating protein binds to activated

PDGFRB and negatively regulates the signaling by hydrolyzing guanosine triphosphate (GTP) bound to RAS, thereby converting activated RAS-GTP to its inactive RAS-guanosine diphosphate form (Fantl et al., 1992). However, RAS-GTPase activating protein does not bind to PDGFRA and thus, the signaling pathways downstream of RAS, like extracellular signal-regulated kinase (ERK), may remain activated for longer after activation by PDGFRA than after activation by PDGFRB (Jurek et al., 2011). In mouse embryonic fibroblasts (MEFs), PDGFRs are downregulated by the mechanistic target of rapamycin kinase signaling pathway through a negative feedback loop. Intriguingly, a cellular peroxidase, peroxiredoxin type II also downregulates PDGFR pathways through a negative feedback loop involving the inhibition of protein tyrosine phosphatase inactivation (Choi et al., 2005). In cardiomyocytes, PDGFRB expression is regulated negatively by microRNA (miRNA)-9 in a negative feedback loop (Zhang et al., 2011).

1.2. EGFR family

EGFR belongs to erythroblastic oncogene B (ERBB) family. This family comprises EGFR, ERBB2, ERBB3, and ERBB4 (Grant et al., 2002).

1.2.1. Role of EGFR family in cancer

The EGFR family members play decisive roles in many different types of cancer. The amplifications or mutations of EGFR are very common in GBMs and these alterations promote cancer cell growth and survival (Cloughesy et al., 2014). One of the well-known mutations in *EGFR* is type III EGFR deletion mutant (*EGFRvIII*) found in many different types of cancer like brain, lung, prostate, ovary, non-small cell lung cancer, etc (Moscatello et al., 1995; Okamoto et al., 2003). EGFRvIII promotes cancer cell growth, invasion, and angiogenesis (Grandis and Sok, 2004). This EGFR mutant has a deletion in the extracellular part (in-frame deletion of exons 2-7) that is necessary for ligand binding. Thus, EGFRvIII is constitutively active without ligand binding (Damstrup et al., 2002). Intriguingly, EGFRvIII is able to dimerize with both EGFR and ERBB2 (Luwor et al., 2004; Tang et al., 2000). It was shown that EGFRvIII promotes

GBMs through enhancer landscape modulation (Liu et al., 2015). Another well-known EGFR point mutation that occurs in the tyrosine kinase domain is exon 21 codon 858 substitution, L858R. This mutation is frequently seen in lung adenocarcinoma patients and responds to EGFR inhibitors like gefitinib and erlotinib (Lynch et al., 2004). This mutation confers the EGFR an oncogenic potential by activating the downstream signaling pathways and antiapoptotic mechanisms (Sordella et al., 2004). Generally speaking, most oncogenic RTKs like EGFR have strong prognostic indications in different types of human cancers. They are significantly associated with poor survival of cancer patients (Nicholson, et al., 2001). EGFR and ERBB2 are good examples of RTKs that often are amplified in different types of cancers (Blume-Jensen and Hunter, 2001). ERBB2 is amplified in many different types of cancer with different degrees of heterogeneity (Grob et al., 2012). DNA amplification is the main mechanism of ERBB2 overexpression that can be detected, for example, in approximately 20% of breast cancers. High expression of this receptor is correlated with poor prognosis and treatment resistance (Moasser, 2007). The synergistic cooperation between ERBB2 and ERBB3 has been reported in human breast cancer (Siegel et al., 1999).

1.2.2. EGFR family signaling pathways

Three layers of diversity generation can be defined in the ERBB signaling network (Figure 3): (1) input layer, (2) signal-processing layer, and (3) output layer.

In the input layer, different ligands, including TGF-α, EGF, epiregulin, β-cellulin, amphiregulin, etc bind to ERBB family receptors. Ligand binding induces a conformational change in EGFR, ERBB3, or ERBB4, to a dimerization competent state. ERBB2 lacks a physiological ligand and is always dimerization competent. Dimerization competent receptors dimerize, leading to the phosphorylation of tyrosine residues located in the kinase domain.

In the signal-processing layer, various adaptor proteins or enzymes such as SRC, CBL, phospholipase C-γ, non-receptor type-11 protein tyrosine

phosphatase, GTPase activating protein, GRB2, GRB7, etc are recruited and activated. GRB2 can recruit different proteins to branch off the signaling pathway. If CBL is recruited, the ligand-EGFR complex is ubiquitinated and sorted to the endosomes while if sun of sevenless is recruited, RAS pathway is activated and branches into phosphatidylinositol 3-kinase (PI3K) or mitogen-activated protein kinase (MAPK) pathways. Endosomes might recycle EGFR back to the cell membrane or for degradation in the lysosome. Downstream cascades such as Ak strain transforming protein (AKT), MAPK, or Jun N-terminal kinase pathways activate various transcription factors like MYC, FBJ murine osteosarcoma viral oncogene homolog (FOS), JUN, etc in the nucleus.

Finally, in the output layer, different cell processes such as growth, apoptosis, migration, adhesion, and differentiation are activated (Yarden and Sliwkowski, 2001).

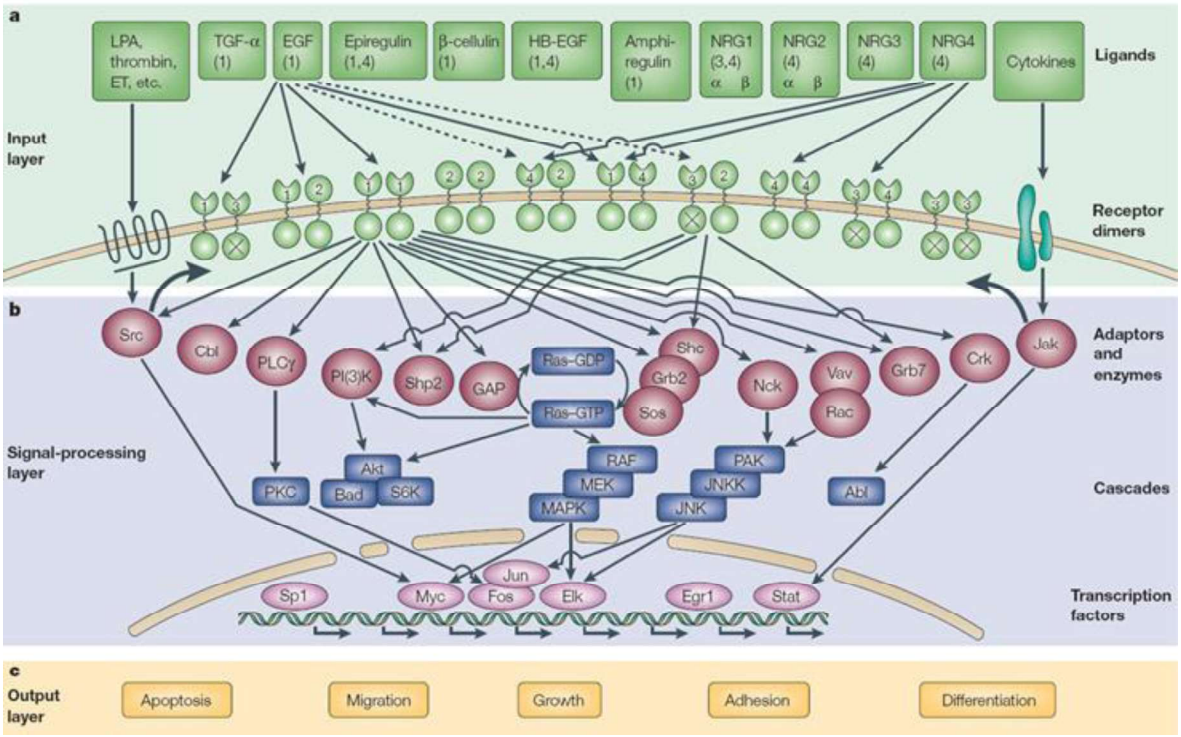

Figure 3. ERBB signaling network (Yarden and Sliwkowski, 2001; used with permission from Nature Reviews, Molecular Cell Biology, Springer Nature).

1.2.3. Negative regulation of EGFR family

RTK feedback regulation has been studied thoroughly in the context of ERBB family signaling pathways. This feedback regulation often takes the form of regulatory feedback loops. Both positive and negative feedback loops have been described. In a feedback loop, a protein, A, activates another downstream protein, B, either at the protein or transcription level. Then, protein B regulates protein A positively or negatively (Avraham and Yarden, 2011).

In ERBB family signaling pathways, regulatory mechanisms are active at all three layers of signaling network described in Figure 3.

In the input layer where the ligands act, for example, steroid hormone receptor activates the transcription of *ERBB* ligand genes. At the cell surface, one type of EGFR ligand, the heparin-binding EGF-like growth factor is cleaved by matrix metallopeptidases that are activated by G-protein-coupled receptors. How specific ligands are regulated in various contexts are highly complex processes that need to be investigated further. The identity of the ligand and receptor (or heterodimer receptors) determine the specificity and strength of intracellular signals. ERBB2-ERBB3 heterodimer has the highest mitogenic and transforming capacity compared to other receptor dimers. The main process to turn off the signaling pathway is related to ligand-mediated receptor endocytosis which acts as a negative feedback loop regulator. However, the signaling is not turned off immediately after endocytosis, as still, the receptor remains active to some extent in the intracellular vesicles.

In the signal-processing layer, there are different ways of signaling regulation. For example, EGFR is not necessarily only activated by its own ligands; EGFR can also be phosphorylated by Janus kinase, which in turn is activated by cytokine receptors. Protein kinase C, another EGFR downstream protein might not be triggered directly by EGFR ligands-receptor complex, instead, for example, PDGF activates protein kinase C and increases threonine and serine phosphorylation of EGFR which in turn compromises tyrosine phosphorylation. In this context, interestingly, PDGF negatively regulates EGFR pathway. Similar

to PDGFRs, EGFR is negatively regulated by the E3 ubiquitin ligase CBL in a negative feedback loop. Thus, activated EGFR signaling triggers CBL activation, which tags the receptor for degradation. The tyrosine phosphorylations can be also regulated in a negative feedback loop by protein tyrosine-specific phosphatases (PTPs) like DEP1. There are various PTPs with important functions in the negative regulation of EGFR endocytic pathway like PTP1B, non-receptor type-2 PTP, non-receptor type-21 PTP, receptor type-K PTP (PTPRK), etc. Interestingly, PTPRK was discovered to be one of the LRIG1-interacting proteins (Huttlin et al., 2015 and 2017). In contrast to the ubiquitin ligase CBL which tags the EGFR for degradation in lysosomes, deubiquitinating enzymes promote the targeting of the EGFR to the recycling pathway. Also downstream of EGFR activation, negative feedback loops are prominent. RAS activated v-Raf murine leukemia viral oncogene homolog (RAF) kinase, for example, is regulated by its downstream effector, ERK through a negative feedback loop. AKT, which also is activated by RAS, also negatively regulates RAF. Intriguingly, miRNAs are also involved in EGFR signaling pathway regulation. For example, the immediate early genes expression is suppressed by immediately downregulated microRNAs such as miR-101 and miR-191. Upon EGF induction, the expression of immediately downregulated microRNAs is downregulated to increase the expression of immediate early genes such as FOS, JUN, and early growth response 1 . Thus, EGFR regulation can be divided into early and late loops. In the early phase, receptor endocytosis or phosphorylation of regulatory proteins play important roles, whereas in the late phase, EGF-induced transcription of new genes occurs. Some of these newly expressed proteins regulate EGFR pathway by a feedback loop (Yarden and Sliwkowski, 2001; Avraham and Yarden, 2011).

Accordingly, negative regulators of EGFR signaling pathways can be divided into early and late regulators. As examples, E-cadherin, and decorin are early-regulators but mitogen-inducible gene 6 (MIG-6), and sprouty are late-regulators. E-cadherin binds to EGFR by its extracellular domain and decreases ligand binding to EGFR by decreasing the receptor mobility at the cell surface. Decorin affects EGFR internalization and degradation mainly through a

caveolar-mediated pathway that is a clathrin-independent pathway. Also, soluble decorin inhibits EGF-mediated EGFR dimerization. MIG-6 binds to EGFR intracellular domain and prevents autophosphorylation of EGFR. *Sprouty* gene is induced by RAS/ERK pathway and the newly expressed sprouty protein downregulates specifically RAS pathway by a negative feedback loop. Sprouty interacts with CBL and targets EGFR for ubiquitination and subsequent degradation. However, the negative regulatory of sprouty is controversial (Ledda and Paratcha, 2007). LRIG1 was shown to behave as a late-regulator of EGFR, at least, in some contexts (Gur et al., 2004).

1.3. MET

MET receptor physiologically plays important roles in survival, growth, and migration in different cell and tissue types (Gherardi et al., 2012).

1.3.1. Role of MET in cancer

Aberrant MET activation occurs in many different tumor types. Moreover, stromal cells overexpress the MET ligand hepatocyte growth factor, thereby sending a tumorigenic message to neighboring cancer cells. Thus, cancer cells hijack MET for their own invasion and metastasis, a process that physiologically happens during embryogenesis. Various aspects of epithelial-mesenchymal transition (EMT), including remote metastasis, migration, and invasion of cancer cells are all related to MET signaling. E-cadherin and integrin proteins are well-known targets in MET downstream signaling and both have critical roles in the EMT process and tumorigenesis (Gherardi et al., 2012). As metastasis has a major impact on cancer patients' outcome, oncogenic MET is generally associated with poor prognosis in cancer (Birchmeier et al., 2003).

1.3.2. MET signaling pathways

MET signaling pathways are largely similar to the PDGFR and EGFR signaling pathways described in previous sections (Trusolino et al., 2010), and are not further discussed, here.

1.3.3. Negative regulation of MET

Regarding negative regulation of MET, receptor internalization and degradation in the lysosome or proteasome constitute a major negative feedback loop as described for EGFR, above. Also, many of negative regulators of EGFR like MIG-6, decorin, sprouty, CBL, E-cadherin, etc also act against MET. MIG-6 binds indirectly to MET via an adaptor protein, GRB2, thereby inhibiting the signaling. Decorin directly binds to the MET receptor with high affinity. Then, CBL is recruited to the receptor complex, resulting in rapid ubiquitination and degradation of MET. The mechanism of action of sprouty, and E-cadherin are similar to what was explained for EGFR (Ledda and Paratcha, 2007), above. Similar to EGFR, the non-receptor type-1 PTP, non-receptor type-2 PTP, receptor type J PTP, etc negatively regulate MET by dephosphorylating tyrosine residues (Sangwan et al., 2008). Signaling crosstalks are also important regarding RTKs regulation by creating both negative and positive feedback loops. One such mechanism concerning MET is the Notch signaling pathway. Activation of Notch culminates in MET downregulation and in turn, MET activation results in transcriptional activation of Notch signaling pathway (Stella et al., 2005).

2. Leucine-rich repeats and immunoglobulin-like domains (LIG) superfamily

The most common protein domain encoded in the human genome is the immunoglobulin-like (Ig) domain which is present in 765 proteins, whereas leucine-rich repeat (LRR) domain is present in 188 proteins (Lander et al., 2001). Both of these domains participate in protein-protein interactions (Williams and Barclay, 1988; Zinn and Ozkan, 2017; Bella et al., 2008). A few proteins (18 proteins) have both Ig and LRR domains and are named LIG proteins (MacLaren et al., 2004; Mandai et al., 2009). Figure 4 shows the structural similarity between the members of this superfamily (Neirinckx et al., 2017).

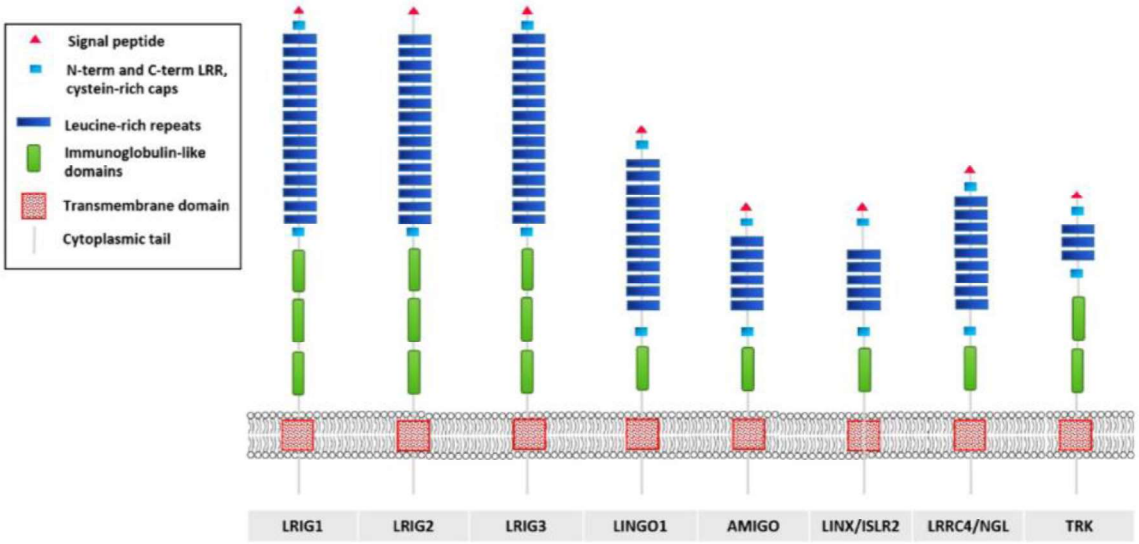

Figure 4. Structural domains in LIG superfamily (Neirinckx et al., 2017; used with permission from Biochimica et Biophysica Acta, Elsevier).

2.1. LRIG1 and LRIG2 discovery

One important subgroup of LIG superfamily is **l**eucine-**r**ich repeats and **i**mmuno**g**lobulin-like domains (LRIG) gene family that consists of three members: LRIG1, LRIG2, and LRIG3. The LRIG proteins are commonly expressed in mammalian tissues. Structurally, all LRIG proteins share an LRR domain consisting of fifteen leucin-rich repeats, three immunoglobulin-like domains, one transmembrane domain, and a cytosolic domain. The least conserved region is the cytosolic domain. *LRIG* gene family is conserved during evolution (Guo et al., 2004). The founding human member, LRIG1 (the former name was LIG1) was discovered by a search for homologs of the insect protein, kekkon-1 (Nilsson et al., 2001). However, it turned out that LRIG1 and kekkon-1 are not true orthologs, although they show structural similarities. LRIG2 was discovered in a search of additional LRIG family members (Guo et al., 2004).

2.2. The role of LIG and LRIG proteins in the development

LIG family genes are involved in neural development and in axonal growth and guidance. All *LIG* genes might interact with different RTKs such as tropomyosin-related kinases (Trks) and rearranged during transfection (Ret) at

different stages of axonal development (Mandai et al., 2009). *Lrig* mRNA expression profiling at embryonic day 10 in mice by using whole-mount *in situ* hybridization technique revealed differential patterns. However, all *Lrigs* could be detected in both central nervous system and peripheral nervous system (Homma et al., 2009). The role of *Lrig* gene family was pinpointed in the mouse inner ear development. In this study, single and double *Lrig* knockout mice were generated and the expression patterns of *Lrigs* were investigated to see differential or overlapping expression patterns. Additionally, some specific features of inner ear functions were analyzed in different *Lrig* genotypes. The obtained data of this study are described below (Del Rio et al., 2013).

2.2.1. Developmental phenotypes of *Lrig1* knockout mice

To study *LRIG1* gene function in physiology, different research groups have used knockout mouse models. Intriguingly, *Lrig1* knockout mice with different backgrounds show differential phenotypes. The first *Lrig1* knockout mouse was developed by Suzuki et al. in 2002 on a mixed genetic background (129S7/SvEV and C57BL/6). The *Lrig1*-deficient-mouse behavior, growth, and fertility appeared normal. However, at three weeks to four months of age, they developed some macroscopic phenotypes on their tail, face, and ear. The tail skin was thicker and some parts of face developed alopecia (loss of hair). Epidermal hyperplasia of tail skin was visualized by using hematoxylin and eosin staining and also immunohistochemical analyses of the keratinocytes (Suzuki et al., 2002). In another study, *Lrig1*-deficient mice were generated in FVB/N background. These mice were smaller than wildtype littermates and showed a drastic severe phenotype, a very big abdomen on day 10, so the mice had to be sacrificed. The authors showed that this phenotype was a result of stem cell niche enlargement. Western blotting showed higher expression of Egfr, p-Egfr, ErbB2, p-ErbB2, ErbB3, p-ErbB3, and Met in *Lrig1* knockout mice compared to the wildtype mice (Wong et al., 2012). In yet another study, *Lrig1*-knockout mice (Lrig1-CreERT2/CreERT2), with a mixed background of 129S7/SvEv and C57BL/6, developed low-grade duodenal tumors (14 of 16 total mice) at 5 to 6 months of age. This study provided the first in vivo evidence

about the role of *Lrig1* as a tumor suppressor gene. In these tumors, adenomatous polyposis coli gene was intact and canonical wingless type pathway was not dysregulated. Adenomatous polyposis coli loss in Lrig1-positive cells led to intestinal adenomas and large tumors in the distal colon. This shows that Lrig1-positive cells that mark intestinal stem cells have the potential to develop into cancer (Powell et al., 2012). *Lrig1*-deficient mice with the same genetic background as was used by Suzuki et al. showed hyperproliferation of tracheal epithelium in the lung. The expression of p-EGFR was increased in epithelial cells in knockout mice compared to wildtype mice (Lu et al., 2013).

To study inner ear development, *Lrig1*-deficient mice (C57BL/6N strain) was also generated. At embryonic day 12.5, Lrig1 showed only limited expression in the cochlea but *Lrig1* knockout mice showed normal inner ear morphogenesis (Del Rio et al., 2013). Some other *Lrig1* knockout mice are described in "*in vivo* cancer models". In the current thesis, we also generated *Lrig1*-deficient mice to study the physiological role of Lrig1 (Paper II).

2.2.2. Developmental phenotypes of *Lrig2* knockout mice

To study inner ear development, Del Rio et al. generated *Lrig2*-deficient mice (C57BL/6N strain). These mice did not show any gross defects. At embryonic day 12.5, *Lrig2* showed ubiquitous expression, in contrast to Lrig1, in the whole early otic epithelium. *Lrig2* single knockouts showed normal inner ear morphogenesis but they did not process sound appropriately (Del Rio et al., 2013). In the current thesis, we also generated *Lrig2*-deficient mice and investigated the role of Lrig2 in the development (Paper I).

