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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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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.

Keywords
LRIG1, LRIG2, PDGFR, glioma, interactome, breast cancer, prognosis

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