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LRIG1 in lung cancer

Prognostic implications and mechanistic studies

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Akademisk avhandling

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Abstract
Lung cancer is the leading cause of cancer-related death worldwide as well as in Sweden. Non-small cell lung cancer (NSCLC) is the predominant form, which is largely subdivided into adenocarcinomas and squamous cell carcinomas. A small minority of NSCLC cases that present with localized small tumors are curable with surgery alone, but adjuvant chemotherapy is recommended in most cases that are treated with surgery, even though it only increases the chance of cure by less than 5%. Therefore, additional biomarkers are needed to guide the clinical decision making in early-stage disease.

The leucine-rich repeats and immunoglobulin-like (LRIG) family of proteins consists of three paralogous transmembrane proteins that are involved in the regulation of growth factor signaling. Of these proteins, LRIG1 is the most studied and it interacts with a wide variety of growth factor receptors and related proteins, including the epidermal growth factor (EGFR) and several others. High levels of LRIG1 have been associated with better survival in a multitude of malignant diseases, including (but not limited to) breast cancer, bladder cancer, cervical cancer, glioma and melanoma. The aim of this thesis was to investigate whether LRIG1 was a prognostic factor in NSCLC as well, and to further characterize the biological role of LRIG proteins in this disease.

To investigate the prognostic impact of LRIG proteins in NSCLC, we stained a tissue microarray (TMA) containing tumor samples from 347 surgically treated early-stage NSCLC patients for LRIG1, LRIG2 and LRIG3. LRIG1 high-expressing adenocarcinoma cases had a large and statistically significant survival benefit of 33 months compared to negative cases. Similarly, an *in silico* analysis of a large gene expression dataset from the Oncomine database showed that high *LRIG1* mRNA expression was linked to better survival as well. Differences in survival persisted even when adjusting for known prognostic factors, meaning that LRIG1 was an independent positive prognostic marker for survival in NSCLC.

A yeast two-hybrid screen was performed to search for proteins interacting with a conserved cytosolic peptide shared between all three mammalian LRIG proteins. This screen yielded hits for the paralogous proteins LIM domain only protein 7 (LMO7) and LIM and calponin homology domains-containing protein 1 (LIMCH1). LRIG1 and LRIG3 were both found to physically interact with LMO7 and LIMCH1 as assessed through immunoprecipitation techniques on overexpressed proteins. For LMO7, this could also be confirmed at endogenous protein levels using the proximity ligation assay. The 347 samples in the previously mentioned TMA were stained for LMO7. In our survival analysis, we observed no significant survival differences when looking at LMO7 alone, but the survival benefit observed for high LRIG1 was found to be limited to the subgroup that also was negative for LMO7. This means that the observed physical interaction between LRIG1 and LMO7 appears to translate to differences in patient survival.

To investigate possible mechanisms underlying the observed association between high LRIG1 expression and a favorable patient survival, a panel of NSCLC cell lines was modified to overexpress LRIG1. Broadly, LRIG1 overexpression resulted in minor decreases in cellular proliferation rates and no effects on chemosensitivity or radiosensitivity. Looking across the panel of NSCLC cell lines, no clear pattern was observed regarding the effects of LRIG1 overexpression on cellular motility. However, LRIG1 overexpression significantly decreased the clonogenic potential in most cell lines. The only cell line in the panel, H1299, that formed hematogenous disseminated disease in immunodeficient mice was used to establish a mixed-population primary tumor of tagged LRIG1 overexpressing cells and control cells. The LRIG1 transduced cells were was found to be enriched in the injected primary tumor, but no significant changes in their relative abundance was observed between the metastatic sites and their corresponding primary tumors.

In summary, we found that LRIG1 was an independent positive prognostic factor in early-stage NSCLC. We identified LMO7 and LIMCH1 as interaction partners for LRIG proteins and showed that the interaction between LMO7 and LRIG1 had implications for the clinical outcome in NSCLC. Furthermore, our mechanistic studies on the effects of LRIG1 overexpression on NSCLC cells suggested that the survival benefit conferred by high LRIG1 expression may be due to differences in metastatic potential. Taken together, the findings in this thesis suggest an important biological role for LRIG proteins in NSCLC.

Keywords
Lung cancer, signaling, LRIG1, prognosis, LRIG2, LRIG3, LMO7, LIMCH1

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