2.2.3. *Lrig1* and *Lrig2* double knockout mice

As mentioned, the role of *Lrig* gene family was investigated in inner ear development. *Lrig1* and *Lrig2* double knockouts die with a high frequency before six weeks of age. However, in live mice, inner ear morphogenesis is almost normal but the mice show a mild phenotype of "circling behavior". This

phenotype is not seen in *Lrig1* or *Lrig2* single knockouts showing the cooperation of two Lrigs. The investigation in this study showed that Lrig1 is needed for sound detection whereas Lrig2 is needed for the proper neuronal response (Del Rio et al., 2013).

2.2.4. LRIG ortholog in C elegans

An LRIG ortholog, SMA-10 is found in *Caenorhabditis elegans (C. elegans)*. SMA-10 binds to bone morphogenetic protein receptors and positively regulates their signaling. Phenotypically, *sma-10* mutants show a reduced body size. Intriguingly, LRIG1 strongly interacts with activin receptor-like kinase (ALK6), a type I bone morphogenetic protein receptor and weakly with other ALKs receptors (Gumienny et al., 2010). Using confocal microscopy, it was shown in the *C. elegans* intestine, that SMA-10 interacts with type I and II bone morphogenetic protein receptors disproportionately (42.1 % to type I and 7% to type II). In *sma-10* mutants, type I receptor endocytic trafficking is disrupted by accumulation in the early endosomes and multivesicular bodies and the receptor is less ubiquitinated. SMA-10 does not mediate receptor recycling to the membrane. Intriguingly, EGFR signaling (LET-23 in *C. elegans*) is not affected in *sma-10* mutant *C. elegans* (Gleason et al., 2017).

3. LRIG1 and LRIG2 in cancer

During the last two decades, *LRIG* gene family has been under investigation. Below, I mention briefly some interesting findings in the field for LRIG1 and LRIG2.

3.1. *LRIG1* and *LRIG2* genes and their expression in human cancer

The genetics, epigenetics, mRNA levels, and protein levels of LRIG1 have been investigated in different cancer types.

At the DNA level, *LRIG1* resides at chromosomal locus 3p14.3 (Nilsson et al., 2001). There is strong evidence that chromosome 3p plays a role in cancer as

loss of heterozygosity (LOH) of this region frequently happen in different malignancies (Knuutila et al., 1999). *LRIG1* loss of heterozygosity (LOH) was investigated in around 170 lung cancer cell lines and LOH was seen in 75% of all cases (Lu et al., 2013). Intriguingly, there is a fragile site approximately 200 kb away from *LRIG1* locus (Rodriguez-Perales et al., 2004). New risk loci in *LRIG1* were reported in colorectal cancer (rs812481) and glioma (rs11706832) (Schumacher et al., 2015; Raskin et al., 2017; Melin et al., 2017). At the epigenetic level, in colorectal cancer, increased methylation in the *LRIG1* promoter is inversely correlated with mRNA and proteins levels (Kou et al., 2015).

At the mRNA level, *LRIG1* is downregulated in lung (Yokdang et al., 2015), prostate, and colorectal cancer cell lines (Hedman et al., 2002), and in astrocytoma (Ye et al., 2009), advanced nasopharyngeal carcinoma (Sheu et al., 2009), and squamous cell carcinoma patients (Boelens et al., 2009) compared to the normal tissues. However, *LRIG1* mRNA overexpression was reported in oligodendroglioma, colorectal, ovarian, and prostate cancers (Lindquist et al., 2014). Moreover, LRIG1 was shown to be upregulated in a cutaneous squamous cell carcinoma cell line treated with keratinocyte growth factor compared to normal epidermal keratinocytes. This might reinforce the concept of negative feedback regulation by LRIG1 (Toriseva et al., 2012).

At the protein level, LRIG1 is downregulated in human breast (Miller et al., 2008) and, bladder (Chang et al., 2013) tumors and in pre-invasive squamous cell lung samples (Lu et al., 2013) compared to the normal tissues. However, in contrast, LRIG1 overexpression was reported in human breast (Miller et al., 2008) and prostate tumors (Thomasson et al., 2011). These data might indicate context-dependent LRIG1 functions. However, most results are in line with a tumor suppressive function of LRIG1 in cancer.

LRIG2 has been less studied than LRIG1 during last years and prior to our work (paper I), it was not clear if LRIG2 was a tumor promoter or suppressor. *LRIG2* chromosomal position is 1p13 (Holmlund et al., 2004) and 1p is frequently lost

in cancer (Knuutila et al., 1999). Recently, LRIG2 was reported to be overexpressed in melanoma compared to the adjacent tissue and promoted melanoma cell proliferation (Chen et al., 2017). On the other hand, a newly published study reported that LRIG2 suppresses endometrial cancer growth. Similar to LRIG1, this might indicate context-dependent functions also of LRIG2.

3.2. LRIG1 and LRIG2 in cancer xenograft models

In xenograft models of lung (Lu et al., 2013), pituitary (Wang et al., 2016), bladder (Li et al., 2011), and breast cancer (Yokdang et al., 2015; Miller et al., 2008), LRIG1 overexpression inhibits tumorigenesis.

LRIG2 has been less studied. However, in a xenograft model of endometrial cancer, silencing the expression of LRIG2 was shown to suppress cancer cells growth by inducing apoptosis (Suh et al., 2018). In a xenograft model of brain cancer by using U87 GBM cell line, LRIG2 overexpression promotes tumor xenograft growth (Xiao et al., 2014).

3.3. LRIG1 and LRIG2 in cell proliferation

Sustained proliferative signaling is one of the original six hallmarks of cancer proposed by Hanahan and Weinberg (Hanahan and Weinberg, 2000). In many cancer studies, LRIG1 was overexpressed by using expression vectors, or downregulated by using small interfering RNAs (siRNAs) or short hairpin RNAs (shRNAs). Depending on the context, LRIG1 overexpression inhibits the proliferation of MCF-7, MDA-MB-231 (Shattuck et al., 2007), MDA-MB-453, and SKBR-3 (Miller et al., 2008) breast cancer cell lines, TW01 head and neck cancer cell line (Sheu et al., 2014), HCT-116 colorectal cancer cell line (Kou et al., 2015), H4 and U251 glioma cell lines (Ye et al., 2009), and T24 and 5637 bladder cancer cell lines (Chang, et al., 2013). Conversely, in some contexts, LRIG1 depletion promotes cancer cells proliferation. For example, LRIG1 downregulation increases the proliferation of U251 cells (Mao et al., 2012), and ZR75-1 breast cancer cell line (Krig et al., 2011).

In A549 and H357 lung cancer cell lines, transduced LRIG1 did not inhibit the cells growth before confluency but post-confluence analysis revealed that LRIG1 inhibited proliferation. Co-immunoprecipitation assay showed a ternary complex consisting of LRIG1, EGFR, and E-cadherin in these cells. The authors investigated the relation between these three proteins and concluded that LRIG1 inhibited density-dependent cell growth after the formation of the ternary complex (Lu et al., 2013).

LRIG2 has been less studied. However, LRIG2 stable transduction of U251 and U87 cell lines promotes cancer cell proliferation (Xiao et al., 2014). Conversely, LRIG2 downregulation inhibits GL15 GBM cell proliferation (Wang et al., 2009).

3.4. LRIG1 and LRIG2 in cell migration

Activating migration or invasion is another of the hallmarks of cancer (Hanahan and Weinberg, 2000). Depending on the context, LRIG1 overexpression inhibits the migration or invasion of MCF-7, MDA-MB-231 (Shattuck et al., 2007), U251, (Mao et al., 2013), T24, and 5637 cells (Chang, et al., 2013). Conversely, LRIG1 downregulation increases the migration of U251 cells (Mao et al., 2012).

EMT is a fundamental process in development that plays an important role in morphogenesis. In this process, epithelial cells are changed into migratory mesenchymal cells. However, cancer cells may hijack this process to progress and disseminate (De Craene and Berx, 2013). Intriguingly, in immortalized human mammary epithelial cells, endogenous level of LRIG1 protein is downregulated during EMT process. In 3D matrigel culture, ectopic expression of LRIG1 in MDA-MB-231 cells that are highly invasive, inhibited mesenchymal markers expression like vimentin or fibronectin. In this cell line, LRIG1 could inhibit cell migration through MET inhibition (Yokdang et al., 2015).

LRIG2 has been less studied. However, it was shown that LRIG2 downregulation inhibits the migration of human umbilical vein endothelial cells in a transwell model co-cultured with U87 and U251 cells (Yang et al., 2017).

3.5. RTKs signaling regulation and LRIG mechanisms of action

Here, I mention briefly some interesting findings regarding RTKs signaling regulation and mechanism of action of LRIG1 and LRIG2.

Kekkon-1 negatively regulates Drosophila melanogaster EGFR during oogenesis (Ghiglione et al., 1999). Based on the structural similarities between LRIG1 and kekkon-1, it was speculated that LRIG1 might be a negative regulator of human EGFR (Nilsson et al., 2001). Accordingly, LRIG1 was shown to negatively regulate EGFR (Gur et al., 2004; Laederich et al., 2004). Interestingly, different research groups have shown that LRIG1 not only negatively regulates EGFR, but also ERBB2, ERBB3, and ERBB4 (Laederich et al., 2004; Gur et al., 2004; Miller et al., 2008), MET (Shattuck et al., 2007), RET (Ledda et al., 2008), TrkB (Alsina et al., 2016), and Recepteur d'Origine Nantais (Bai et al., 2012). Moreover, in paper I, we showed that LRIG1 negatively regulated also PDGFRA (Rondahl et al., 2013). However, the mechanisms involved are largely unknown, or controversial in some cases.

Below, first, I mention some suggested LRIG1 mechanisms of action regarding EGFR, MET, and RET signaling regulation. Next, briefly, I describe some interesting findings related to LRIG1 mechanism of action.

One of the main regulators of EGFR is CBL which is an E3 ubiquitin ligase that tags the receptor for degradation (Shtiegman and Yarden, 2003). Thus, Gur et al., tested if CBL plays a role in LRIG1 function. They showed that the cytosolic part of LRIG1 interacts with CBL and brings it to the EGFR. They concluded that LRIG1 function is dependent on CBL (Gur et al., 2004). However, Stutz et al. could not reproduce this finding and instead demonstrated that LRIG1 function is not dependent on CBL (Stutz et al. 2008). Among ERBB family members, only EGFR is regulated physiologically by CBL and not the other members (Levkowitz et al., 1996). In human embryonic kidney 293T (HEK293T) cells, co-immunoprecipitation demonstrated that LRIG1 interacts with all ERBB family members, but not with fibroblast growth factor receptor. One of the common EGFR rearrangements in GBM is EGFRvIII, which is

constitutively active and escapes common negative regulatory mechanisms. Intriguingly, LRIG1 downregulates EGFRvIII in U87-EGFRvIII cell line. LRIG1 also inhibits proliferation and motility of U87-EGFRvIII cells to some extent (Stutz et al., 2008).

For MET signaling regulation, the LRIG1 mechanism of action is largely dependent on lysosomal and not proteasomal degradation pathway (Shattuck et al., 2007). LRIG1-mediated MET degradation is regulated by ubiquitin specific protease 8 which regulates ubiquitin modification and LRIG1 post-translational stability (Oh et al., 2014). Apparently, the downregulation of MET by LRIG1 does not involve CBL function (Shattuck et al., 2007; Lee et al., 2014).

For RET signaling regulation, various mechanisms of LRIG1 action have been shown to operate (Ledda et al., 2008). LRIG1 does not downregulate RET protein expression level but the downstream signaling is downregulated. LRIG1, after binding to RET, restricts ligand binding, receptor phosphorylation, MAPK activation, and RET access to lipid raft domains. Also here, LRIG1 functions without the involvement of CBL.

LRIG1 mechanism of action has also been investigated through structure-function analyses. Thus, LRIG1 LRR or immunoglobulin-like domain can bind to EGFR but only LRR deletion disrupts LRIG1 function. LRIG1 and EGFR interactions happen both in the presence and absence of EGF ligand (Gur et al., 2004). LRIG1 LRR domain was confirmed in another study to be necessary for its negative regulatory function (Alsina et al., 2016). However, at least in some studies, LRIG1 ectodomain and full-length proteins do not downregulate EGFR levels despite the downregulation of downstream signaling (Yi et al., 2010; Johansson et al., 2013). Of note, in one study, the specific direct interaction between LRIG1 and EGFR could not be proven (Xu et al., 2015).

As already mentioned, the LRIG proteins share similar structural domains. Thus, it is not surprising if we speculate that mechanistically, they can interact with the same RTKs or with each other. Interestingly, co-transfection studies in HEK293T cells showed that LRIG1 and LRIG3 are interacting and functionally

oppose each other. More detailed experiments demonstrated that the LRIG1-LRIG3 interaction does not depend on the presence of EGFR and intriguingly, LRIG1 decreased LRIG3 protein half-life in these cells. Deletion studies of LRIG1 domains revealed interactions are disrupted only when the ectodomain is deleted. However, LRIG1 function is disrupted only when the LRR domain is deleted. In this study, receptor degradation was shown to be dependent on the lysosomal pathway and not on CBL or poly-ubiquitination (Rafidi et al., 2013). Therefore, it is important to know if LRIG2 also interacts with other LRIG family members. In paper III, we gained insights about this question.

In addition to RTKs signaling pathways, the connection between estrogen signaling and LRIG1 has been investigated. In two breast cancer cell lines, ZR75-1 and MCF-7, E2 (17β-estradiol) ligand treatment induces LRIG1 expression at the mRNA and protein levels. Thus, estrogen receptor binding sites are positioned at 23 to 80 kb from *LRIG1* transcription start site and within *LRIG1* introns. Interestingly, in ZR75-1 cell line, ERBB2 activation suppresses estrogen receptor and in turn, inhibits the induction of LRIG1 by E2 ligand. E2 ligand treatment increases ZR75-1 cell proliferation and siRNAs against LRIG1, increases this proliferation further. Interestingly, LRIG1 depletion did not change estrogen receptor expression level. However, these findings could not be repeated with MCF-7 cell line, demonstrating the context-dependency of LRIG1 action in breast cancer cell lines (Krig et al., 2011). Of note, in paper IV, we investigated the association of *LRIG1* copy number (CN) alterations (CNAs) with estrogen-receptor status.

LRIG2 has been less studied and its mechanism of action is not clear yet. Co-transfection assays in HEK293T cells showed that LRIG2, in contrast to LRIG1, does not have any significant effect on the expression of EGFR and ERBB2 (Rafidi et al. 2013). In paper I, we obtained similar results, showing a lack of effects of LRIG1 on PDGFRA (Rondahl et al. 2013). Additionally, LRIG2 interacts with EGFR in U251 and U87 GBM cell lines and increases EGFR signaling (Xiao et al., 2014). However, in paper III, interestingly, we showed that LRIG2 might modulate PDGFRA expression levels, indeed. In this paper,

LRIG2 was identified as one of the LRIG1-interacting proteins (Faraz et al., 2018), which is further discussed in the corresponding section.

Taken together, a lot of questions remain unanswered regarding the exact mechanisms of LRIG1 and LRIG2 functions. Thus, in papers I, II, and III, we found it of importance to investigate further the function of LRIG1 and LRIG2 in cancer cells and their mechanisms of action.

3.6. LRIG1 and LRIG2 as prognostic and predictive factors

Biological markers (biomarkers) might be categorized into diagnostic, prognostic, and predictive. A prognostic marker predicts the possible outcome of a disease, like progression or relapse risk regardless of given treatments (Sawyers, 2008). A predictive marker on the other hand, predicts the response to a given treatment. Movements toward personalized medicine have been already started (Duffy and Crown, 2008) and important aspects in this context are prognosis and prediction (Sawyers, 2008). In cancer treatments and interventions, proper prognostic and predictive markers help to maximize treatment efficacy and minimize toxicity (Conley and Taube, 2004).

Both LRIG1 and LRIG2 have been investigated as prognostic markers in different studies (reviewed in Lindquist et al., 2014). Below I will briefly mention interesting studies regarding the prognostic value of DNA, mRNA, and protein of LRIG1 or LRIG2 in different cancer types.

At the DNA level, in advanced nasopharyngeal carcinoma, LRIG1 homozygous deletion was reported in 30% (12/40) of samples. In this study, a significant association was found between *LRIG1* locus deletion and poor clinical outcome (Sheu et al., 2009). At the epigenetic level, in cervical cancer, the hypermethylated *LRIG1* promoter was correlated with a worse prognosis (Lando et al., 2015).

At the mRNA level, a meta-analysis was performed to find new prognostic markers in ovarian serous carcinoma. In patients with stage 3 and 4 disease, low

level of *LRIG1* mRNA expression was correlated with a poor prognosis (Willis et al., 2016). In another study, investigation of publicly available expression database of estrogen receptor-positive breast cancer revealed that patients with lower expression of *LRIG1* had a worse prognosis than patients with higher expression of *LRIG1* (Krig et al., 2011).

At the protein level, lower expression of LRIG1 is associated with a poor outcome in squamous cell carcinoma (Jensen et al., 2008). Moreover, higher expression of LRIG1 is associated with a better outcome in cutaneous squamous cell carcinoma (Tanemura et al., 2005), early-stage invasive cervical cancer (Lindström et al., 2008), cervical adenocarcinoma (Muller et al., 2013), non-small cell lung cancer (An et al., 2015; Kvarnbrink et al., 2015), hepatocellular carcinoma (Yang et al., 2016), and primary vaginal carcinoma (Ranhem et al., 2017). Unexpectedly, in sebaceous gland tumors, LRIG1 was reported to be overexpressed in poorly differentiated samples. The authors suggested that higher expression of LRIG1 is a negative prognostic marker in this context (Punchera et al., 2016). This is somewhat surprising and could be attributed to the technical variation in LRIG1 analysis.

LRIG2 has been also investigated as a prognostic marker. High expression of the LRIG2 protein is correlated with poor prognosis in early-stage subcutaneous cell carcinoma of the uterine cervix (Hedman et al., 2010) and non-small cell lung cancer (Wang and Song, 2014). Moreover, subcellular localization of LRIG2 was shown to affect patients' outcome. For example, perinuclear staining of LRIG2 in astrocytoma was correlated with better prognosis (Gue et al., 2006) and in stages II and III oligodendrogliomas, cytoplasmic LRIG2 expression was correlated with poor prognosis (Holmlund et al., 2009).

4. Brain tumors

Mammalian brain contains neurons, glial, endothelial, and other cells. One of the main functions of glial cells is to support the neurons. There are four various main subtypes of glial cells: (1) oligodendrocytes, (2) astrocytes, (3) microglia, (4) NG2-glia which are the progenitors (Azevedo et al., 2009). Oligodendrocytes

support the neurons by generating myelin (Nave, 2010) and disturbed functions are involved in some neurodegeneration diseases, such as amyotrophic lateral sclerosis and Alzheimer's disease (Ettle et al., 2016). Astrocytes have different functions for neuron homeostasis and are needed for formation, maintenance, and elimination of synapses during development (Chung et al., 2015). Their role has been shown in some neurodevelopmental or neurological diseases like Amyotrophic lateral sclerosis, Alzheimer's disease, etc (Phatnani and Maniatis, 2015). Microglia control the homeostasis of the neurons by growth factors, cytokines, and chemokines. They also regulate the development, plasticity, and function of synapses. Moreover, they are the phagocytes of the brain and interact with various immune cells in the CNS (Wolf et al., 2017).

Nervous system tumors (NSTs) cover less than 2% of all malignancies. Children and adults (aged 45-70) have the highest peaks of incidence. More than 90% of NSTs are primarily located in the brain. Gliomas, the so-called "diffuse gliomas", account for 40-60% of all primary brain tumors and, can be divided into oligodendroglioma, astrocytoma, and ependymoma subtypes. Other NST types are meningiomas (20-35%), and schwannomas (5-10%). The rare types of NSTs consist of pituitary adenomas, medulloblastomas, and tumors of the spine or peripheral nerves (Boyle and Levin, 2008).

The detailed etiology of NSTs is not known. However, some well-known risk factors are ionizing radiation and chemicals, such as nitrosourea. Regarding the environmental risk factors, there are a lot of speculations (such as tobacco smoke or some occupations) but most studies are not conclusive (Boyle and Levin, 2008). There are some well-known inherited syndromes that are associated with NSTs and might be considered as genetic risk factors: (1) Li-Fraumeni, (2) Cowden, (3) Ataxia telangiectasia, (4) Gorlin, (5) Familial adenomatous polyposis and hereditary nonpolyposis colorectal cancer, (6) Neurofibromatosis types 1 and 2, etc (Vijapura, 2017).

4.1. Brain tumors classification

4.1.1. World Health Organization (WHO) classification of glioma tumors

The well-known brain tumor classification comes from the World Health Organization (WHO). This classification was initially based on the tumor cells histology resembling the original glial cells and the malignancy of the tumor by grade definitions. Grade I defines low proliferative tumors that usually is curable by surgery. Grade II is often low proliferative with a diffuse growth pattern that might develop into higher grades. Grade III or anaplastic tumors are more proliferative tumors with nuclear atypia. Grade 4 or GBM is a highly aggressive, proliferative with great cellular atypia and neo-angiogenesis. Grades I and II are called low-grade glioma and grades III and IV are called high-grade gliomas. WHO Grade II or III gliomas can develop into higher grade tumors with poor prognosis. The diffuse gliomas and especially high-grade gliomas have a poor prognosis. Most of the patients diagnosed with grade 4 glioma die within 9 to 12 months (Louis et al., 2007).

During the last decades, a lot of molecular information about glioma tumor has been collected. The 2007 classification and earlier versions were mostly based on histology and the interpretations were often varied by different neuropathologists based on their observations (intra and interobserver variability). To resolve these issues, classification of tumors of the central nervous system was updated in 2016 by breaking the “century-old principle of diagnosis”. The new classification is based on identities including Isocitrate dehydrogenase (*IDH*) mutations and 1p/19q codeletion status. (Louis et al., 2016). Figure 5 shows the updated version of the mentioned system based on more genetic characteristics (Park et al., 2017).

Recently, The Cancer Genome Atlas (TCGA) research network analyzed low-grade glioma patients to integrate the genome-wide data from DNA, RNA, and protein platforms. Three molecular classes were defined based on *IDH*, 1p/19q co-deletion, and tumor protein 53 (TP53) status. The authors could classify and stratify gliomas by using molecular profiling more distinctly regarding clinical outcomes compared to WHO classification (Cancer Genome Atlas Research et al., 2015). Molecular profiling techniques including methylation signatures

(CpG islands methylation profiles) have defined more distinct subsets of gliomas (Ceccarelli et al., 2016).

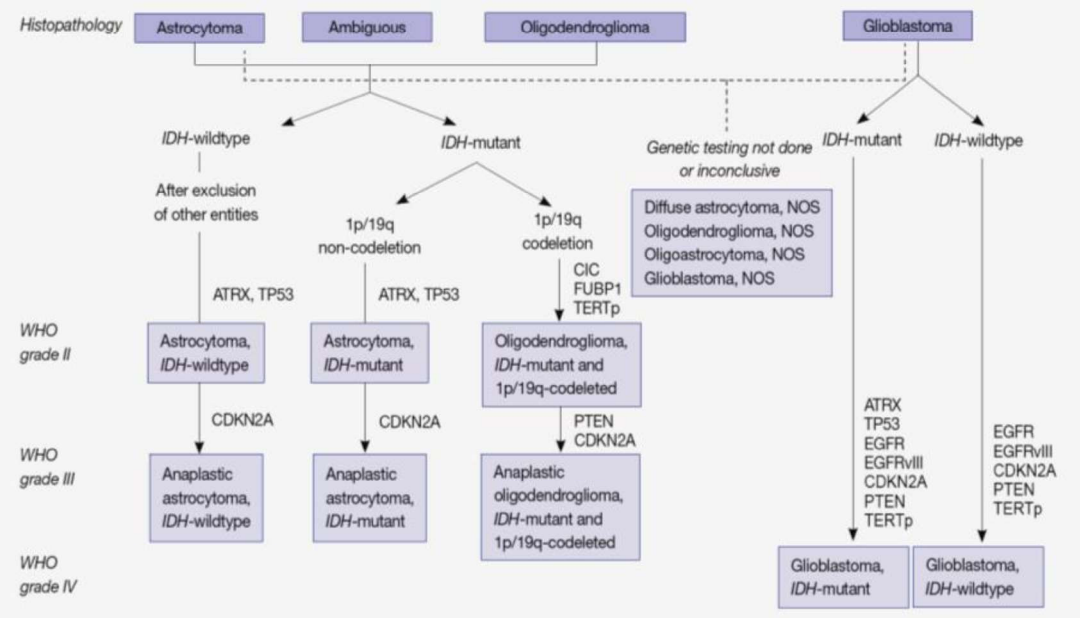

Figure 5. A simplified classification of diffuse gliomas based on histological and genetic characteristics (Park et al., 2017; This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License).

4.2. Oligodendroglioma

Oligodendroglioma represents 5-20% of all gliomas and approximately 55% of patients belong to age group 40 to 65 (Cairncross et al., 1994). Patients with features of oligodendroglioma seem to have a better prognosis compared to other types of gliomas (Van den Bent et al., 2008).

4.2.1. Molecular biology

1p/19q loss (co-deletion of the short arm of chromosome 1, and long arm of chromosome 19) is the most common chromosomal change in oligodendroglioma (Bigner et al., 1999). This loss is associated with chemosensitivity (Cairncross et al, 1998). A couple of genes located on 1p and 19q arms, such as cyclin-dependent kinase inhibitor 2A, tumor protein 73, and

paternally-expressed gene 3 are suggested to have important roles in the genesis of oligodendroglioma (Reifenberger and Louis, 2003; Trouillard et al., 2004).

TP53 mutations are also found in oligodendroglioma (TCGA, 2015). *TP53* promoter is methylated in 74% (31/42) of oligodendroglioma patients (Amatya et al., 2005).

IDH1 and 2 mutations are seen in approximately 70% of gliomas including oligodendroglioma. The most frequent mutation occurs in amino acid 132 located in the active site of IDH (Yan et al., 2009). IDH converts isocitrate to 2-oxoglutarate by producing nicotinamide adenine dinucleotide phosphate (Cupp and McAlister-Henn, 1991). Surprisingly, the mutant IDH obtains a gain of function and converts 2-oxoglutarate into 2-hydroxyglutarate, which acts as an oncometabolite (Dang et al., 2009).
Regarding other essential genes, activated PDGFR signaling pathway and co-expression of ligand and receptor are common in oligodendroglioma (Nister et al., 1988; Hermanson et al., 1992; Martinho et al., 2009). EGFR amplification, often seen in oligodendroglioma is negatively correlated with 1p/19q loss status (Horbinski et al., 2011). 10q loss is one of the most common chromosomal changes in all gliomas. One important gene located on 10q is PTEN, which has shown to be a tumor suppressor gene (Sasaki et al., 2001).

Another gene, O-6-methylguanine-DNA methyltransferase (*MGMT*) has been studied intensively in cancer research. MGMT is a transferase enzyme that plays a very important role in DNA repair. MGMT recognizes the DNA adduct at the *O6* position of guanine and removes the alkyl groups (Pegg et al., 1995). MGMT promoter hypermethylation frequently occurs in oligodendrogliomas (Kaloshi et al., 2008).

Other studies have shown mutations on telomerase reverse transcriptase (*TERT*) promoter in diffuse gliomas including oligodendroglioma (Koelsche et al., 2013). The mutations are related to higher glioma progression (Vinagre et al., 2014).

4.3. Glioblastoma

Glioblastoma multiform (GBM, WHO grade IV) is the most common type of primary brain tumor. GBM can be divided into primary and secondary GBMs. Primary GBM consists 90% of all cases. Secondary GBM is transformed from low-grade tumors (Furnari et al., 2007).

4.3.1. Molecular biology

Some main pathways seem to be involved in the pathobiology of GBM. Various RTKs and two well-known tumor suppressor genes, *TP53* and retinoblastoma 1 have an important role in the pathogenesis of GBM (Furnari et al., 2007).

Integrative analyses of genetic alterations show three core signaling pathways in GBM (Figure 6). RTK/RAS/PI3K, TP53, and retinoblastoma signaling pathways are altered in 88%, 87%, and 78% of GBM samples, respectively (TCGA, 2008). Based on gene expression profiling from 200 patients, TCGA classifies GBM samples to four different subtypes: (1) Classical, (2) Mesenchymal, (3) Proneural, and (4) Neural. *EGFR* amplification or mutation, PDGFA or fibroblast growth factor receptor 3 activation, homozygous deletion of cyclin-dependent kinase inhibitor 2A, and high expression of nestin (a neural precursor and stem cell marker) are detected often in the classical subtype. Also, Notch and Sonic hedgehog signaling pathways are very active. Interestingly, *TP53* mutations were not common in a subset of the classical group. This subtype was strongly associated with astrocytic signature in mice. In the mesenchymal subtype, hemizygous deletions of neurofibromin 1, and high expression of MET, cluster of differentiation 44, microglia markers, tumor necrosis factor superfamily and nuclear factor-κB signaling pathways are common. It seems EMT, and inflammatory processes play important roles in this group. This subtype is very similar to the cultured astroglial signature. In proneural subtype, the main events are *PDGFRA* and *IDH1* alterations. *PDGFRA* amplifications are detected almost in all GBMs but in proneural subtype, it has a higher rate. *TP53* and *PIK3CA* mutations, high expression of oligodendrocyte development markers, PDGFRA, oligodendrocyte transcription factor-2, and proneural development genes, SRY-related HMG-box, and

transcription factor 4 are prominent observations. Gene ontology search proves a cell cycle signature in this subtype. Most secondary GBMs are found in this group. The neural subtype is marked by expression of neuron markers (Verhaak et al., and TCGA, 2010).

EGFR gene rearrangements occur often in GBM. One of the most common rearrangements is a deletion of exons 2-7 in *EGFR* gene. This variant, named EGFRvIII is constitutively active, cannot bind to ligands, and plays important roles in tumor growth, survival, and treatment resistance (Nicholas et al., 2006).

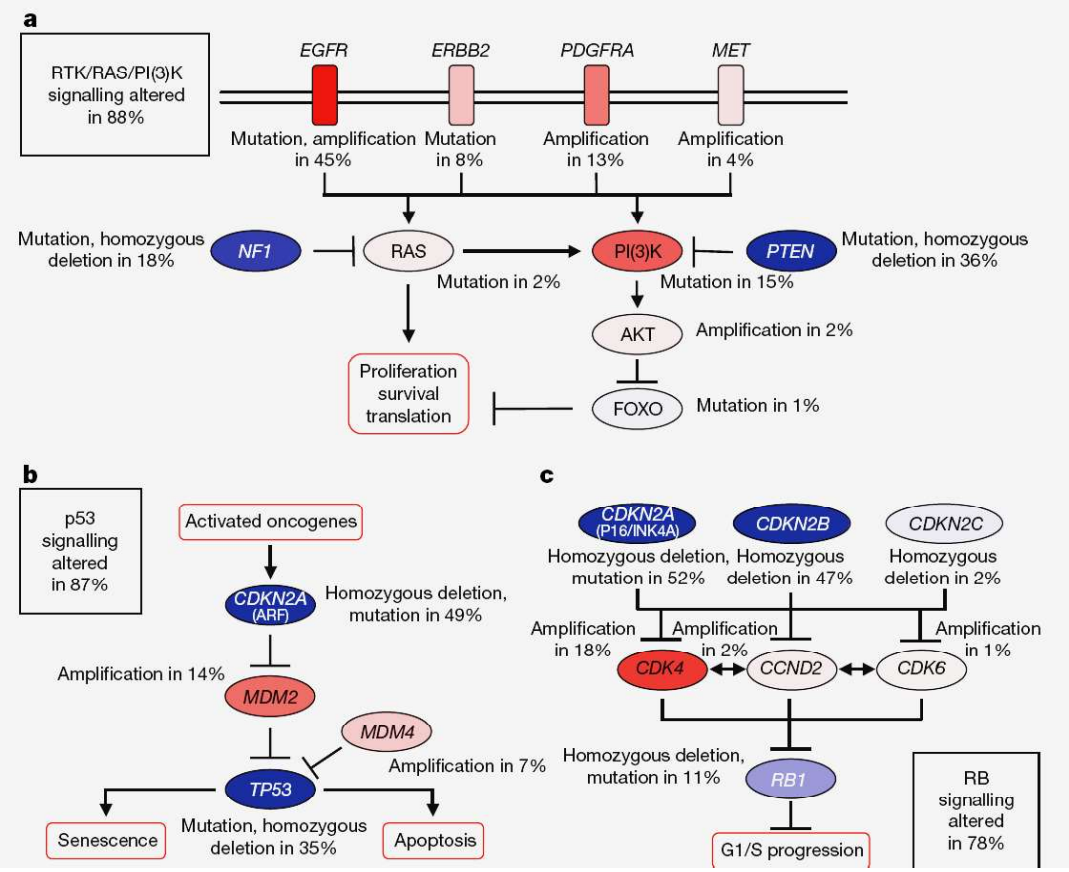

Figure 6. Core signaling pathways in GBM (TCGA, 2008; used with permission from Nature, Springer Nature).

Noushmehr et al. identified a glioma-CpG island methylator phenotype that was associated with IDH1 mutations, patient's age, and their clinical outcome. In this cohort, two genes, retinol binding protein 1, and G0/G1 switch gene 2 show the strongest evidence for promoter methylation effect in glioma-CpG island

methylator phenotype tumors. Intriguingly, they reported only 17% concordance between genes promoter hypermethylation and their expression levels (Noushmehr et al., and TCGA, 2010).
Generally, promoter methylation occurs more often in secondary GBMs compared to primary GBMs (Ohgaki and Kleihues, 2007). Like oligodendroglioma, *MGMT* hypermethylation is detected in most GBMs (Herman and Baylin, 2003).

4.4. Prognostic and predictive markers in glioma tumors

A prospective randomized study shows that *IDH1* mutations have a very strong prognostic value in anaplastic oligodendroglioma but cannot predict the outcome of patients to the procarbazine, lomustine, and vincristine (PCV) chemotherapy. 1p/19q co-deletions have also prognostic significance but not predictive value (Van den Bent et al., 2010).

In GBM, both *MGMT* promoter hypermethylation and *IDH* mutations seem to have prognostic and to some extent predictive values. TP53, EGFR, PDGFR, PTEN, and 1p/19q markers have prognostic but not predictive value (Karsy et al., 2015). TERT promoter mutations are also suggested to have a prognostic value (Killela et al., 2014).

4.5. Treatment

Primary treatment of oligodendroglioma includes PCV or in some patients temozolomide in the postsurgical situation. Combination of radiotherapy and chemotherapy have been shown to increase the response rate and survival figures in some clinical situations. In anaplastic oligodendroglioma (grade III), and oligoastrocytoma (grade III), if the tumor exhibits 1p/19q co-deletion, there is strong evidence for increased response to PCV treatment. For high-grade gliomas (anaplastic gliomas, grade III tumors) and astrocytoma grade IV (GBM) surgery, irradiation, and chemotherapy (temozolomide) during and after radiotherapy are the main options for the treatment. Radiotherapy is usually given as 2 Gy daily fraction on weekdays up to 56-60 Gy. Temozolomide is given daily, even during weekends (42 days) and after radiotherapy with 6 courses

every fourth week. In large tumors located in sensitive areas and in old fragile patients, cytotoxic agents or radiotherapy as single treatment may be an option. Bevacizumab might be used as salvage therapy for glioma treatment (Weller et al., 2017).

A new modality, tumor treating fields for the treatment of GBM are under implementation in the clinic (Rick et al., 2018). The use of other agents, signaling pathways inhibitors, and immunotherapy are under intensive investigations (Davis, 2016).

5. Breast cancer

Breast cancer is one of the most common types of cancer in the world (Siegel et al., 2017). Although about 90% of the patients survive for more than 5 years, it is the second cause of death in cancer after lung cancer in Sweden in women (Swedish cancer registry, 2016).

Age, geography, age at menarche and menopause, age at first pregnancy, family history, radiation, oral contraceptive, hormone replacement therapy, and lifestyle (diet, weight, alcohol consumption, smoking) are established and probable risk factors for breast cancer development (Kaminska et al., 2015).

Mammography with a biopsy of the suspected tissue is the gold standard method for breast cancer diagnosis. However, a new toolbox has been developed to diagnose breast cancer such as digital breast tomosynthesis mammography, contrast-enhanced digital mammography, ultrasonography, magnetic resonance imaging, and liquid biopsies including molecular biological methods like real-time PCR or microarrays (De Abreu et al., 2013).

5.1. Clinical and Histological Classification

Human breast is a cutaneous exocrine gland that consists of skin and subcutaneous tissue in which ducts, lobules, and supporting stroma reside (Jesinger, 2014). Breast cancer can be divided into two broad groups: (1) *in situ* carcinoma that can be ductal or lobular, and (2) invasive carcinoma that can be categorized into more subgroups such as infiltrating ductal, medullary,

mucinous, invasive lobular, ductal lobular, and tubular tumors (Malhotra et al., 2010).

Breast cancer can be also categorized based on stage or grade. Stage is one part of diagnosis to determine the size and the spread of the tumor. One of the staging systems of breast cancer is TNM system in which T, N, and M stand for tumor (tumor size), node (nodal status), and metastasis (distant metastasis). The American Joint Committee on Cancer updates this system periodically (Edge and Compton, 2010). Histological grading determines the degree of malignancy, similar to glioma, that is also relevant to prognosis. There are different scoring systems to grade a breast cancer. The Nottingham grading system (Elston-Ellis) considers three parameters: (1) differentiation of tumor, (2) nuclear features, (3) mitotic count. Each parameter will be scored from 1 to 3. In total, the sum of all these three variables gives the grade of a breast cancer (Elston and Ellis, 1991).

Clinical classification categorizes breast cancer into three main therapeutic subtypes: (1) the estrogen receptor positive group which is the most common type, (2) the *ERBB2*-amplified group, and (3) triple-negative breast cancer group which is sometimes called basal-like cancer (TCGA, 2012).

Perou *et al.* classified breast cancer by using cDNA microarray technique into different "intrinsic molecular subtypes" based on the gene expression patterns of 8102 genes. Their hierarchical clustering analysis divided breast cancer into different subtypes as ERBB2-enriched (10-15%), luminal A (40%), luminal B (20%), basal-like (15-20%), etc (Perou et al., 2000).

Inherited tumor syndromes are significant risks for breast cancer. Mostly, the genes involved in these syndromes are tumor suppressors and DNA repair genes like breast cancer A1 and A2, ataxia telangiectasia mutated (ataxia telangiectasia), and *TP53* (Li-Fraumeni) (Nielsen et al., 2016).

5.2. Molecular classification

Human breast cancer is highly heterogeneous (Ronnov-Jessen et al., 1996). The breast cancer research community has put a lot of efforts into clarification of the molecular heterogeneity of the disease during the last years.

In such an effort, TCGA analyzed and integrated a large dataset of information from different molecular platforms such as DNA CN, DNA methylation, exome sequencing, mRNA arrays, microRNA sequencing, and targeted proteomics by using reverse phase protein array. This study confirmed the previous "molecular intrinsic subtypes" identities and provided additional detailed information. *TP53,* retinoblastoma 1*, PIK3CA, PTEN, AKT1,* cyclin-dependent kinase inhibitor 1B*,* etc were the most significantly mutated genes. The summary of this research is shown in table 2 (TCGA, 2012).

Table 2. Breast cancer subtypes characteristics (TCGA, 2012; used with permission from Nature, Springer Nature).

Subtype	Luminal A	Luminal B	Basal-like	HER2E
$ER^+/HER2^-$ (%)	87	82	10	20
$HER2^+$ (%)	7	15	2	68
TNBCs (%)	2	1	80	9
TP53 pathway	*TP53* mut (12%); gain of *MDM2* (14%)	*TP53* mut (32%); gain of *MDM2* (31%)	*TP53* mut (84%); gain of *MDM2* (14%)	*TP53* mut (75%); gain of *MDM2* (30%)
PIK3CA/PTEN pathway	*PIK3CA* mut (49%); *PTEN* mut/loss (13%); *INPP4B* loss (9%)	PIK3CA mut (32%) PTEN mut/loss (24%) INPP4B loss (16%)	PIK3CA mut (7%); PTEN mut/loss (35%); INPP4B loss (30%)	PIK3CA mut (42%); PTEN mut/loss (19%); INPP4B loss (30%)
RB1 pathway	Cyclin D1 amp (29%); *CDK4* gain (14%); low expression of *CDKN2C*; high expression of *RB1*	Cyclin D1 amp (58%); *CDK4* gain (25%)	*RB1* mut/loss (20%); cyclin E1 amp (9%); high expression of *CDKN2A*; low expression of *RB1*	Cyclin D1 amp (38%); *CDK4* gain (24%)
mRNA expression	High ER cluster; low proliferation	Lower ER cluster; high proliferation	Basal signature; high proliferation	HER2 amplicon signature; high proliferation
Copy number	Most diploid; many with quiet genomes; 1q, 8q, 8p11 gain; 8p, 16q loss; 11q13.3 amp (24%)	Most aneuploid; many with focal amp; 1q, 8q, 8p11 gain; 8p, 16q loss; 11q13.3 amp (51%); 8p11.23 amp (28%)	Most aneuploid; high genomic instability; 1q, 10p gain; 8p, 5q loss; *MYC* focal gain (40%)	Most aneuploid; high genomic instability; 1q, 8q gain; 8p loss; 17q12 focal *ERRB2* amp (71%)
DNA mutations	*PIK3CA* (49%); *TP53* (12%); *GATA3* (14%); *MAP3K1* (14%)	*TP53* (32%); *PIK3CA* (32%); *MAP3K1* (5%)	*TP53* (84%); *PIK3CA* (7%)	*TP53* (75%); *PIK3CA* (42%); *PIK3R1* (8%)
DNA methylation	–	Hypermethylated phenotype for subset	Hypomethylated	–
Protein expression	High oestrogen signalling; high MYB; RPPA reactive subtypes	Less oestrogen signalling; high FOXM1 and MYC; RPPA reactive subtypes	High expression of DNA repair proteins, PTEN and INPP4B loss signature (pAKT)	High protein and phospho-protein expression of EGFR and HER2

Percentages are based on 466 tumour overlap list. Amp, amplification; mut, mutation.

5.3. Prognostic and predictive markers

Various prognostic and predictive biomarkers have been recognized as shown in table 3 (Nicolini et al., 2017). However, these days only a few biomarkers are used in the routine clinics such as estrogen, progesterone, and ERBB2 receptors status as well as proliferative markers (Nowsheen et al., 2012).

Table 3. Breast cancer prognostic and predictive biomarkers (Nicolini et al., 2017; used with permission from Elsevier).

Biomarker category	Prognosis		Prediction of	
	Worse	Better	Response	Resistance
Conventional; in tissue				
Lymph-nodes (N)	[a]Positive	[a]Negative	NA	NA
Tumor size (T)	[b] > 2 cm	[b] ≤2 cm	NA	NA
Tumor grade (G) (Nottingham score)	2–3	1	NA	NA
ER	Negative	Positive	[c]Positive	[c]Negative
PR	Negative	Positive	[c]Positive	[c]Negative
Ki67	> 25%	< 10%	NA	NA
HER2/neu	Positive	Negative	[d]Positive	[d]Negative
Oncotype DX RS	> 30	< 18	[e] > 30 (CT)	[e] < 18 (CT)
Mammaprint	High risk	Low risk	[e]High risk (CT)	[e]Low risk (CT)
uPA ans PAI test	High	Low	NA	NA
Investigational, in tissue and in circulation				
miRNAs				
CTCs	[f] ≥ 1/7.5 mL [g] ≥ 5/7.5 mL	[f] < 1/7.5 mL [g] < 5/7.5 mL	NA (ongoing trials)	NA (ongoing trials)
ctDNA (and ESR1 mutational status)	present	absent	NA (ongoing trials)	NA (ongoing trials)

Also see text. RS: recurrence score, low risk < 18, intermediate risk 18–30, high risk > 30; NA: not applicable; CT: chemotherapy; CTCs: circulating tumor cells; ctDNA: circulating tumor DNA.

[a] Direct correlation with the number of the involved lymph-nodes.

[b] In spite of the current ASCO and NCCI guideline this cut-off value is reported in a recent large study.

[c] To antiestrogens.

[d] To anti-HER2 mAbs/TKIs.

[e] In adjuvant setting, high-risk scored patients.

[f] More than 1 tumor cell per 7.5 mL of blood in adjuvant setting.

[g] More than 5 tumor cells per 7.5 mL in metastatic setting.

5.4. Treatment

For early-stage breast cancer, breast-conserving therapy is the first choice which generally includes breast surgery plus radiotherapy. Adjuvant therapy, radiotherapy, chemotherapy, endocrine therapy or the combinations are used to eradicate the micrometastatic disease. For ERBB2-positive breast cancer, a monoclonal antibody, trastuzumab is used (Sledge et al., 2014). In the advanced disease or when the tumor recurs, chemotherapy, endocrine therapy, signal pathways inhibitors such as trastuzumab or some of the recently introduced drugs like specific cell cycle inhibitors, palbociclib or ribociclib are used (Ramos-Esquivel et al., 2018). Poly ADP ribose polymerase enzyme inhibitors

are also used in the treatment of breast cancer. Many cancer cells are more dependent on this enzyme than regular cells, making an attractive target for cancer therapy (Turk and Wisinski, 2018).

Specific aims:

- To investigate the roles of LRIG1 and LRIG2 in gliomagenesis (Paper I and II).

- To identify functionally important LRIG1-interacting proteins (Paper III).

- To investigate the importance of *LRIG1* CNAs in breast cancer prognosis (Paper IV).

Materials and Methods

Generation of *Lrig1*- and *Lrig2*-deficient mice (I, II)

First, conditional *Lrig1*- and *Lrig2*-deficient mice were generated by inserting lox P sites of exon 1 of *Lrig1* or on both sides of exon 12 of *Lrig2* (I and II). Briefly, targeting vectors containing the lox P sites were electroporated into embryonic stem cells derived from 129Sv/J mice. Selected embryonic stem colonies were injected into C57BL/6 blastocysts to generate chimeric mice, which were used to obtain germ line transmission of the conditional allele. Thereafter, *Lrig1* and *Lrig2*-knockout mice were generated by removing the floxed exons through mating of the mice with OzCre mice (Ozgene), which ubiquitously express Cre recombinase. Western blotting and reverse transcription polymerase chain reaction (RT-PCR) were used to confirm the phenotypes of mice.

RNA isolation and real-time RT-PCR (I, II, III)

RNA was extracted by using RNAqueos kit (I and II) and PARIS kit (III). Contaminating DNA was removed by using TURBO DNA-free kit. Quantitative real-time RT-PCR was performed using 20 ng (I and II) or 40 ng (III) of total RNA and qscript One-Step quantitative RT-PCR kit. *RN18S* (*18S rRNA*) was used as the reference gene to normalize the data.

Droplet digital PCR (ddPCR) (IV)

In ddPCR, every sample is partitioned into approximately 20,000 separate droplets and PCR reactions occur inside each droplet. After the completion of the PCR, all droplets are analyzed and scored as positive or negative. Poisson statistics is then used to calculate the number of templates in the original sample. The probes were labeled with fluorophores at their 5' ends and quenchers at their 3' ends. The DNA substrate was digested with HindIII before the PCR. Then, the ddPCR reaction mixture was prepared, droplets were

generated, and the PCR was run. After PCR amplification, QX200 droplet reader (Bio-Rad) was used to obtain the data. The data were filtered out if they had low quality or showed high variation. If the *LRIG1*/reference gene ratio was higher than 1.15, we considered this as an *LRIG1* gain and if the ratio was lower than 0.85, we defined this as an *LRIG1* loss. For *ERBB2*, the American Society of Clinical Oncology/College of American Pathologists guideline, stating that if the *ERBB2*/reference gene ratio is higher than 2, the tumor is defined as being *ERBB2*- positive, was followed. The researcher was blind to the clinical data when performing the ddPCR analysis.

Cell culture, transfections, and transductions (I, II, III)

In the current thesis, primary mouse embryonic fibroblasts (MEFs), DF-1 chicken fibroblasts, HEK293T, HEK293, Lenti-X 293, TB101, and TB107 cells were used. Primary MEFs were taken from E12.5 or E13.5 mouse embryos resulting from matings of *Lrig2* heterozygote mice. DF-1 chicken fibroblasts transformed with PDGFB-expressing RCAS virus were kindly provided by Lene Uhrbom (Uppsala University, Sweden). The MEFs and DF-1 cells were cultured in DMEM containing 10% fetal bovine serum (FBS), 50 μg/ml gentamycin, MEM non-essential amino acids, and 50 μM 2-mercaptoethanol. HEK293T, HEK293, and Lenti-X 293 cells were obtained from American Type Culture Collection, the European Collection of Authenticated Cell Cultures, and Clontech, respectively. These cells were cultured in DMEM supplemented with 10% FBS and 50 μg/ml gentamycin. For HEK293T and HEK293 cells, BD Pure-Coat amine cell culture plates were used because the cells tended to detach easily when cultured on standard cell culture plastics. TB101 and TB107 were established from two different GBM (after informed consent and approval from ethics committee). These primary GBM cell lines were cultured in a neural stem cell medium on laminin-coated plates. All cell lines were cultured at 37 °C in a humidified atmosphere containing 5% CO_2. For transient transfections, X-tremeGene 9 or X-tremeGene HP DNA transfection reagents were used. The cells were seeded the day before transfection, and on the day of transfection, DNA vectors and reagents were mixed and added to the cell culture. The cells

were lysed after 2 (I and II) or 4 days (III) by using cell extraction buffer. Lentiviral particles were used to transduce glioma cell lines. The viral particles were produced by using Lenti-X 293 cells transiently co-transfected with relevant expression vectors and Lenti-X HTX packaging mix by using Xfect transfection reagent. In studies I and II, DF-1 chicken fibroblasts producing RCAS-PDGFB-HA retroviruses were used to transduce mouse brain cells after intracranial injection.

Generation of *LRIG1*-null cells (III)

To generate *LRIG1*-null HEK293T cells, CRISPR-Cas9 technology was used. In the final knockout clone, clone 3, the deletion of the entire *LRIG1* gene, spanning exon 1 to exon 19, of both alleles, was confirmed by Sanger DNA sequencing (III).

Glioma mouse models

We used the replication-competent avian retrovirus (RCAS)/*tv-a* gene transfer system to generate gliomas in mice. Mice do not normally express the RCAS receptor, tv-a, so RCAS viruses can normally not infect mouse cells. However, the Ntv-a transgenic mouse strain expresses the tv-a receptor under the control of the nestin promoter, resulting in expression of tv-a by neural progenitor cells. Thereby, the virus can specifically infect the neural progenitor cells in the Ntv-a mice. The RCAS vector used in our study had been engineered to include a cDNA encoding human PDGFB (Holland, 2001; Fomchenko, and Holland, 2006). Tumors were induced by injecting RCAS-*PDGFB*-producing DF-1 cells into the brains of newborn Ntv-a mice. This protocol results in the development of oligodendroglioma-like or GBM-like tumors at 12 weeks-of-age (Uhrbom et al., 1998).

In the orthotopic xenograft model, the primary human GBM cell lines, TB101 and TB107, were injected into the frontal lobe of eight-week-old BALB/cA nude mice. Four months after injection, the mice were euthanized and the brains

were analyzed by immunohistochemistry for human vimentin, which marked the human glioma cells.

Plasmids, shRNAs, and doxycycline-inducible system (I, II, III)

Expression vectors used in this thesis included: *Myc-LRIG1, pcDNA3-PDGFRA, pcDNA3.1, p3xFLAG-LRIG2, p3x-FLAG-CMV-13, pLVX-LRIG1-TRE3G, pLVX-TRE3G,* and *pLVX-Tet3G*. The inducible expression vectors used were based on the Tet-On 3G system. In the presence of doxycycline, Tet-on 3G protein expressed from the *pLVX-TRE3G* vector is able to bind to the *TRE3G* promoter and activate transcription of *LRIG1* from the *pLVX-LRIG1-TRE3G* expression vector. The advantage of this system is that the cDNA cloned into the vector is induced by doxycycline addition and the expression can be adjusted by the doxycycline concentration (Zhou et al., 2006). In paper III, short hairpin RNAs (shRNAs) were used to downregulate the endogenous levels of LRIG1 interactors. First, bacterial glycerol stocks were purchased and then shRNA vectors were prepared by using mini, midi, or maxiprep kits. The complete list of shRNAs used can be found in supplementary Table S1 in paper III.

Cell and tissue lysis, Western blotting, and phospho-RTK assay (I, II, III)

Mouse embryos, tissues, or cells were lysed in a lysis buffer containing 1% Triton X-100, 50 mM Tris-HCl, pH 7.5 and 150 mM NaCl (I) or a modified radioimmunoprecipitation assay buffer containing 50 mM Tris-HCl, pH 7.4, 1% Triton X-100, 0.2% sodium deoxycholate, 0.2% sodium-dodecyl-sulfate, 1 mM sodium ethylene diamine tetra acetate, 1 mM phenylmethylsulfonylfluoride (II) supplemented with protease inhibitor. The cells (III) were lysed in an ice-cold cell extraction buffer supplemented with the EDTA-free protease inhibitor and 1 mM phenylmethylsulfonyl fluoride. The supernatants were collected after centrifugation. Protein concentrations were measured by using a BCA protein assay kit. Samples were mixed with a denaturing sample buffer, reducing agent, and incubated at 100°C for 5 minutes. Equal amounts of proteins were loaded and separated on a 3–8% Tris-Acetate or 10% Bis-Tris gel. The proteins in the

gels were electro-transferred to polyvinylidene difluoride or nitrocellulose membranes. To block the membranes, 5% or 2% nonfat dry milk in Tris-buffered saline with tween (200mM Tris, pH 7.4, 150mM NaCl, 0.1% Tween 20) or Odyssey blocking buffer was used. The blots were incubated with specific primary antibodies according to the manufacturers' instructions followed by horseradish peroxidase-conjugated or fluorescent secondary antibodies. ECL Select kit was used to detect horseradish peroxidase-conjugated secondary antibodies and the images were obtained with a ChemiDoc Touch Imaging System. For fluorescent secondary antibodies, the Odyssey CLx imager was used to obtain the images. The data were analyzed by Image Lab software or Image Studio Lite software. As an internal loading control, actin protein was used. RTKs phosphorylation screening was performed by using a human Phospho-RTK array kit.

Flow cytometry (III)

HEK293 cells were detached and dissociated by incubation with Accutase enzyme mixture for 10 minutes. The detached cells were centrifuged and washed with cold phosphate-buffered saline (PBS). Thereafter, the cells were incubated on ice for 30 minutes with primary antibody diluted in fluorescence-activated cell sorting (FACS) buffer (3% FBS and 0.02% NaN_3 in PBS). After a wash step, the cells were incubated with the fluorescent secondary antibody. The cells were washed again, resuspended in FACS buffer including propidium iodide (0.1 µg/ml), and analyzed using a BD Accuri C6 flow cytometer. The main population of cells was gated and 10,000 events were acquired. FlowJo software was used to analyze the data and prepare FACS graphs.

Immunohistochemistry (II)

The mouse brains were fixed in 4% paraformaldehyde overnight and then transferred to 70% ethanol. The fixed tissues were embedded in paraffin, cut into 4 µm slices, incubated at 60°C for 2 hours, treated with xylene, and water. A citrate buffer was used to retrieve the epitopes. Then, the slides were labeled with an anti-vimentin antibody.

Proximity ligation assays (I)

Proximity ligation assay was used to detect phosphorylated Pdgfra or Pdgfrb in primary MEF cells. Mouse anti-phospho-tyrosine antibody was combined with rabbit anti-Pdgfra or rabbit anti-Pdgfrb antibody. Proximity ligation assay can detect epitopes that are in close proximity to each other, i.e., less than 40 nm apart. We used the secondary antibodies, reagents, and protocols provided by the manufacturer of the proximity ligation assays kit (Olink, Uppsala).

In Situ hybridization (I, II)

In situ RNA hybridization for *Lrig1* and *Lrig2* mRNAs was performed by using RNAscope kit. This method is an improvement of the traditional *in situ* hybridization technique. Briefly, cells or tissues were fixed and permeabilized. Then, RNAscope target probes were allowed to bind their target mRNA, followed by hybridization of multiple signal amplification molecules, and staining of the slides.

Migration assay (II)

A modified Boyden assay was used to analyze the migratory potential of primary GBM cell lines. The upper chamber has a microporous membrane that allowed the cells to migrate through it, down to the bottom chamber. Cells that had migrated from the upper to the lower chamber were stained and counted manually under a microscope.

Confocal microscopy (I, III)

Confocal laser scanning microscopy was used to visualize primary cilia in MEF cells (I) and to study colocalization of paraoxonase 2 (PON2) and LRIG1 in HEK293 cells (III). In confocal microscopy, the sample is visualized by a focused laser beam in the sample at a definite point. The light emitted from this point passes a pinhole and is detected by a detector. The light from out-of-focus planes in the sample cannot pass through the pinhole so the image in confocal

microscopy is prepared point by point (Thorn, 2015). The cells were cultured on glass coverslips. After transfection, the cells were fixed with 4% paraformaldehyde, permeabilized, and blocked with PBS including 5% FBS and 0.1% Triton X-100. Thereafter, the cells were incubated with specific primary antibodies and fluorescent secondary antibodies. To stain nuclei, DAPI was used. The images were acquired with a Zeiss LSM 710 confocal microscope.

Yeast two-hybrid (Y2H) screen (II)

Yeast two-hybrid (Y2H) system was developed by Fields and Song in 1989. This technique is based on a transcription factor that consists of two different domains, a DNA-binding domain and an activation domain. In the Y2H system, these two domains are separated and fused to other proteins. The DNA-binding and activation domains are fused to bait and prey, respectively. When the bait and prey proteins interact, then the activation domain is brought to the DNA-bound DNA-binding domain, which triggers the transcription of a reporter gene (Fields and Song, 1989) (Figure 7).

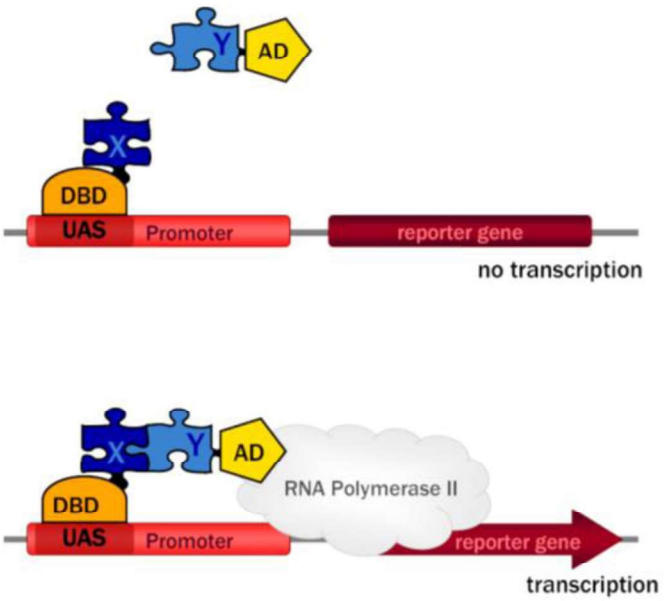

Figure 7. Y2H system. X and Y (blue) show two different proteins. The activation domain (AD) and DNA-binding domain (DBD) are fused to a bait and prey, respectively. If an interaction occurs between the bait and prey, the reporter gene will be transcribed (Fields and Song, 1989; This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-

In our project (paper III), the entire cytosolic domain of LRIG1 was cloned into the bait vector. DSY-1 yeast cells were co-transformed with the *LRIG1*-bait vector and a human whole-brain cDNA library cloned into the prey vector. The positive interactors were determined by sequencing.

In silico analyses (III)

TCGA database was used to download RNAseq and normalized counts data for all solid tumors. Spearman correlation test was used to analyze the correlation between *LRIG1* and LRIG1 interactors at the mRNA level.

Patients and tumor samples (IV)

The patients included in study IV were diagnosed with primary breast carcinoma between 1987 and 1999. Overall survival (OS) was calculated from diagnosis date to the date of death or the date of the last follow-up. Metastasis-free survival (MFS) was calculated from the date of diagnosis to the date of metastasis or the last follow-up. The last follow-up time for OS and MFS were June, 30, 2017 and December, 31, 2013, respectively. The TNM system was used for patient staging. Patients who were recorded with metastasis within six months after diagnosis date, were categorized as stage IV. The treatment of patients was given based on the guidelines of the North Swedish Breast Cancer Group. After pathology evaluation, representative tumor tissue was frozen in liquid nitrogen. Frozen tumor tissue was homogenized, and then suspended in cold standard receptor buffer (10 mmol/L Tris, pH 7.4; 1.5 mmol/L ethylenediaminetetraacetic acid; 10 mmol/L sodium molybdate; 1.0 mmol/L monothioglycerol). The samples were centrifuged at 20,000 x g and the supernatants (also called cytosols) were collected and stored at -70 °C. The pellets were analyzed by the Burton method to evaluate cell concentrations.

Statistical analyses (I, II, III, IV)

SPSS software was used for all statistical analyses except in paper III in which data were analyzed by using Excel. If the data had a normal distribution, the

mean of two or multiple groups was compared by Student's t test or ANOVA, respectively. If the data were not normally distributed, the mean of two or multiple groups were compared by Mann-Whitney or Kruskal-Wallis test, respectively. For correlation studies, non-parametric data were analyzed by Spearman correlation test. To describe the association between categorical data, Chi-square test was used if the test assumptions were not violated, otherwise the data were analyzed by Fisher's exact test. To analyze OS and MFS, log-rank test was used and the data were illustrated with Kaplan-Meier survival curves. For multivariate analysis (IV), Cox regression analysis was used. In all statistical analyses, the significance level was set to p-values less than 0.05 or, in cases with many multiple comparisons (more than 15 comparison tests), less than 0.01.

Studies approval and ethics (I, II, IV)

The housing of mice and all animal experiments were conducted according to the European Communities Council Directive (86/609/EEC). The experimental protocols were approved by the Regional Ethics Committee of Umeå University, Umeå, Sweden (registration numbers, A12-07, A25-07, A5-2010, A52-10, A41-10, and A42-10) (I and II). The project in paper IV was approved with registration number Um dnr 02-455.

Results and discussion

Paper I

Lrig2-deficient mice are protected against PDGFB-induced glioma

To investigate the physiological and molecular role of Lrig2 in mouse development and in a mouse model of PDGF-induced gliomagenesis, we generated *Lrig2*-deficient mice. *Lrig2* exon 12 (*Lrig2E12*) was removed and this resulted in a frameshift in the open reading frame of *Lrig2*, resulting in multiple stop codons. Thus, it is possible that a truncated version of Lrig2 was expressed in the knockout mice. Regrettably, this possibility could not be tested because of a lack of suitable reagents, i.e., specific antibodies against the region encoded by *Lrig2* exon 1-11. The identities of wildtype, heterozygote, and knockout *Lrig2* mice were confirmed at the DNA, mRNA, and protein levels by Southern blotting, RT-PCR, and Western blotting, respectively. To obtain a homogenous genetic background, *Lrig2* mice were back-crossed onto a C57BL/6 background for five generations.

Lrig2 knockout mice had an increased mortality rate compared to wildtype or heterozygous mice and they grew slower, initially, however, at 15-18 weeks of age, the body weights were the same among the genotypes. The knockout mice did not show any macroscopic anatomical defects or difference in organ weights. Myelin sheaths also appeared to be normal.

In the mouse cancer model of gliomagenesis, *Ntv-a* transgenic mice with different *Lrig2* genotypes were transduced with PDGFB-encoding RCAS viruses. In this model, most of the resulting tumors are of lower WHO grades (grades II and III), with similarities to human oligodendroglioma and a few tumors are of higher WHO grade (IV), with similarities to human GBM. The tumor incidence and grade were dependent on the *Lrig2* genotype. *Lrig2* knockout mice showed a lower glioma incidence and a higher proportion of low-grade tumors than

wildtype and heterozygous mice. This difference was not dependent on RCAS virus transduction efficiency.

To investigate the molecular mechanisms behind the observed differences in PDGFB-induced gliomas between the *Lrig2* genotypes, we established a series of MEF lines from mice of different *Lrig2* genotypes and analyzed them with regard to Pdgf signaling. Intriguingly, no difference was observed between the genotypes with regard to Pdgfr levels or phosphorylation levels of Pdgfrs, Akt, or Erk1/2 after PDGF-BB treatment. However, the PDGF-BB-induced induction of the immediate-early genes *Fos* and early growth response 2 differed among the different *Lrig2* genotypes. *Lrig2* knockout MEFs showed a more rapid induction-kinetics of *Fos* and early growth response 2 than wildtype and heterozygous MEFs did. Co-transfection of *LRIG2* and *PDGFRA* In HEK293 cells revealed no effect of LRIG2 expression on PDGFRA level. However, LRIG1 negatively downregulated PDGFRA expression levels in a dose-dependent manner. Taken together, these results indicate that Lrig2 affects Pdgfr signaling downstream of receptor activation.

In summary, we concluded that Lrig2 is important in mouse development and PDGF-induced gliomagenesis, possibly via modulating Pdgfr downstream signaling.

Paper II

Lrig1 is a haploinsufficient tumor suppressor gene in malignant glioma

To investigate the role of Lrig1 in the mouse model of PDGF-induced gliomagenesis, we generated *Lrig1*-deficient mice. Disruption of *Lrig1* was achieved by deleting *Lrig1* exon 1, which contains the predicted ATG start codon. Quantitative RT-PCR and Western blotting were used to evaluate the *Lrig1* mRNA and protein levels in brains of the mice. Both mRNA and protein expression levels of Lrig1 were lower in heterozygous than in wildtype mice, and neither Lrig1 mRNA or protein was detected in the knockout mice. PDGF-

induced gliomas were generated according to the same method as described in paper I. The incidence of generated tumors was similar among different *Lrig1* genotypes but, intriguingly, *Lrig1* heterozygous mice had a significantly higher incidence of high-grade tumors than wildtype mice had. This showed that *Lrig1* is a haploinsufficient tumor suppressor of PDGFB-induced experimental glioma in mice.

Next, we asked if ectopic expression of LRIG1 can decrease glioma malignancy *in vivo*. In a xenograft model, we stably transduced a primary GBM cell line, TB107, with lentiviral vectors encoding LRIG1 (*pLVX-LRIG1)* or an empty control vector. The transduced cells were injected orthotopically into BALB/cA nude mice. The tumor incidence was similar for the cell line that overexpressed LRIG1 and the control cell line. However, the LRIG1 overexpressing tumors showed a strikingly reduced invasiveness. The reduction in invasiveness *in vivo* was mirrored by a reduced migratory capacity of TB107 cells *in vitro* that had been transduced with an inducible *LRIG1* allele. Intriguingly, induced LRIG1 overexpression downregulated the phosphorylation level of MET, but had no effect on the phosphorylation level of EGFR. To further investigate the role of MET in the LRIG1-mediated suppression of TB107 cell migration, a pharmacological inhibitor of MET, PHA-665752, was used. The results demonstrated that LRIG1 suppressed the migration of TB107 cells partly through negative regulation of MET. Strikingly, LRIG1 overexpression had no effect on the migration of TB101 cells. Accordingly, TB101 cells did not display any phosphorylated MET, which possibly accounted for the lack of effect on migration by LRIG1 in these cells. On the other hand, LRIG1 suppressed the proliferation of TB101. The mechanism behind this anti-proliferative effect of LRIG1 remains unexplored.

The lack of an effect of LRIG1 overexpression on p-EGFR levels in TB101 or TB107 cells was surprising. This result contrasted with several previous studies showing that LRIG1 downregulates EGFR. It will be important to investigate if LRIG1 and EGFR directly interact or if they interact indirectly through a protein complex including other interactors. If such mediators are missing or disrupted,

then the LRIG1 effect might be compromised. In paper III, we identified various LRIG1 interactors and showed that some of them can regulate the function of LRIG1. In the HEK293T cell context, LRIG1 did not directly interact with EGFR; instead, PON2 and PTPRK may mediate an interaction between LRIG1 and EGFR. It will be interesting to investigate the importance of these LRIG1-interactors also in TB101 and TB107 GBM cells.

In summary, in this study, we showed that *Lrig1* is a haploinsufficient tumor suppressor gene in PDGFB-induced gliomagenesis in mice and that LRIG1 could inhibit glioma cell invasion and migration, partly through MET inhibition.

Paper III

A protein interaction network centered on leucine-rich repeats and immunoglobulin-like domains 1 (LRIG1) regulates growth factor receptors

In the cell, proteins are not functioning in isolation instead they function through interactions with other proteins. Some proteins also interact with DNA, RNA, lipids, or small molecules. To understand the function of proteins at the cellular level, systems biology methods are needed. In this perspective, protein-protein interaction maps or interactomes can be useful.

The mechanisms of LRIG1 function remain largely unknown. Different RTKs are downregulated by LRIG1 and some mechanisms have been suggested. However, the unbiased identification of LRIG1-interacting proteins may help to better understand the mechanisms of LRIG1 function and how LRIG1 acts in different contexts. To identify LRIG1-interacting proteins and analyze their effects on LRIG1 function, we performed a Y2H screen, mined the data from the Bio-Plex (biophysical interactions of ORFeome-based complexes) protein interaction data, and developed a triple co-transfection system which we used to evaluate the functional importance of the identified LRIG1-interacting proteins.

In our Y2H screen, the cytosolic tail of LRIG1 was used as the bait to screen a human adult whole-brain cDNA library. Thereby, we found the LRIG1 interactors glutaredoxin 3 (GLRX3) and zinc finger and BTB domain–containing 16 (ZBTB16).

In the Bio-Plex project, a lentiviral library consisting of 13,000 open reading frames or baits was used to co-immunoprecipitate endogenous proteins from HEK293T cells. After co-immunoprecipitation (affinity purification), mass spectrometry technique was used to analyze and identify the interacting proteins. An "extracting software" named comparative proteomic analysis software suite (*CompPASS*) was used to filter out non-specific interactors. In this dataset, 18 proteins were discovered as LRIG1 high confidence interacting proteins: calcium activated nucleotidase 1 (CANT1), canopy fibroblast growth factor signaling regulator 3 (CNPY3), CNPY4, galactosyl-3-sulfonyl-transferase-1 (GAL3ST1), glycosylphosphatidylinositol anchored molecule-like (GML), major histocompatibility complex, class II, DR (HLA-DRA), major histocompatibility complex, class I, E (HLA-E), LRIG2, LRIG3, leucine-rich repeat containing 40 (LRRC40), mitochondrial fission regulator 1 like (MTFR1L), paraoxonase 2 (PON2), protein tyrosine phosphatase, receptor type K (PTPRK), RAB4A, member RAS oncogene family (RAB4A), scavenger receptor class A member 3 (SCARA3), secretoglobin family 2A member 2 (SCGB2A2), scribbled planar cell polarity protein (SCRIB), and tubulin beta 8 class VIII (TUBB8). To investigate the functional importance of all identified LRIG1-interacting proteins, we established a transient triple co-transfection system. In paper I, we showed that LRIG1 downregulates PDGFRA when *LRIG1* and *PDGFRA* are co-transfected in HEK293 cells. In the triple co-transfection system, we also included shRNAs to downregulate the endogenous levels of the LRIG1 interactors and evaluated the PDGFRA expression level as the functional read out. We used a mixture of five different shRNAs against all interactors because we aimed to target all potential splice variants of respective interactor and to reach a high level of transient downregulation. LRIG1-mediated down-regulation of PDGFRA expression was blunted by shRNA mixtures against *PON2* and *GAL3ST1*. We saw also the same trend with shRNAs against *RAB4A*

and *GLRX3*. The opposite was seen with shRNAs against *LRIG2, CNPY3, HLA-DRA, GML, CNPY4, LRRC40*, and *LRIG3*; i.e., these shRNA mixtures seemed to enhance the LRIG1-mediated down-regulation of PDGFRA. The transferrin receptor protein was used as a control for possible non-specific effects on the expression or stability of unrelated transmembrane proteins. Intriguingly, LRIG1 itself showed more or less the same pattern as PDGFRA. That is, the shRNAs that increased LRIG1-mediated down-regulation of PDGFRA expression also decreased LRIG1 expression level and vice versa. One interpretation of these results is that both LRIG1 and its PDGFRA target are degraded together, as previously shown for LRIG1 and EGFR (Gur et al., 2004).

Because we identified antibodies that could recognize endogenous PON2 and RAB4, we analyzed these interactors further. Of note, we failed to identify antibodies that could recognize some other endogenous LRIG1-interacting proteins. The quantification of the PON2 and RAB4A protein levels showed a relatively modest extent of downregulation by the shRNA mixtures (~20%). However, it should be noted that only about 50% of the cells were successfully transfected by the shRNAs, whereas in Western blotting, all the cells are analyzed, thereby diluting out any specific effects. Nevertheless, using the different shRNAs individually, a perfect match was seen between the level of RAB4A downregulation and decreased LRIG1-mediated down-regulation of PDGFRA. One important consideration is related to various spliced variants of RAB4A, PON2, and the other LRIG1 interactors. Regarding RAB4A, there are at least two different spliced variants and not all individual shRNAs can target both variants. To investigate if all interactors spliced variants have the same function, more experiments are needed. As it is clear from PON2 and RAB4A, not all individual shRNAs are functional and this is another reason why we chose a mixture of shRNAs in our screen. A weakness of the study is that we did not show that all shRNA mixtures induced an efficient downregulation of their respective target protein. Therefore, our negative results regarding eight of the 19 interactors need to be validated by future experiments. We purchased the shRNAs from Sigma Aldrich and many of those shRNAs were already validated in HEK293T or other cell lines (See paper III, Table S1). However, the low

endogenous level of some LRIG1 interactors makes it difficult to use Western blotting for validation of the shRNA effects on the protein level. Of note, by using quantitative real-time RT-PCR, we could show that the shRNA mixtures against *GAL3ST1, GLRX3*, and *ZBTB16* induced an efficient downregulation at the mRNA level (data not shown). However, mRNA and protein levels may not necessarily correlate in all cases.

One important question is whether the monitored PDGFRA expression level changes were dependent on LRIG1. In principle, in our triple co-transfection system, the respective shRNA could also affect PDGFRA levels directly, without the involvement of LRIG1. To address this question, we generated *LRIG1*-null HEK293T cells and repeated the same triple co-transfection experiments with or without LRIG1 overexpression. These experiments showed for PON2 and ZBTB16, that the effect of their shRNA-mediated downregulation on PDGFRA expression was dependent on LRIG1 expression. To exclude LRIG1 independent effects, this experiment needs to be repeated for all LRIG1-interactors.

The LRIG1-interacting proteins could be classified into three groups according to the effects of their shRNAs on the LRIG1-mediated down-regulation of PDGFRA seen in the triple co-transfection system: (1) *RAB4A, PON2, GLRX3*, and *GAL3ST1* whose shRNAs resulted in increased levels of PDGFRA and/or LRIG1; (2) *MTFR1L, SCRIB, PTPRK, CANT1, TUBB8, SCARA3, and HLA-E* whose shRNAs resulted in no significant change in PDGFRA or LRIG1 levels; (3) *ZBTB16, LRIG2, CNPY3, HLA-DRA, GML, CNPY4, LRRC40*, and *LRIG3* whose shRNAs resulted in decreased levels of PDGFRA and/or LRIG1. Regarding the genes in the second group, which did not affect PDGFRA levels, we cannot rule out the possibility that these genes affect LRIG1 function in other manners.

To investigate if the expression of *LRIG1* is associated with the expression level of LRIG1-interacting proteins, we performed an *in silico* analysis. We downloaded RNAseq count data from TCGA for *LRIG1* and its interactors in different cancer types and normal tissues. Spearman correlation analysis revealed a consistent positive correlation between *LRIG1* expression and the

expression of *ZBTB16* and *PTPRK* and a negative correlation between *LRIG1* expression and the expression of *GLRX3*. The correlations were stronger in normal tissues than in cancer tissues. This might imply that in cancer cells the relations between LRIG1 and its interactors are disrupted.

Here, briefly, I describe the proteins not belonging to the LRIG family which we identified as LRIG1 interactors with functional importance:

RAB4A. shRNAs against *RAB4A* did not affect the PDGFRA level significantly but affected the LRIG1 level. RAB4A is a master regulator in the regulation of endocytosis. It plays important roles in recycling from early endosomes, transport to early endosomes, and homotypic fusion of early endosomes. RAB4A affects both recycling and degradative endosomal trafficking (McCaffrey et al., 2001) by recruitment of adaptor proteins to early endosomes (d'Souza et al., 2014). Intriguingly, in PDGFR signaling pathway, recycling of PDGFRB is dependent on RAB4A (Hellberg et al., 2009) and deregulation of RAB4A alters PDGFR trafficking (Chamberlain et al., 2010). Rab4A also regulates EGFR signaling pathway (Goueli et al., 2012). RAB4A has dual roles both in slow-recycling and fast-recycling routes of receptors recycling back to the plasma membrane (Deneka et al., 2003; Sheff et al., 1999).

PON2. shRNAs against *PON2* affected both the PDGFRA and LRIG1 expression levels significantly, in a manner suggesting that PON2 supports LRIG1 function. We were able to use confocal microscopy for PON2 as we had a good antibody that was compatible with this technique. This analysis revealed that PON2 was mostly localized in the cytoplasm in HEK293 cells and partly colocalized with LRIG1. PON2 has previously been reported to localize to the endoplasmic reticulum, mitochondria, and peri-nuclear region (Aviram and Rosenblat, 2004; Horke et al., 2007; Rothem et al., 2007). In response to oxidative stress, PON2 localization changes to the plasma membrane (Hagmann et al., 2014). *PON2* belongs to the *PON* gene family. PON2 is a type II transmembrane protein that protects against lipid peroxidation and regulates the content of glucosylceramide. The name PON comes from the paraoxon substrate that is

hydrolyzed by a paraoxonase. However, PON2 in contrast to PON1 cannot hydrolyze paraoxon (Primo-Parmo et al., 1996). PON2 has a lactonase and an anti-oxidant activity. Still it is not clear if these two enzymatic functions are related to each other. PON2 can promote cell survival by reducing cellular oxidative damage (Altenhöfer et al., 2010). Specifically, PON2 is an important antioxidant in the central nervous system (Garrick et al., 2016). Moreover, PON2 also has an anti-apoptotic function (Horke et al., 2007). PON2 is upregulated in cancer and supports tumor progression (Furlong et al., 2016). Recently, PON2 was shown to be a metabolic regulator of pancreatic ductal adenocarcinoma tumor growth and metastasis. Inactivation of TP53 in pancreatic ductal adenocarcinoma cells results in PON2 overexpression which leads to cancer progression. In this case, PON2 regulates glucose transport by glucose transporter 1 protein which is important for cancer cell metabolism (Nagarajan et al., 2017).

GLRX3. shRNAs against GLRX3 did not affect the PDGFRA or LRIG1 expression levels significantly. However, *in silico* analysis of the TCGA data sets showed inverse correlations between the transcripts encoding *LRIG1* and *GLRX3*. GLRX3 (other aliases: PICOT or TXNL2) was identified as a protein that interacts with protein kinase C θ isoform by using Y2H system (Witte et al., 2000). Deletion of *GLRX3* in mice is lethal (Cheng et al., 2011) and GLRX3 plays an important role in iron homeostasis and hemoglobin maturation (Haunhorst et al., 2013). As an antioxidant enzyme, GLRX3 regulates cellular stress responses (Qu et al., 2011). High expression of GLRX3 was reported in Hodgkin's lymphoma (Ohayon et al., 2009) and in human colon and lung carcinoma cells (Cha and Kim, 2009). GLRX3 is also overexpressed in nasopharyngeal carcinoma cells where it promotes cell growth and metastasis through the EGFR signaling pathway (He et al., 2016).

GAL3ST1. shRNAs against *GAL3ST1* affected both the PDGFRA and LRIG1 expression levels significantly, in a manner suggesting that GAL3ST1 supports LRIG1 function. GAL3ST1 (also known as CST) is a sulfotransferase that catalyzes the transfer of a sulfate group to one of the carbons in

galactosylceramide. The produced sulfatide is a plasma membrane sulfolipid (Goldberg, 1961). Sulfation is also a common modification of glycoproteins (Seko et al., 2001). GAL3ST1 is overexpressed in ovarian carcinoma cells (Liu et al., 2010) and high enzymatic activity was reported also in human renal cell carcinoma cells, which results in elevated levels of sulfatide (Honke et al., 1998). GAL3ST1 activity is increased in renal cell carcinoma cells by EGFR and MET signaling pathways (Kobayashi et al., 1993 and 1994).

PTPRK, shRNAs against *PTPRK* did not affect PDGFRA or LRIG1 expression levels. However, *in silico* analysis of TCGA data sets showed consistent correlations between the transcripts encoding LRIG1 and PTPRK in both normal and cancer tissues. PTPRK is a protein tyrosine phosphatase. Protein tyrosine phosphorylation is one of the most well-known protein modifications that control normal and cancer cell growth (Hunter, 2014). Protein tyrosine phosphatases play important roles in cell homeostasis (Fischer et al., 1991). PTPRK connects homophilic cell-cell contact to cellular signaling (Sap et al., 1994). In early studies, *PTPRK* gene was shown to be a target of the TGF-β signaling pathway (Flavell et al., 2008). PTPRK promotes localization of β-catenin/E-cadherin complex in cell-cell contact surfaces in adherence junctions. In this scenario, the β-catenin pool in the cytosol and in turn, the transcriptional activity of β-catenin in the nucleus, is limited after its stabilization at adherence junctions (Novellino et al., 2008). In breast cancer, low expression of PTPRK was associated with poor prognosis. *In vitro* analyses demonstrated that PTPRK downregulation in breast cancer cells is associated with increased cell proliferation, adhesion, and migration (Sun et al., 2013). PTPRK dephosphorylates EGFR in Chinese hamster ovary cells and primary human keratinocytes (Xu et al., 2005).

ZBTB16. shRNAs against *ZBTB16* affected PDGFRA expression levels significantly, in a manner suggesting that ZBTB16 opposes LRIG1 function. However, the effect size on PDGFRA level was modest and when the experiment was repeated in *LRIG1*-null HEK293T cells, the effect was only of borderline significance. Intriguingly, *in silico* analysis of the TCGA data sets showed

consistent correlation between the *LRIG1* and *ZBTB16* transcripts. ZBTB16 is a highly conserved protein, from yeast to human, during evolution (Avantaggiato et al., 1995). ZBTB16 has been shown to be a negative regulator of cell cycle progression through MYC suppression (Yeyati et al., 1999). In malignant melanoma and gallbladder cancer, *ZBTB16* behaves as a tumor suppressor gene (Felicetti et al., 2004; Shen et al., 2018). Recently, both *LRIG1* and *ZBTB16* were shown to be glioma susceptibility risk alleles (Melin et al., 2017). ZBTB16 has dual roles both as a transcription factor (Mathew et al., 2012) and as an adaptor involved in E3 ubiquitin ligase complex (Xu et al., 2003).

CNPY3 and CNPY4. shRNAs against CNPY3 or CNPY4 affected both the PDGFRA and LRIG1 expression levels significantly, in a manner suggesting that CNPY3 and CNPY4 oppose LRIG1 function. Information about the functions of CNPY3 and CNPY4 is scarce. CNPY3 (also known as PRAT4A) acts as a co-chaperone for a HSP90 paralog, gp96, in the proper folding of Toll-like receptors. Gp96 resides in endoplasmic reticulum and can directly interact with CNPY3 and form a complex *in vitro* and *in vivo* (Liu et al., 2010). CNPY4 (also known as PRAT4B) is also a co-chaperone. In HEK293 cells, CNPY4 downregulation decrease Toll-like receptor 4 expression at the cell surface (Konno et al., 2006). It is possible that CNPY3 and CNPY4 are needed for proper folding and expression of both PDGFRA and LRIG1, however, this hypothesis needs to be tested experimentally.

HLA-DRA. shRNAs against *HLA-DRA* affected both the PDGFRA and LRIG1 expression levels significantly, in a manner suggesting that HLA-DRA opposes the function of LRIG1. HLA-DRA is an MHC class II molecule which play central roles in antigen presentation in the immune system (Rosenthal and Shevach, 1973). How HLA-DRA might regulate the function of LRIG1 remains to be explored.

GML. shRNAs against *GML* affected both the PDGFRA and LRIG1 expression levels significantly, in a manner suggesting that GML opposes the function of LRIG1. GML has been shown to sensitize esophageal cancer cells to ionizing

radiation in a TP53-dependent manner and triggering apoptosis (Kagawa et al., 1997). Ectopic expression of GML in a human GBM cell line, T98G suppressed proliferation of the cells by inducing a G2/M arrest (Ueda et al., 1999). In a colorectal cancer cell line, GML expression increased the sensitivity of the cells to mitomycin C (Hashimoto et al., 2001). Strikingly, to my knowledge, no studies have been published about GML during the last 15 years.

LRRC40. shRNAs against *LRRC40* affected both the PDGFRA and LRIG1 expression levels significantly, in a manner suggesting that LRRC40 opposes the function of LRIG1. LRRC40 is predicted to be a cytoplasmic or nuclear protein, however, to my knowledge, no function of LRRC40 has been reported. On the other hand, LRRC40 seems to be highly conserved during evolution, suggesting an important function (NCBI, Basic Local Alignment Search Tool).

It will be interesting to see how our functional LRIG1 interactome will be translated into cancer biology. The findings from our *in vitro* study should also be extended to studies *in vivo*. In any case, I believe our suggested LRIG1 functional interactome has opened up a new direction for LRIG research as most of the identified LRIG1 interactors in this study were not known to interact with LRIG1, previously. Future studies will reveal the physiological importance of these LRIG1-interacting proteins.

Paper IV

LRIG1 gene copy number analysis by ddPCR and correlation to clinical factors in breast cancer

Previous *LRIG1* CN analyses using FISH technique demonstrated loss and gain of *LRIG1* in approximately 3.5% and 34% of the tumors, respectively (Ljuslinder et al., 2005; Ljuslinder et al., 2009). Later, another investigation that used molecular inversion probes and included 971 early-stage breast tumors showed *LRIG1* loss and gain in 8.9% and 3.9% of the tumors, respectively (Thompson et al., 2014). Importantly, in the latter study, it was shown that *LRIG1* loss could predict both early and late relapse of early-stage breast cancer. To validate or

refute the results of these studies, we investigated *LRIG1* CN in a breast cancer cohort by using the ddPCR technique. This technique is very sensitive and powerful to detect CN alterations (CNAs) in cancer samples. First, suitable reference genes were selected by a literature review and TCGA data mining. Moreover, ddPCR assays were designed for six different loci of *LRIG1* gene. We tested six different *LRIG1*/reference assays in twelve healthy individuals. Four of six pairs of *LRIG1*/reference assays demonstrated *LRIG1* CN ratios very close to 1, thereby validating these assays.

To compare the ddPCR method with FISH, we used four pairs of *LRIG1*/reference assays to quantify *LRIG1*/reference gene ratios in 34 tumors that had been analyzed by FISH, previously. Strikingly, no significant correlation was observed between the results obtained with ddPCR and FISH. In six of the tumors, FISH detected *LRIG1* gains that were normal by ddPCR and in 3 tumors, FISH showed *LRIG1* normal CN that were detected as a loss by ddPCR. One aspect of the observed discordance between FISH and ddPCR might be related to the tumor ploidy and the reference gene used. In contrast to the ddPCR analyses, no reference gene was used in the FISH study.

In 423 breast tumor cytosols, we analyzed *LRIG1* and *ERBB2* CN ratios by using ddPCR. We detected *LRIG1* loss and gain in 18.2% and 12.5% of the tumors, respectively. By using Fisher's exact test, we analyzed the possible correlations between *LRIG1* CNAs and other clinicopathological parameters (Table 1, paper IV). We found significant associations between *LRIG1* CN status and steroid receptor status, ERBB2 status, tumor subtype, tumor grade, nodal status, and tumor types. The *LRIG1* loss was more common among estrogen receptor-negative or ERBB2-positive tumors than among estrogen receptor-positive or ERBB2-negative tumors. Thus, it can be speculated that there is a selection against LRIG1 expression in estrogen receptor-negative and ERBB2-positive tumors. *LRIG1* loss was more common in higher tumor grades or node-positive patients. These data are in line with a tumor suppressive function of LRIG1 in breast cancer.

To investigate the relationships between *LRIG1* CNAs and patient's outcome, we analyzed overall survival and metastasis-free survival both in all patients and in early-stage patients. Generally speaking, patients with both *LRIG1* loss and gain had a poor outcome. However, in a Cox regression model including all variables affecting patients' survival, only tumor subtype and nodal status behaved as independent prognostic factors, whereas *LRIG1* loss or gain did not. Disappointingly, this result suggests that *LRIG1* CN analyses may not add any important prognostic information to the clinical parameters that are already routinely evaluated in breast cancer.

To summarize paper IV, we successfully designed and validated ddPCR assays to quantify relative *LRIG1* CNs in breast cancer cytosols and demonstrated that *LRIG1* CNAs were associated with several clinical parameters. These results are consistent with possible important biological functions of LRIG1 in breast cancer, however, LRIG1 CN ratios did not prove to be independent prognostic factors in the breast cancer cohort analyzed.

Conclusions

- Lrig2 plays an important role in PDGFB-induced gliomagenesis *in vivo* and modulates downstream Pdgfr signaling *in vitro* (paper I).
- *Lrig1* functions as a haploinsufficient tumor suppressor gene in PDGFB-induced gliomagenesis (paper II).
- LRIG1 inhibits glioma cell migration, partly through MET inhibition, and proliferation, in a cell context-dependent manner (paper II).
- Functionally important LRIG1-interacting proteins include: RAB4A, PON2, GLRX3, GAL3ST1, PTPRK, ZBTB16, LRIG2, CNPY3, HLA-DRA, GML, CNPY4, LRRC40, and LRIG3 (paper III).
- *LRIG1* gene aberrations may be important determinants of breast cancer biology but do not seem to predict the risk of relapse in early-stage breast cancer (paper IV).

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References

Alsina, F.C., Hita, F.J., Fontanet, P.A., Irala, D., Hedman, H., Ledda, F., and Paratcha, G. (2016). Lrig1 is a cell-intrinsic modulator of hippocampal dendrite complexity and BDNF signaling. EMBO reports 17, 601-616.

Altenhofer, S., Witte, I., Teiber, J.F., Wilgenbus, P., Pautz, A., Li, H., Daiber, A., Witan, H., Clement, A.M., Forstermann, U., et al. (2010). One enzyme, two functions: PON2 prevents mitochondrial superoxide formation and apoptosis independent from its lactonase activity. The Journal of biological chemistry 285, 24398-24403.

Amatya, V.J., Naumann, U., Weller, M., and Ohgaki, H. (2005). TP53 promoter methylation in human gliomas. Acta neuropathologica 110, 178-184.

An, Y., Zhao, Z., Ou, P., and Wang, G. (2015). Expression of LRIG1 is Associated With Good Prognosis for Human Non-small Cell Lung Cancer. Medicine 94, e2081.

Andrae, J., Gallini, R., and Betsholtz, C. (2008). Role of platelet-derived growth factors in physiology and medicine. Genes & development 22, 1276-1312.

Avantaggiato, V., Pandolfi, P.P., Ruthardt, M., Hawe, N., Acampora, D., Pelicci, P.G., and Simeone, A. (1995). Developmental analysis of murine Promyelocyte Leukemia Zinc Finger (PLZF) gene expression: implications for the neuromeric model of the forebrain organization. The Journal of neuroscience: the official journal of the Society for Neuroscience 15, 4927-4942.

Aviram, M., and Rosenblat, M. (2004). Paraoxonases 1, 2, and 3, oxidative stress, and macrophage foam cell formation during atherosclerosis development. Free radical biology & medicine 37, 1304-1316.

Avraham, R., and Yarden, Y. (2011). Feedback regulation of EGFR signalling: decision making by early and delayed loops. Nature reviews Molecular cell biology 12, 104-117.

Azevedo, F.A., Carvalho, L.R., Grinberg, L.T., Farfel, J.M., Ferretti, R.E., Leite, R.E., Jacob Filho, W., Lent, R., and Herculano-Houzel, S. (2009). Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. The Journal of comparative neurology 513, 532-541.

Bai, L., McEachern, D., Yang, C.Y., Lu, J., Sun, H., and Wang, S. (2012). LRIG1 modulates cancer cell sensitivity to Smac mimetics by regulating TNFalpha expression and receptor tyrosine kinase signaling. Cancer research 72, 1229-1238.

Bella, J., Hindle, K.L., McEwan, P.A., and Lovell, S.C. (2008). The leucine-rich repeat structure. Cellular and molecular life sciences: CMLS 65, 2307-2333.

Bigner, S.H., Matthews, M.R., Rasheed, B.K., Wiltshire, R.N., Friedman, H.S., Friedman, A.H., Stenzel, T.T., Dawes, D.M., McLendon, R.E., and Bigner, D.D. (1999). Molecular genetic aspects of oligodendrogliomas including analysis by comparative genomic hybridization. The American journal of pathology 155, 375-386.

Birchmeier, C., Birchmeier, W., Gherardi, E., and Vande Woude, G.F. (2003). Met, metastasis, motility and more. Nature reviews Molecular cell biology 4, 915-925.

Blume-Jensen, P., and Hunter, T. (2001). Oncogenic kinase signalling. Nature 411, 355-365.

Boelens, M.C., van den Berg, A., Fehrmann, R.S., Geerlings, M., de Jong, W.K., te Meerman, G.J., Sietsma, H., Timens, W., Postma, D.S., and Groen, H.J. (2009). Current smoking-specific gene expression signature in normal bronchial epithelium is enhanced in squamous cell lung cancer. The Journal of pathology 218, 182-191.

Boyle P, Levin B (2008) World Cancer Report 2008. IARC Nonserial Publication. World Health Organisation: Lyon.

Cairncross, G., Macdonald, D., Ludwin, S., Lee, D., Cascino, T., Buckner, J., Fulton, D., Dropcho, E., Stewart, D., Schold, C., Jr., et al. (1994). Chemotherapy for anaplastic oligodendroglioma. National

Cancer Institute of Canada Clinical Trials Group. Journal of clinical oncology: official journal of the American Society of Clinical Oncology 12, 2013-2021.

Cairncross, J.G., Ueki, K., Zlatescu, M.C., Lisle, D.K., Finkelstein, D.M., Hammond, R.R., Silver, J.S., Stark, P.C., Macdonald, D.R., Ino, Y., et al. (1998). Specific genetic predictors of chemotherapeutic response and survival in patients with anaplastic oligodendrogliomas. Journal of the National Cancer Institute 90, 1473-1479.

Cancer Genome Atlas, N. (2012). Comprehensive molecular portraits of human breast tumours. Nature 490, 61-70.

Cancer Genome Atlas Research, N. (2008). Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 455, 1061-1068.

Cancer Genome Atlas Research, N., Brat, D.J., Verhaak, R.G., Aldape, K.D., Yung, W.K., Salama, S.R., Cooper, L.A., Rheinbay, E., Miller, C.R., Vitucci, M., et al. (2015). Comprehensive, Integrative Genomic Analysis of Diffuse Lower-Grade Gliomas. The New England journal of medicine 372, 2481-2498.

Ceccarelli, M., Barthel, F.P., Malta, T.M., Sabedot, T.S., Salama, S.R., Murray, B.A., Morozova, O., Newton, Y., Radenbaugh, A., Pagnotta, S.M., et al. (2016). Molecular Profiling Reveals Biologically Discrete Subsets and Pathways of Progression in Diffuse Glioma. Cell 164, 550-563.

Cha, M.K., and Kim, I.H. (2009). Preferential overexpression of glutaredoxin3 in human colon and lung carcinoma. Cancer epidemiology 33, 281-287.

Chamberlain, M.D., Oberg, J.C., Furber, L.A., Poland, S.F., Hawrysh, A.D., Knafelc, S.M., McBride, H.M., and Anderson, D.H. (2010). Deregulation of Rab5 and Rab4 proteins in p85R274A-expressing cells alters PDGFR trafficking. Cellular signalling 22, 1562-1575.

Chang, L., Shi, R., Yang, T., Li, F., Li, G., Guo, Y., Lang, B., Yang, W., Zhuang, Q., and Xu, H. (2013). Restoration of LRIG1 suppresses bladder cancer cell growth by directly targeting EGFR activity. Journal of experimental & clinical cancer research: CR 32, 101.

Chen, L., Wang, W., Zhu, S., Jin, X., Wang, J., Zhu, J., and Zhou, Y. (2017). MicroRNA-590-3p enhances the radioresistance in glioblastoma cells by targeting LRIG1. Experimental and therapeutic medicine 14, 1818-1824.

Chen, W., Zhang, J., Xu, H., Dai, J., and Zhang, X. (2017). The negative regulation of miR-149-5p in melanoma cell survival and apoptosis by targeting LRIG2. American journal of translational research 9, 4331-4340.

Cheng, N.H., Zhang, W., Chen, W.Q., Jin, J., Cui, X., Butte, N.F., Chan, L., and Hirschi, K.D. (2011). A mammalian monothiol glutaredoxin, Grx3, is critical for cell cycle progression during embryogenesis. The FEBS journal 278, 2525-2539.

Choi, M.H., Lee, I.K., Kim, G.W., Kim, B.U., Han, Y.H., Yu, D.Y., Park, H.S., Kim, K.Y., Lee, J.S., Choi, C., et al. (2005). Regulation of PDGF signalling and vascular remodelling by peroxiredoxin II. Nature 435, 347-353.

Chung, W.S., Allen, N.J., and Eroglu, C. (2015). Astrocytes Control Synapse Formation, Function, and Elimination. Cold Spring Harbor perspectives in biology 7, a020370.

Claesson-Welsh, L., Eriksson, A., Moren, A., Severinsson, L., Ek, B., Ostman, A., Betsholtz, C., and Heldin, C.H. (1988). cDNA cloning and expression of a human platelet-derived growth factor (PDGF) receptor specific for B-chain-containing PDGF molecules. Molecular and cellular biology 8, 3476-3486.

Cloughesy, T.F., Cavenee, W.K., and Mischel, P.S. (2014). Glioblastoma: from molecular pathology to targeted treatment. Annual review of pathology 9, 1-25.

Conley, B.A., and Taube, S.E. (2004). Prognostic and predictive markers in cancer. Disease markers 20, 35-43.

Corless, C.L., Barnett, C.M., and Heinrich, M.C. (2011). Gastrointestinal stromal tumours: origin and molecular oncology. Nature reviews Cancer 11, 865-878.

Cupp, J.R., and McAlister-Henn, L. (1991). NAD(+)-dependent isocitrate dehydrogenase. Cloning, nucleotide sequence, and disruption of the IDH2 gene from Saccharomyces cerevisiae. The Journal of biological chemistry 266, 22199-22205.

Dai, C., Celestino, J.C., Okada, Y., Louis, D.N., Fuller, G.N., and Holland, E.C. (2001). PDGF autocrine stimulation dedifferentiates cultured astrocytes and induces oligodendrogliomas and oligoastrocytomas from neural progenitors and astrocytes in vivo. Genes & development 15, 1913-1925.

Damstrup, L., Wandahl Pedersen, M., Bastholm, L., Elling, F., and Skovgaard Poulsen, H. (2002). Epidermal growth factor receptor mutation type III transfected into a small cell lung cancer cell line is predominantly localized at the cell surface and enhances the malignant phenotype. International journal of cancer 97, 7-14.

Dang, L., White, D.W., Gross, S., Bennett, B.D., Bittinger, M.A., Driggers, E.M., Fantin, V.R., Jang, H.G., Jin, S., Keenan, M.C., et al. (2009). Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature 462, 739-744.

Davis, M.E. (2016). Glioblastoma: Overview of Disease and Treatment. Clinical journal of oncology nursing 20, S2-8.

De Abreu, F.B., Wells, W.A., and Tsongalis, G.J. (2013). The emerging role of the molecular diagnostics laboratory in breast cancer personalized medicine. The American journal of pathology 183, 1075-1083.

De Craene, B., and Berx, G. (2013). Regulatory networks defining EMT during cancer initiation and progression. Nature reviews Cancer 13, 97-110.

Del Rio, T., Nishitani, A.M., Yu, W.M., and Goodrich, L.V. (2013). In vivo analysis of Lrig genes reveals redundant and independent functions in the inner ear. PLoS genetics 9, e1003824.

Deneka, M., Neeft, M., and van der Sluijs, P. (2003). Regulation of membrane transport by rab GTPases. Critical reviews in biochemistry and molecular biology 38, 121-142.

Duffy, M.J., and Crown, J. (2008). A personalized approach to cancer treatment: how biomarkers can help. Clinical chemistry 54, 1770-1779.

D'Souza, R.S., Semus, R., Billings, E.A., Meyer, C.B., Conger, K., and Casanova, J.E. (2014). Rab4 orchestrates a small GTPase cascade for recruitment of adaptor proteins to early endosomes. Current biology: CB 24, 1187-1198.

Edge, S.B., and Compton, C.C. (2010). The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. Annals of surgical oncology 17, 1471-1474.

Elston, C.W., and Ellis, I.O. (1991). Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology 19, 403-410.

Ettle, B., Schlachetzki, J.C.M., and Winkler, J. (2016). Oligodendroglia and Myelin in Neurodegenerative Diseases: More Than Just Bystanders? Molecular neurobiology 53, 3046-3062.

Farooqi, A.A., and Siddik, Z.H. (2015). Platelet-derived growth factor (PDGF) signalling in cancer: rapidly emerging signalling landscape. Cell biochemistry and function 33, 257-265.

Felicetti, F., Bottero, L., Felli, N., Mattia, G., Labbaye, C., Alvino, E., Peschle, C., Colombo, M.P., and Care, A. (2004). Role of PLZF in melanoma progression. Oncogene 23, 4567-4576.

Fischer, E.H., Charbonneau, H., and Tonks, N.K. (1991). Protein tyrosine phosphatases: a diverse family of intracellular and transmembrane enzymes. Science 253, 401-406.

Flavell, J.R., Baumforth, K.R., Wood, V.H., Davies, G.L., Wei, W., Reynolds, G.M., Morgan, S., Boyce, A., Kelly, G.L., Young, L.S., et al. (2008). Down-regulation of the TGF-beta target gene, PTPRK, by the Epstein-Barr virus encoded EBNA1 contributes to the growth and survival of Hodgkin lymphoma cells. Blood 111, 292-301.

Fomchenko, E.I., and Holland, E.C. (2006). Mouse models of brain tumors and their applications in preclinical trials. Clinical cancer research: an official journal of the American Association for Cancer Research 12, 5288-5297.

Fredriksson, L., Li, H., and Eriksson, U. (2004). The PDGF family: four gene products form five dimeric isoforms. Cytokine & growth factor reviews 15, 197-204.

Furlong, C.E., Marsillach, J., Jarvik, G.P., and Costa, L.G. (2016). Paraoxonases-1, -2 and -3: What are their functions? Chemico-biological interactions 259, 51-62.

Furnari, F.B., Fenton, T., Bachoo, R.M., Mukasa, A., Stommel, J.M., Stegh, A., Hahn, W.C., Ligon, K.L., Louis, D.N., Brennan, C., et al. (2007). Malignant astrocytic glioma: genetics, biology, and paths to treatment. Genes & development 21, 2683-2710.

Garrick, J.M., Dao, K., de Laat, R., Elsworth, J., Cole, T.B., Marsillach, J., Furlong, C.E., and Costa, L.G. (2016). Developmental expression of paraoxonase 2. Chemico-biological interactions 259, 168-174.

Gherardi, E., Birchmeier, W., Birchmeier, C., and Vande Woude, G. (2012). Targeting MET in cancer: rationale and progress. Nature reviews Cancer 12, 89-103.

Ghiglione, C., Carraway, K.L., 3rd, Amundadottir, L.T., Boswell, R.E., Perrimon, N., and Duffy, J.B. (1999). The transmembrane molecule kekkon 1 acts in a feedback loop to negatively regulate the activity of the Drosophila EGF receptor during oogenesis. Cell 96, 847-856.

Gleason, R.J., Vora, M., Li, Y., Kane, N.S., Liao, K., and Padgett, R.W. (2017). C. elegans SMA-10 regulates BMP receptor trafficking. PloS one 12, e0180681.

Goldberg, I.H. (1961). The sulfolipids. Journal of lipid research 2, 103-109.

Goueli, B.S., Powell, M.B., Finger, E.C., and Pfeffer, S.R. (2012). TBC1D16 is a Rab4A GTPase activating protein that regulates receptor recycling and EGF receptor signaling. Proceedings of the National Academy of Sciences of the United States of America 109, 15787-15792.

Grandis, J.R., and Sok, J.C. (2004). Signaling through the epidermal growth factor receptor during the development of malignancy. Pharmacology & therapeutics 102, 37-46.

Grant, S., Qiao, L., and Dent, P. (2002). Roles of ERBB family receptor tyrosine kinases, and downstream signaling pathways, in the control of cell growth and survival. Frontiers in bioscience: a journal and virtual library 7, d376-389.

Grassot, J., Mouchiroud, G., and Perriere, G. (2003). RTKdb: database of Receptor Tyrosine Kinase. Nucleic acids research 31, 353-358.

Grob, T.J., Kannengiesser, I., Tsourlakis, M.C., Atanackovic, D., Koenig, A.M., Vashist, Y.K., Klose, H., Marx, A.H., Koops, S., Simon, R., et al. (2012). Heterogeneity of ERBB2 amplification in adenocarcinoma, squamous cell carcinoma and large cell undifferentiated carcinoma of the lung. Modern pathology: an official journal of the United States and Canadian Academy of Pathology, Inc 25, 1566-1573.

Gumienny, T.L., Macneil, L., Zimmerman, C.M., Wang, H., Chin, L., Wrana, J.L., and Padgett, R.W. (2010). Caenorhabditis elegans SMA-10/LRIG is a conserved transmembrane protein that enhances bone morphogenetic protein signaling. PLoS genetics 6, e1000963.

Guo, D., Holmlund, C., Henriksson, R., and Hedman, H. (2004). The LRIG gene family has three vertebrate paralogs widely expressed in human and mouse tissues and a homolog in Ascidiacea. Genomics 84, 157-165.

Guo, D., Nilsson, J., Haapasalo, H., Raheem, O., Bergenheim, T., Hedman, H., and Henriksson, R. (2006). Perinuclear leucine-rich repeats and immunoglobulin-like domain proteins (LRIG1-3) as prognostic indicators in astrocytic tumors. Acta neuropathologica 111, 238-246.

Gur, G., Rubin, C., Katz, M., Amit, I., Citri, A., Nilsson, J., Amariglio, N., Henriksson, R., Rechavi, G., Hedman, H., et al. (2004). LRIG1 restricts growth factor signaling by enhancing receptor ubiquitylation and degradation. The EMBO journal 23, 3270-3281.

Hagmann, H., Kuczkowski, A., Ruehl, M., Lamkemeyer, T., Brodesser, S., Horke, S., Dryer, S., Schermer, B., Benzing, T., and Brinkkoetter, P.T. (2014). Breaking the chain at the membrane: paraoxonase 2 counteracts lipid peroxidation at the plasma membrane. FASEB journal: official publication of the Federation of American Societies for Experimental Biology 28, 1769-1779.

Hanahan, D., and Weinberg, R.A. (2000). The hallmarks of cancer. Cell 100, 57-70.

Hashimoto, Y., Ueda, K., Minami, K., and Watatani, M. (2001). The potential clinical value of GML and the p53 gene as a predictor of chemosensitivity for colorectal cancer. International journal of clinical oncology 6, 90-96.

Haunhorst, P., Hanschmann, E.M., Brautigam, L., Stehling, O., Hoffmann, B., Muhlenhoff, U., Lill, R., Berndt, C., and Lillig, C.H. (2013). Crucial function of vertebrate glutaredoxin 3 (PICOT) in iron homeostasis and hemoglobin maturation. Molecular biology of the cell 24, 1895-1903.

He, F., Wei, L., Luo, W., Liao, Z., Li, B., Zhou, X., Xiao, X., You, J., Chen, Y., Zheng, S., et al. (2016). Glutaredoxin 3 promotes nasopharyngeal carcinoma growth and metastasis via EGFR/Akt pathway and independent of ROS. Oncotarget 7, 37000-37012.

Hedman, H., and Henriksson, R. (2007). LRIG inhibitors of growth factor signalling - double-edged swords in human cancer? European journal of cancer 43, 676-682.

Hedman, H., Lindstrom, A.K., Tot, T., Stendahl, U., Henriksson, R., and Hellberg, D. (2010). LRIG2 in contrast to LRIG1 predicts poor survival in early-stage squamous cell carcinoma of the uterine cervix. Acta oncologica 49, 812-815.

Hedman, H., Nilsson, J., Guo, D., and Henriksson, R. (2002). Is LRIG1 a tumour suppressor gene at chromosome 3p14.3? Acta oncologica 41, 352-354.

Heldin, C.H. (2012). Autocrine PDGF stimulation in malignancies. Upsala journal of medical sciences 117, 83-91.

Heldin, C.H., and Westermark, B. (1999). Mechanism of action and in vivo role of platelet-derived growth factor. Physiological reviews 79, 1283-1316.

Hellberg, C., Schmees, C., Karlsson, S., Ahgren, A., and Heldin, C.H. (2009). Activation of protein kinase C alpha is necessary for sorting the PDGF beta-receptor to Rab4a-dependent recycling. Molecular biology of the cell 20, 2856-2863.

Herman, J.G., and Baylin, S.B. (2003). Gene silencing in cancer in association with promoter hypermethylation. The New England journal of medicine 349, 2042-2054.

Hermanson, M., Funa, K., Hartman, M., Claesson-Welsh, L., Heldin, C.H., Westermark, B., and Nister, M. (1992). Platelet-derived growth factor and its receptors in human glioma tissue: expression of messenger RNA and protein suggests the presence of autocrine and paracrine loops. Cancer research 52, 3213-3219.

Hoch, R.V., and Soriano, P. (2003). Roles of PDGF in animal development. Development 130, 4769-4784.

Holbro, T., Civenni, G., and Hynes, N.E. (2003). The ErbB receptors and their role in cancer progression. Experimental cell research 284, 99-110.

Holland, E.C. (2001). Gliomagenesis: genetic alterations and mouse models. Nature reviews Genetics 2, 120-129.

Holmlund, C., Haapasalo, H., Yi, W., Raheem, O., Brannstrom, T., Bragge, H., Henriksson, R., and Hedman, H. (2009). Cytoplasmic LRIG2 expression is associated with poor oligodendroglioma patient survival. Neuropathology: official journal of the Japanese Society of Neuropathology 29, 242-247.

Holmlund, C., Nilsson, J., Guo, D., Starefeldt, A., Golovleva, I., Henriksson, R., and Hedman, H. (2004). Characterization and tissue-specific expression of human LRIG2. Gene 332, 35-43.

Homma, S., Shimada, T., Hikake, T., and Yaginuma, H. (2009). Expression pattern of LRR and Ig domain-containing protein (LRRIG protein) in the early mouse embryo. Gene expression patterns: GEP 9, 1-26.

Honke, K., Tsuda, M., Hirahara, Y., Miyao, N., Tsukamoto, T., Satoh, M., and Wada, Y. (1998). Cancer-associated expression of glycolipid sulfotransferase gene in human renal cell carcinoma cells. Cancer research 58, 3800-3805.

Horbinski, C., Hobbs, J., Cieply, K., Dacic, S., and Hamilton, R.L. (2011). EGFR expression stratifies oligodendroglioma behavior. The American journal of pathology 179, 1638-1644.

Horke, S., Witte, I., Wilgenbus, P., Kruger, M., Strand, D., and Forstermann, U. (2007). Paraoxonase-2 reduces oxidative stress in vascular cells and decreases endoplasmic reticulum stress-induced caspase activation. Circulation 115, 2055-2064.

Hunter, T. (2014). The genesis of tyrosine phosphorylation. Cold Spring Harbor perspectives in biology 6, a020644.

Jensen, K.B., Collins, C.A., Nascimento, E., Tan, D.W., Frye, M., Itami, S., and Watt, F.M. (2009). Lrig1 expression defines a distinct multipotent stem cell population in mammalian epidermis. Cell stem cell 4, 427-439.

Jensen, K.B., Jones, J., and Watt, F.M. (2008). A stem cell gene expression profile of human squamous cell carcinomas. Cancer letters 272, 23-31.

Jesinger, R.A. (2014). Breast anatomy for the interventionalist. Techniques in vascular and interventional radiology 17, 3-9.

Johansson, M., Oudin, A., Tiemann, K., Bernard, A., Golebiewska, A., Keunen, O., Fack, F., Stieber, D., Wang, B., Hedman, H., et al. (2013). The soluble form of the tumor suppressor Lrig1 potently inhibits in vivo glioma growth irrespective of EGF receptor status. Neuro-oncology 15, 1200-1211.

Jurek, A., Heldin, C.H., and Lennartsson, J. (2011). Platelet-derived growth factor-induced signaling pathways interconnect to regulate the temporal pattern of Erk1/2 phosphorylation. Cellular signalling 23, 280-287.

Kagawa, K., Inoue, T., Tokino, T., Nakamura, Y., and Akiyama, T. (1997). Overexpression of GML promotes radiation-induced cell cycle arrest and apoptosis. Biochemical and biophysical research communications 241, 481-485.

Kaloshi, G., Everhard, S., Laigle-Donadey, F., Marie, Y., Navarro, S., Mokhtari, K., Idbaih, A., Ducray, F., Thillet, J., Hoang-Xuan, K., et al. (2008). Genetic markers predictive of chemosensitivity and outcome in gliomatosis cerebri. Neurology 70, 590-595.

Kaminska, M., Ciszewski, T., Lopacka-Szatan, K., Miotla, P., and Staroslawska, E. (2015). Breast cancer risk factors. Przeglad menopauzalny = Menopause review 14, 196-202.

Karlsson, S., Kowanetz, K., Sandin, A., Persson, C., Ostman, A., Heldin, C.H., and Hellberg, C. (2006). Loss of T-cell protein tyrosine phosphatase induces recycling of the platelet-derived growth factor (PDGF) beta-receptor but not the PDGF alpha-receptor. Molecular biology of the cell 17, 4846-4855.

Karsy, M., Neil, J.A., Guan, J., Mahan, M.A., Colman, H., and Jensen, R.L. (2015). A practical review of prognostic correlations of molecular biomarkers in glioblastoma. Neurosurgical focus 38, E4.

Kazlauskas, A., and Cooper, J.A. (1989). Autophosphorylation of the PDGF receptor in the kinase insert region regulates interactions with cell proteins. Cell 58, 1121-1133.

Killela, P.J., Pirozzi, C.J., Healy, P., Reitman, Z.J., Lipp, E., Rasheed, B.A., Yang, R., Diplas, B.H., Wang, Z., Greer, P.K., et al. (2014). Mutations in IDH1, IDH2, and in the TERT promoter define clinically distinct subgroups of adult malignant gliomas. Oncotarget 5, 1515-1525.

Knuutila, S., Aalto, Y., Autio, K., Bjorkqvist, A.M., El-Rifai, W., Hemmer, S., Huhta, T., Kettunen, E., Kiuru-Kuhlefelt, S., Larramendy, M.L., et al. (1999). DNA copy number losses in human neoplasms. The American journal of pathology 155, 683-694.

Kobayashi, T., Honke, K., Gasa, S., Kato, N., Miyazaki, T., and Makita, A. (1993). Epidermal growth factor elevates the activity levels of glycolipid sulfotransferases in renal-cell-carcinoma cells. International journal of cancer 55, 448-452.

Kobayashi, T., Honke, K., Gasa, S., Miyazaki, T., Tajima, H., Matsumoto, K., Nakamura, T., and Makita, A. (1994). Hepatocyte growth factor elevates the activity levels of glycolipid sulfotransferases in renal cell carcinoma cells. European journal of biochemistry 219, 407-413.

Koelsche, C., Sahm, F., Capper, D., Reuss, D., Sturm, D., Jones, D.T., Kool, M., Northcott, P.A., Wiestler, B., Bohmer, K., et al. (2013). Distribution of TERT promoter mutations in pediatric and adult tumors of the nervous system. Acta neuropathologica 126, 907-915.

Kohler, N., and Lipton, A. (1974). Platelets as a source of fibroblast growth-promoting activity. Experimental cell research 87, 297-301.

Konno, K., Wakabayashi, Y., Akashi-Takamura, S., Ishii, T., Kobayashi, M., Takahashi, K., Kusumoto, Y., Saitoh, S., Yoshizawa, Y., and Miyake, K. (2006). A molecule that is associated with Toll-like receptor 4 and regulates its cell surface expression. Biochemical and biophysical research communications 339, 1076-1082.

Kou, C., Zhou, T., Han, X., Zhuang, H., and Qian, H. (2015). LRIG1, a 3p tumor suppressor, represses EGFR signaling and is a novel epigenetic silenced gene in colorectal cancer. Biochemical and biophysical research communications 464, 519-525.

Krig, S.R., Frietze, S., Simion, C., Miller, J.K., Fry, W.H., Rafidi, H., Kotelawala, L., Qi, L., Griffith, O.L., Gray, J.W., et al. (2011). Lrig1 is an estrogen-regulated growth suppressor and correlates with longer relapse-free survival in ERalpha-positive breast cancer. Molecular cancer research: MCR 9, 1406-1417.

Kvarnbrink, S., Karlsson, T., Edlund, K., Botling, J., Lindquist, D., Jirstrom, K., Micke, P., Henriksson, R., Johansson, M., and Hedman, H. (2015). LRIG1 is a prognostic biomarker in non-small cell lung cancer. Acta oncologica 54, 1113-1119.

Laederich, M.B., Funes-Duran, M., Yen, L., Ingalla, E., Wu, X., Carraway, K.L., 3rd, and Sweeney, C. (2004). The leucine-rich repeat protein LRIG1 is a negative regulator of ErbB family receptor tyrosine kinases. The Journal of biological chemistry 279, 47050-47056.

Lander, E.S., Linton, L.M., Birren, B., Nusbaum, C., Zody, M.C., Baldwin, J., Devon, K., Dewar, K., Doyle, M., FitzHugh, W., et al. (2001). Initial sequencing and analysis of the human genome. Nature 409, 860-921.

Lando, M., Fjeldbo, C.S., Wilting, S.M., B, C.S., Aarnes, E.K., Forsberg, M.F., Kristensen, G.B., Steenbergen, R.D., and Lyng, H. (2015). Interplay between promoter methylation and chromosomal loss in gene silencing at 3p11-p14 in cervical cancer. Epigenetics 10, 970-980.

Lechleider, R.J., Sugimoto, S., Bennett, A.M., Kashishian, A.S., Cooper, J.A., Shoelson, S.E., Walsh, C.T., and Neel, B.G. (1993). Activation of the SH2-containing phosphotyrosine phosphatase SH-PTP2 by its binding site, phosphotyrosine 1009, on the human platelet-derived growth factor receptor. The Journal of biological chemistry 268, 21478-21481.

Ledda, F., and Paratcha, G. (2007). Negative Regulation of Receptor Tyrosine Kinase (RTK) Signaling: A Developing Field. Biomarker insights 2, 45-58.

Ledda, F., Bieraugel, O., Fard, S.S., Vilar, M., and Paratcha, G. (2008). Lrig1 is an endogenous inhibitor of Ret receptor tyrosine kinase activation, downstream signaling, and biological responses to GDNF. The Journal of neuroscience: the official journal of the Society for Neuroscience 28, 39-49.

Lee, J.M., Kim, B., Lee, S.B., Jeong, Y., Oh, Y.M., Song, Y.J., Jung, S., Choi, J., Lee, S., Cheong, K.H., et al. (2014). Cbl-independent degradation of Met: ways to avoid agonism of bivalent Met-targeting antibody. Oncogene 33, 34-43.

Levkowitz, G., Klapper, L.N., Tzahar, E., Freywald, A., Sela, M., and Yarden, Y. (1996). Coupling of the c-Cbl protooncogene product to ErbB-1/EGF-receptor but not to other ErbB proteins. Oncogene 12, 1117-1125.

Lindquist, D., Kvarnbrink, S., Henriksson, R., and Hedman, H. (2014). LRIG and cancer prognosis. Acta oncologica 53, 1135-1142.

Lindstrom, A.K., Ekman, K., Stendahl, U., Tot, T., Henriksson, R., Hedman, H., and Hellberg, D. (2008). LRIG1 and squamous epithelial uterine cervical cancer: correlation to prognosis, other

tumor markers, sex steroid hormones, and smoking. International journal of gynecological cancer: official journal of the International Gynecological Cancer Society 18, 312-317.

Liu, B., Yang, Y., Qiu, Z., Staron, M., Hong, F., Li, Y., Wu, S., Li, Y., Hao, B., Bona, R., et al. (2010). Folding of Toll-like receptors by the HSP90 paralogue gp96 requires a substrate-specific cochaperone. Nature communications 1, 79.

Liu, F., Hon, G.C., Villa, G.R., Turner, K.M., Ikegami, S., Yang, H., Ye, Z., Li, B., Kuan, S., Lee, A.Y., et al. (2015). EGFR Mutation Promotes Glioblastoma through Epigenome and Transcription Factor Network Remodeling. Molecular cell 60, 307-318.

Liu, T., Cheng, W., Lai, D., Huang, Y., and Guo, L. (2010). Characterization of primary ovarian cancer cells in different culture systems. Oncology reports 23, 1277-1284.

Ljuslinder, I., Golovleva, I., Henriksson, R., Grankvist, K., Malmer, B., and Hedman, H. (2009). Co-incidental increase in gene copy number of ERBB2 and LRIG1 in breast cancer. Breast cancer research: BCR 11, 403.

Ljuslinder, I., Malmer, B., Golovleva, I., Thomasson, M., Grankvist, K., Hockenstrom, T., Emdin, S., Jonsson, Y., Hedman, H., and Henriksson, R. (2005). Increased copy number at 3p14 in breast cancer. Breast cancer research: BCR 7, R719-727.

Louis, D.N., Ohgaki, H., Wiestler, O.D., Cavenee, W.K., Burger, P.C., Jouvet, A., Scheithauer, B.W., and Kleihues, P. (2007). The 2007 WHO classification of tumours of the central nervous system. Acta neuropathologica 114, 97-109.

Lu, L., Teixeira, V.H., Yuan, Z., Graham, T.A., Endesfelder, D., Kolluri, K., Al-Juffali, N., Hamilton, N., Nicholson, A.G., Falzon, M., et al. (2013). LRIG1 regulates cadherin-dependent contact inhibition directing epithelial homeostasis and pre-invasive squamous cell carcinoma development. The Journal of pathology 229, 608-620.

Luwor, R.B., Zhu, H.J., Walker, F., Vitali, A.A., Perera, R.M., Burgess, A.W., Scott, A.M., and Johns, T.G. (2004). The tumor-specific de2-7 epidermal growth factor receptor (EGFR) promotes cells survival and heterodimerizes with the wild-type EGFR. Oncogene 23, 6095-6104.

Lynch, T.J., Bell, D.W., Sordella, R., Gurubhagavatula, S., Okimoto, R.A., Brannigan, B.W., Harris, P.L., Haserlat, S.M., Supko, J.G., Haluska, F.G., et al. (2004). Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. The New England journal of medicine 350, 2129-2139.

MacLaren, C.M., Evans, T.A., Alvarado, D., and Duffy, J.B. (2004). Comparative analysis of the Kekkon molecules, related members of the LIG superfamily. Development genes and evolution 214, 360-366.

Malhotra, G.K., Zhao, X., Band, H., and Band, V. (2010). Histological, molecular and functional subtypes of breast cancers. Cancer biology & therapy 10, 955-960.

Mandai, K., Guo, T., St Hillaire, C., Meabon, J.S., Kanning, K.C., Bothwell, M., and Ginty, D.D. (2009). LIG family receptor tyrosine kinase-associated proteins modulate growth factor signals during neural development. Neuron 63, 614-627.

Mao, F., Wang, B., Xi, G., Sun, W., Zhang, H., Ye, F., Guo, D., and Lei, T. (2012). Effects of RNAi-mediated gene silencing of LRIG1 on proliferation and invasion of glioma cells. Journal of Huazhong University of Science and Technology Medical sciences, 32, 227-232.

Mao, F., Wang, B., Xiao, Q., Xi, G., Sun, W., Zhang, H., Ye, F., Wan, F., Guo, D., Lei, T., et al. (2013). A role for LRIG1 in the regulation of malignant glioma aggressiveness. International journal of oncology 42, 1081-1087.

Martinho, O., Longatto-Filho, A., Lambros, M.B., Martins, A., Pinheiro, C., Silva, A., Pardal, F., Amorim, J., Mackay, A., Milanezi, F., et al. (2009). Expression, mutation and copy number analysis of platelet-derived growth factor receptor A (PDGFRA) and its ligand PDGFA in gliomas. British journal of cancer 101, 973-982.

Mathew, R., Seiler, M.P., Scanlon, S.T., Mao, A.P., Constantinides, M.G., Bertozzi-Villa, C., Singer, J.D., and Bendelac, A. (2012). BTB-ZF factors recruit the E3 ligase cullin 3 to regulate lymphoid effector programs. Nature 491, 618-621.

McCaffrey, M.W., Bielli, A., Cantalupo, G., Mora, S., Roberti, V., Santillo, M., Drummond, F., and Bucci, C. (2001). Rab4 affects both recycling and degradative endosomal trafficking. FEBS letters 495, 21-30.

Melin, B.S., Barnholtz-Sloan, J.S., Wrensch, M.R., Johansen, C., Il'yasova, D., Kinnersley, B., Ostrom, Q.T., Labreche, K., Chen, Y., Armstrong, G., et al. (2017). Genome-wide association study of glioma subtypes identifies specific differences in genetic susceptibility to glioblastoma and non-glioblastoma tumors. Nature genetics 49, 789-794.

Miller, J.K., Shattuck, D.L., Ingalla, E.Q., Yen, L., Borowsky, A.D., Young, L.J., Cardiff, R.D., Carraway, K.L., 3rd, and Sweeney, C. (2008). Suppression of the negative regulator LRIG1 contributes to ErbB2 overexpression in breast cancer. Cancer research 68, 8286-8294.

Miyake, S., Mullane-Robinson, K.P., Lill, N.L., Douillard, P., and Band, H. (1999). Cbl-mediated negative regulation of platelet-derived growth factor receptor-dependent cell proliferation. A critical role for Cbl tyrosine kinase-binding domain. The Journal of biological chemistry 274, 16619-16628.

Moasser, M.M. (2007). The oncogene HER2: its signaling and transforming functions and its role in human cancer pathogenesis. Oncogene 26, 6469-6487.

Moscatello, D.K., Holgado-Madruga, M., Godwin, A.K., Ramirez, G., Gunn, G., Zoltick, P.W., Biegel, J.A., Hayes, R.L., and Wong, A.J. (1995). Frequent expression of a mutant epidermal growth factor receptor in multiple human tumors. Cancer research 55, 5536-5539.

Muller, S., Lindquist, D., Kanter, L., Flores-Staino, C., Henriksson, R., Hedman, H., and Andersson, S. (2013). Expression of LRIG1 and LRIG3 correlates with human papillomavirus status and patient survival in cervical adenocarcinoma. International journal of oncology 42, 247-252.

Nagarajan, A., Dogra, S.K., Sun, L., Gandotra, N., Ho, T., Cai, G., Cline, G., Kumar, P., Cowles, R.A., and Wajapeyee, N. (2017). Paraoxonase 2 Facilitates Pancreatic Cancer Growth and Metastasis by Stimulating GLUT1-Mediated Glucose Transport. Molecular cell 67, 685-701 e686.

Nave, K.A. (2010). Myelination and support of axonal integrity by glia. Nature 468, 244-252.

Neirinckx, V., Hedman, H., and Niclou, S.P. (2017). Harnessing LRIG1-mediated inhibition of receptor tyrosine kinases for cancer therapy. Biochimica et biophysica acta 1868, 109-116.

Nicholas, M.K., Lukas, R.V., Jafri, N.F., Faoro, L., and Salgia, R. (2006). Epidermal growth factor receptor - mediated signal transduction in the development and therapy of gliomas. Clinical cancer research: an official journal of the American Association for Cancer Research 12, 7261-7270.

Nicholson, R.I., Gee, J.M., and Harper, M.E. (2001). EGFR and cancer prognosis. European journal of cancer 37 Suppl 4, S9-15.

Nicolini, A., Ferrari, P., and Duffy, M.J. (2017). Prognostic and predictive biomarkers in breast cancer: Past, present and future. Seminars in cancer biology.

Nielsen, F.C., van Overeem Hansen, T., and Sorensen, C.S. (2016). Hereditary breast and ovarian cancer: new genes in confined pathways. Nature reviews Cancer 16, 599-612.

Nilsson, J., Vallbo, C., Guo, D., Golovleva, I., Hallberg, B., Henriksson, R., and Hedman, H. (2001). Cloning, characterization, and expression of human LIG1. Biochemical and biophysical research communications 284, 1155-1161.

Nister, M., Libermann, T.A., Betsholtz, C., Pettersson, M., Claesson-Welsh, L., Heldin, C.H., Schlessinger, J., and Westermark, B. (1988). Expression of messenger RNAs for platelet-derived growth factor and transforming growth factor-alpha and their receptors in human malignant glioma cell lines. Cancer research 48, 3910-3918.

Noskovicova, N., Petrek, M., Eickelberg, O., and Heinzelmann, K. (2015). Platelet-derived growth factor signaling in the lung. From lung development and disease to clinical studies. American journal of respiratory cell and molecular biology 52, 263-284.

Noushmehr, H., Weisenberger, D.J., Diefes, K., Phillips, H.S., Pujara, K., Berman, B.P., Pan, F., Pelloski, C.E., Sulman, E.P., Bhat, K.P., et al. (2010). Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. Cancer cell 17, 510-522.

Novellino, L., De Filippo, A., Deho, P., Perrone, F., Pilotti, S., Parmiani, G., and Castelli, C. (2008). PTPRK negatively regulates transcriptional activity of wild type and mutated oncogenic beta-catenin and affects membrane distribution of beta-catenin/E-cadherin complexes in cancer cells. Cellular signalling 20, 872-883.

Nowsheen, S., Aziz, K., Panayiotidis, M.I., and Georgakilas, A.G. (2012). Molecular markers for cancer prognosis and treatment: have we struck gold? Cancer letters 327, 142-152.

Oh, Y.M., Lee, S.B., Choi, J., Suh, H.Y., Shim, S., Song, Y.J., Kim, B., Lee, J.M., Oh, S.J., Jeong, Y., et al. (2014). USP8 modulates ubiquitination of LRIG1 for Met degradation. Scientific reports 4, 4980.

Ohayon, A., Babichev, Y., Pasvolsky, R., Dong, G., Sztarkier, I., Benharroch, D., Altman, A., and Isakov, N. (2010). Hodgkin's lymphoma cells exhibit high expression levels of the PICOT protein. Journal of immunotoxicology 7, 8-14.

Ohgaki, H., and Kleihues, P. (2007). Genetic pathways to primary and secondary glioblastoma. The American journal of pathology 170, 1445-1453.

Okamoto, I., Kenyon, L.C., Emlet, D.R., Mori, T., Sasaki, J., Hirosako, S., Ichikawa, Y., Kishi, H., Godwin, A.K., Yoshioka, M., et al. (2003). Expression of constitutively activated EGFRvIII in non-small cell lung cancer. Cancer science 94, 50-56.

Oster, S.K., Marhin, W.W., Asker, C., Facchini, L.M., Dion, P.A., Funa, K., Post, M., Sedivy, J.M., and Penn, L.Z. (2000). Myc is an essential negative regulator of platelet-derived growth factor beta receptor expression. Molecular and cellular biology 20, 6768-6778.

Park, S.H., Won, J., Kim, S.I., Lee, Y., Park, C.K., Kim, S.K., and Choi, S.H. (2017). Molecular Testing of Brain Tumor. Journal of pathology and translational medicine 51, 205-223.

Pegg, A.E., Dolan, M.E., and Moschel, R.C. (1995). Structure, function, and inhibition of O6-alkylguanine-DNA alkyltransferase. Progress in nucleic acid research and molecular biology 51, 167-223.

Perou, C.M., Sorlie, T., Eisen, M.B., van de Rijn, M., Jeffrey, S.S., Rees, C.A., Pollack, J.R., Ross, D.T., Johnsen, H., Akslen, L.A., et al. (2000). Molecular portraits of human breast tumours. Nature 406, 747-752.

Peters, K.G., Escobedo, J.A., Fantl, W.J., and Williams, L.T. (1992). Interactions of PDGF and FGF receptors with cytoplasmic signaling molecules. Cold Spring Harbor symposia on quantitative biology 57, 63-66.

Phatnani, H., and Maniatis, T. (2015). Astrocytes in neurodegenerative disease. Cold Spring Harbor perspectives in biology 7.

Pietras, K., Sjoblom, T., Rubin, K., Heldin, C.H., and Ostman, A. (2003). PDGF receptors as cancer drug targets. Cancer cell 3, 439-443.

Powell, A.E., Wang, Y., Li, Y., Poulin, E.J., Means, A.L., Washington, M.K., Higginbotham, J.N., Juchheim, A., Prasad, N., Levy, S.E., et al. (2012). The pan-ErbB negative regulator Lrig1 is an intestinal stem cell marker that functions as a tumor suppressor. Cell 149, 146-158.

Primo-Parmo, S.L., Sorenson, R.C., Teiber, J., and La Du, B.N. (1996). The human serum paraoxonase/arylesterase gene (PON1) is one member of a multigene family. Genomics 33, 498-507.

Punchera, J., Barnes, L., and Kaya, G. (2016). Lrig1 Expression in Human Sebaceous Gland Tumors. Dermatopathology 3, 44-54.

Qu, Y., Wang, J., Ray, P.S., Guo, H., Huang, J., Shin-Sim, M., Bukoye, B.A., Liu, B., Lee, A.V., Lin, X., et al. (2011). Thioredoxin-like 2 regulates human cancer cell growth and metastasis via redox homeostasis and NF-kappaB signaling. The Journal of clinical investigation 121, 212-225.

Rafidi, H., Mercado, F., 3rd, Astudillo, M., Fry, W.H., Saldana, M., Carraway, K.L., 3rd, and Sweeney, C. (2013). Leucine-rich repeat and immunoglobulin domain-containing protein-1 (Lrig1) negative regulatory action toward ErbB receptor tyrosine kinases is opposed by leucine-rich repeat and immunoglobulin domain-containing protein 3 (Lrig3). The Journal of biological chemistry 288, 21593-21605.

Ramos-Esquivel, A., Hernandez-Steller, H., Savard, M.F., and Landaverde, D.U. (2018). Cyclin-dependent kinase 4/6 inhibitors as first-line treatment for post-menopausal metastatic hormone receptor-positive breast cancer patients: a systematic review and meta-analysis of phase III randomized clinical trials. Breast cancer.

Ranhem, C., Lillsunde Larsson, G., Hedman, H., Lindquist, D., Karlsson, M.G., Hellstrom, A.C., Ostensson, E., Sorbe, B., Hellman, K., and Andersson, S. (2017). Expression of LRIG proteins as possible prognostic factors in primary vaginal carcinoma. PloS one 12, e0183816.

Raskin, L., Guo, Y., Du, L., Clendenning, M., Rosty, C., Colon Cancer Family, R., Lindor, N.M., Gruber, S.B., and Buchanan, D.D. (2017). Targeted sequencing of established and candidate colorectal cancer genes in the Colon Cancer Family Registry Cohort. Oncotarget 8, 93450-93463.

Reifenberger, G., and Louis, D.N. (2003). Oligodendroglioma: toward molecular definitions in diagnostic neuro-oncology. Journal of neuropathology and experimental neurology 62, 111-126.

Rick, J., Chandra, A., and Aghi, M.K. (2018). Tumor treating fields: a new approach to glioblastoma therapy. Journal of neuro-oncology.

Robinson, D.R., Wu, Y.M., and Lin, S.F. (2000). The protein tyrosine kinase family of the human genome. Oncogene 19, 5548-5557.

Rodriguez-Perales, S., Melendez, B., Gribble, S.M., Valle, L., Carter, N.P., Santamaria, I., Conde, L., Urioste, M., Benitez, J., and Cigudosa, J.C. (2004). Cloning of a new familial t(3;8) translocation associated with conventional renal cell carcinoma reveals a 5 kb microdeletion and no gene involved in the rearrangement. Human molecular genetics 13, 983-990.

Rondahl, V., Holmlund, C., Karlsson, T., Wang, B., Faraz, M., Henriksson, R., and Hedman, H. (2013). Lrig2-deficient mice are protected against PDGFB-induced glioma. PloS one 8, e73635.

Ronnov-Jessen, L., Petersen, O.W., and Bissell, M.J. (1996). Cellular changes involved in conversion of normal to malignant breast: importance of the stromal reaction. Physiological reviews 76, 69-125.

Rosenthal, A.S., and Shevach, E.M. (1973). Function of macrophages in antigen recognition by guinea pig T lymphocytes. I. Requirement for histocompatible macrophages and lymphocytes. The Journal of experimental medicine 138, 1194-1212.

Ross, R., Glomset, J., Kariya, B., and Harker, L. (1974). A platelet-dependent serum factor that stimulates the proliferation of arterial smooth muscle cells in vitro. Proceedings of the National Academy of Sciences of the United States of America 71, 1207-1210.

Rothem, L., Hartman, C., Dahan, A., Lachter, J., Eliakim, R., and Shamir, R. (2007). Paraoxonases are associated with intestinal inflammatory diseases and intracellularly localized to the endoplasmic reticulum. Free radical biology & medicine 43, 730-739.

Sangwan, V., Paliouras, G.N., Abella, J.V., Dube, N., Monast, A., Tremblay, M.L., and Park, M. (2008). Regulation of the Met receptor-tyrosine kinase by the protein-tyrosine phosphatase 1B and T-cell phosphatase. The Journal of biological chemistry 283, 34374-34383.

Sap, J., Jiang, Y.P., Friedlander, D., Grumet, M., and Schlessinger, J. (1994). Receptor tyrosine phosphatase R-PTP-kappa mediates homophilic binding. Molecular and cellular biology 14, 1-9.

Sasaki, H., Zlatescu, M.C., Betensky, R.A., Ino, Y., Cairncross, J.G., and Louis, D.N. (2001). PTEN is a target of chromosome 10q loss in anaplastic oligodendrogliomas and PTEN alterations are associated with poor prognosis. The American journal of pathology 159, 359-367.

Sawyers, C.L. (2008). The cancer biomarker problem. Nature 452, 548-552.

Schumacher, F.R., Schmit, S.L., Jiao, S., Edlund, C.K., Wang, H., Zhang, B., Hsu, L., Huang, S.C., Fischer, C.P., Harju, J.F., et al. (2015). Genome-wide association study of colorectal cancer identifies six new susceptibility loci. Nature communications 6, 7138.

Seko, A., Hara-Kuge, S., and Yamashita, K. (2001). Molecular cloning and characterization of a novel human galactose 3-O-sulfotransferase that transfers sulfate to gal beta 1-->3galNAc residue in O-glycans. The Journal of biological chemistry 276, 25697-25704.

Shattuck, D.L., Miller, J.K., Laederich, M., Funes, M., Petersen, H., Carraway, K.L., 3rd, and Sweeney, C. (2007). LRIG1 is a novel negative regulator of the Met receptor and opposes Met and Her2 synergy. Molecular and cellular biology 27, 1934-1946.

Sheff, D.R., Daro, E.A., Hull, M., and Mellman, I. (1999). The receptor recycling pathway contains two distinct populations of early endosomes with different sorting functions. The Journal of cell biology 145, 123-139.

Shen, H., Zhan, M., Zhang, Y., Huang, S., Xu, S., Huang, X., He, M., Yao, Y., Man, M., and Wang, J. (2018). PLZF inhibits proliferation and metastasis of gallbladder cancer by regulating IFIT2. Cell death & disease 9, 71.

Sheu, J.J., Lee, C.C., Hua, C.H., Li, C.I., Lai, M.T., Lee, S.C., Cheng, J., Chen, C.M., Chan, C., Chao, S.C., et al. (2014). LRIG1 modulates aggressiveness of head and neck cancers by regulating EGFR-MAPK-SPHK1 signaling and extracellular matrix remodeling. Oncogene 33, 1375-1384.

Sheu, J.J., Lee, C.H., Ko, J.Y., Tsao, G.S., Wu, C.C., Fang, C.Y., Tsai, F.J., Hua, C.H., Chen, C.L., and Chen, J.Y. (2009). Chromosome 3p12.3-p14.2 and 3q26.2-q26.32 are genomic markers for prognosis of advanced nasopharyngeal carcinoma. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 18, 2709-2716.

Shih, A.H., Dai, C., Hu, X., Rosenblum, M.K., Koutcher, J.A., and Holland, E.C. (2004). Dose-dependent effects of platelet-derived growth factor-B on glial tumorigenesis. Cancer research 64, 4783-4789.

Shtiegman, K., and Yarden, Y. (2003). The role of ubiquitylation in signaling by growth factors: implications to cancer. Seminars in cancer biology 13, 29-40.

Siegel, P.M., Ryan, E.D., Cardiff, R.D., and Muller, W.J. (1999). Elevated expression of activated forms of Neu/ErbB-2 and ErbB-3 are involved in the induction of mammary tumors in transgenic mice: implications for human breast cancer. The EMBO journal 18, 2149-2164.

Siegel, R.L., Miller, K.D., and Jemal, A. (2017). Cancer Statistics, 2017. CA: a cancer journal for clinicians 67, 7-30.

Sledge, G.W., Mamounas, E.P., Hortobagyi, G.N., Burstein, H.J., Goodwin, P.J., and Wolff, A.C. (2014). Past, present, and future challenges in breast cancer treatment. Journal of clinical oncology: official journal of the American Society of Clinical Oncology 32, 1979-1986.

Sordella, R., Bell, D.W., Haber, D.A., and Settleman, J. (2004). Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. Science 305, 1163-1167.

Stella, M.C., Trusolino, L., Pennacchietti, S., and Comoglio, P.M. (2005). Negative feedback regulation of Met-dependent invasive growth by Notch. Molecular and cellular biology 25, 3982-3996.

Stutz, M.A., Shattuck, D.L., Laederich, M.B., Carraway, K.L., 3rd, and Sweeney, C. (2008). LRIG1 negatively regulates the oncogenic EGF receptor mutant EGFRvIII. Oncogene 27, 5741-5752.

Suh, D.S., Park, S.E., Jin, H., Lee, K., and Bae, J. (2018). LRIG2 is a growth suppressor of Hec-1A and Ishikawa endometrial adenocarcinoma cells by regulating PI3K/AKT- and EGFR-mediated apoptosis and cell-cycle. Oncogenesis 7, 3.

Sun, P.H., Ye, L., Mason, M.D., and Jiang, W.G. (2013). Protein tyrosine phosphatase kappa (PTPRK) is a negative regulator of adhesion and invasion of breast cancer cells, and associates with poor prognosis of breast cancer. Journal of cancer research and clinical oncology 139, 1129-1139.

Suzuki, Y., Miura, H., Tanemura, A., Kobayashi, K., Kondoh, G., Sano, S., Ozawa, K., Inui, S., Nakata, A., Takagi, T., et al. (2002). Targeted disruption of LIG-1 gene results in psoriasiform epidermal hyperplasia. FEBS letters 521, 67-71.

Swedish Cancer Registry,
http://www.socialstyrelsen.se/register/halsodataregister/cancerregistret/.

Tallquist, M., and Kazlauskas, A. (2004). PDGF signaling in cells and mice. Cytokine & growth factor reviews 15, 205-213.

Tanemura, A., Nagasawa, T., Inui, S., and Itami, S. (2005). LRIG-1 provides a novel prognostic predictor in squamous cell carcinoma of the skin: immunohistochemical analysis for 38 cases. Dermatologic surgery: official publication for American Society for Dermatologic Surgery [et al] 31, 423-430.

Tang, C.K., Gong, X.Q., Moscatello, D.K., Wong, A.J., and Lippman, M.E. (2000). Epidermal growth factor receptor vIII enhances tumorigenicity in human breast cancer. Cancer research 60, 3081-3087.

Thomasson, M., Wang, B., Hammarsten, P., Dahlman, A., Persson, J.L., Josefsson, A., Stattin, P., Granfors, T., Egevad, L., Henriksson, R., et al. (2011). LRIG1 and the liar paradox in prostate cancer: a study of the expression and clinical significance of LRIG1 in prostate cancer. International journal of cancer 128, 2843-2852.

Thompson, P.A., Ljuslinder, I., Tsavachidis, S., Brewster, A., Sahin, A., Hedman, H., Henriksson, R., Bondy, M.L., and Melin, B.S. (2014). Loss of LRIG1 locus increases risk of early and late relapse of stage I/II breast cancer. Cancer research 74, 2928-2935.

Toriseva, M., Ala-aho, R., Peltonen, S., Peltonen, J., Grenman, R., and Kahari, V.M. (2012). Keratinocyte growth factor induces gene expression signature associated with suppression of malignant phenotype of cutaneous squamous carcinoma cells. PloS one 7, e33041.

Trouillard, O., Aguirre-Cruz, L., Hoang-Xuan, K., Marie, Y., Delattre, J.Y., and Sanson, M. (2004). Parental 19q loss and PEG3 expression in oligodendrogliomas. Cancer genetics and cytogenetics 151, 182-183.

Trusolino, L., Bertotti, A., and Comoglio, P.M. (2010). MET signalling: principles and functions in development, organ regeneration and cancer. Nature reviews Molecular cell biology 11, 834-848.

Turk, A.A., and Wisinski, K.B. (2018). PARP inhibitors in breast cancer: Bringing synthetic lethality to the bedside. Cancer.

Ueda, K., Miyoshi, Y., Tokino, T., Watatani, M., and Nakamura, Y. (1999). Induction of apoptosis in T98G glioblastoma cells by transfection of GML, a p53 target gene. Oncology research 11, 125-132.

Uhrbom, L., Hesselager, G., Nister, M., and Westermark, B. (1998). Induction of brain tumors in mice using a recombinant platelet-derived growth factor B-chain retrovirus. Cancer research 58, 5275-5279.

Valgeirsdottir, S., Eriksson, A., Nister, M., Heldin, C.H., Westermark, B., and Claesson-Welsh, L. (1995). Compartmentalization of autocrine signal transduction pathways in Sis-transformed NIH 3T3 cells. The Journal of biological chemistry 270, 10161-10170.

Van den Bent, M.J., Dubbink, H.J., Marie, Y., Brandes, A.A., Taphoorn, M.J., Wesseling, P., Frenay, M., Tijssen, C.C., Lacombe, D., Idbaih, A., et al. (2010). IDH1 and IDH2 mutations are prognostic but not predictive for outcome in anaplastic oligodendroglial tumors: a report of the European Organization for Research and Treatment of Cancer Brain Tumor Group. Clinical cancer research: an official journal of the American Association for Cancer Research 16, 1597-1604.

Van den Bent, M.J., Reni, M., Gatta, G., and Vecht, C. (2008). Oligodendroglioma. Critical reviews in oncology/hematology 66, 262-272.

Verhaak, R.G., Hoadley, K.A., Purdom, E., Wang, V., Qi, Y., Wilkerson, M.D., Miller, C.R., Ding, L., Golub, T., Mesirov, J.P., et al. (2010). Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer cell 17, 98-110.

Vijapura, C., Saad Aldin, E., Capizzano, A.A., Policeni, B., Sato, Y., and Moritani, T. (2017). Genetic Syndromes Associated with Central Nervous System Tumors. Radiographics: a review publication of the Radiological Society of North America, Inc 37, 258-280.

Vinagre, J., Pinto, V., Celestino, R., Reis, M., Populo, H., Boaventura, P., Melo, M., Catarino, T., Lima, J., Lopes, J.M., et al. (2014). Telomerase promoter mutations in cancer: an emerging molecular biomarker? Virchows Archiv: an international journal of pathology 465, 119-133.

Wang, B., Han, L., Chen, R., Cai, M., Han, F., Lei, T., and Guo, D. (2009). Downregulation of LRIG2 expression by RNA interference inhibits glioblastoma cell (GL15) growth, causes cell cycle

redistribution, increases cell apoptosis and enhances cell adhesion and invasion in vitro. Cancer biology & therapy 8, 1018-1023.

Wang, G., Wu, J., and Song, H. (2014). LRIG2 expression and prognosis in non-small cell lung cancer. Oncology letters 8, 667-672.

Wang, X., He, X.J., Xu, H.Q., Chen, Z.W., and Fan, H.H. (2016). Inhibition of subcutaneously implanted human pituitary tumor cells in nude mice by LRIG1. Genetics and molecular research: Genetics and molecular research 15 (2).

Wang, Y., Pennock, S.D., Chen, X., Kazlauskas, A., and Wang, Z. (2004). Platelet-derived growth factor receptor-mediated signal transduction from endosomes. The Journal of biological chemistry 279, 8038-8046.

Weller, M., van den Bent, M., Tonn, J.C., Stupp, R., Preusser, M., Cohen-Jonathan-Moyal, E., Henriksson, R., Le Rhun, E., Balana, C., Chinot, O., et al. (2017). European Association for Neuro-Oncology (EANO) guideline on the diagnosis and treatment of adult astrocytic and oligodendroglial gliomas. The Lancet Oncology 18, e315-e329.

Westermark, B., and Wasteson, A. (1976). A platelet factor stimulating human normal glial cells. Experimental cell research 98, 170-174.

Williams, A.F., and Barclay, A.N. (1988). The immunoglobulin superfamily--domains for cell surface recognition. Annual review of immunology 6, 381-405.

Willis, S., Villalobos, V.M., Gevaert, O., Abramovitz, M., Williams, C., Sikic, B.I., and Leyland-Jones, B. (2016). Single Gene Prognostic Biomarkers in Ovarian Cancer: A Meta-Analysis. PloS one 11, e0149183.

Witte, S., Villalba, M., Bi, K., Liu, Y., Isakov, N., and Altman, A. (2000). Inhibition of the c-Jun N-terminal kinase/AP-1 and NF-kappaB pathways by PICOT, a novel protein kinase C-interacting protein with a thioredoxin homology domain. The Journal of biological chemistry 275, 1902-1909.

Wolf, S.A., Boddeke, H.W., and Kettenmann, H. (2017). Microglia in Physiology and Disease. Annual review of physiology 79, 619-643.

Wong, V.W., Stange, D.E., Page, M.E., Buczacki, S., Wabik, A., Itami, S., van de Wetering, M., Poulsom, R., Wright, N.A., Trotter, M.W., et al. (2012). Lrig1 controls intestinal stem-cell homeostasis by negative regulation of ErbB signalling. Nature cell biology 14, 401-408.

Wu, E., Palmer, N., Tian, Z., Moseman, A.P., Galdzicki, M., Wang, X., Berger, B., Zhang, H., and Kohane, I.S. (2008). Comprehensive dissection of PDGF-PDGFR signaling pathways in PDGFR genetically defined cells. PloS one 3, e3794.

Xiao, Q., Tan, Y., Guo, Y., Yang, H., Mao, F., Xie, R., Wang, B., Lei, T., and Guo, D. (2014). Soluble LRIG2 ectodomain is released from glioblastoma cells and promotes the proliferation and inhibits the apoptosis of glioblastoma cells in vitro and in vivo in a similar manner to the full-length LRIG2. PloS one 9, e111419.

Xu, L., Wei, Y., Reboul, J., Vaglio, P., Shin, T.H., Vidal, M., Elledge, S.J., and Harper, J.W. (2003). BTB proteins are substrate-specific adaptors in an SCF-like modular ubiquitin ligase containing CUL-3. Nature 425, 316-321.

Xu, Y., Soo, P., Walker, F., Zhang, H.H., Redpath, N., Tan, C.W., Nicola, N.A., Adams, T.E., Garrett, T.P., Zhang, J.G., et al. (2015). LRIG1 extracellular domain: structure and function analysis. Journal of molecular biology 427, 1934-1948.

Xu, Y., Tan, L.J., Grachtchouk, V., Voorhees, J.J., and Fisher, G.J. (2005). Receptor-type protein-tyrosine phosphatase-kappa regulates epidermal growth factor receptor function. The Journal of biological chemistry 280, 42694-42700.

Yan, H., Parsons, D.W., Jin, G., McLendon, R., Rasheed, B.A., Yuan, W., Kos, I., Batinic-Haberle, I., Jones, S., Riggins, G.J., et al. (2009). IDH1 and IDH2 mutations in gliomas. The New England journal of medicine 360, 765-773.

Yang, B., Dai, C., Tan, R., Zhang, B., Meng, X., Ye, J., Wang, X., Wei, L., He, F., and Chen, Z. (2016). Lrig1 is a positive prognostic marker in hepatocellular carcinoma. OncoTargets and therapy 9, 7071-7079.

Yang, H.K., Chen, H., Mao, F., Xiao, Q.G., Xie, R.F., and Lei, T. (2017). Downregulation of LRIG2 expression inhibits angiogenesis of glioma via EGFR/VEGF-A pathway. Oncology letters 14, 4021-4028.

Yarden, Y., and Sliwkowski, M.X. (2001). Untangling the ErbB signalling network. Nature reviews Molecular cell biology 2, 127-137.

Yarden, Y., and Ullrich, A. (1988). Growth factor receptor tyrosine kinases. Annual review of biochemistry 57, 443-478.

Ye, F., Gao, Q., Xu, T., Zeng, L., Ou, Y., Mao, F., Wang, H., He, Y., Wang, B., Yang, Z., et al. (2009). Upregulation of LRIG1 suppresses malignant glioma cell growth by attenuating EGFR activity. Journal of neuro-oncology 94, 183-194.

Yeyati, P.L., Shaknovich, R., Boterashvili, S., Li, J., Ball, H.J., Waxman, S., Nason-Burchenal, K., Dmitrovsky, E., Zelent, A., and Licht, J.D. (1999). Leukemia translocation protein PLZF inhibits cell growth and expression of cyclin A. Oncogene 18, 925-934.

Yi, W., Holmlund, C., Nilsson, J., Inui, S., Lei, T., Itami, S., Henriksson, R., and Hedman, H. (2011). Paracrine regulation of growth factor signaling by shed leucine-rich repeats and immunoglobulin-like domains 1. Experimental cell research 317, 504-512.

Yokdang, N., Hatakeyama, J., Wald, J.H., Simion, C., Tellez, J.D., Chang, D.Z., Swamynathan, M.M., Chen, M., Murphy, W.J., Carraway Iii, K.L., et al. (2015). LRIG1 opposes epithelial-to-mesenchymal transition and inhibits invasion of basal-like breast cancer cells. Oncogene 35, 2932-2947.

Zhang, J., Chintalgattu, V., Shih, T., Ai, D., Xia, Y., and Khakoo, A.Y. (2011). MicroRNA-9 is an activation-induced regulator of PDGFR-beta expression in cardiomyocytes. Journal of molecular and cellular cardiology 51, 337-346.

Zhou, X., Vink, M., Klaver, B., Berkhout, B., and Das, A.T. (2006). Optimization of the Tet-On system for regulated gene expression through viral evolution. Gene therapy 13, 1382-1390.

Zinn, K., and Ozkan, E. (2017). Neural immunoglobulin superfamily interaction networks. Current opinion in neurobiology 45, 99-105.