Cell signaling pathway crosstalk in fine-tuning cell fate: an in vitro and in silico case study on the Reelin-NOTCH interactions during lymphangiogenesis

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Summary

Cancer is one of the leading causes of mortality worldwide. Its progression highly depends on interactions between cancer cells and other biological entities and systems, particularly the vascular and lymphatic systems. Traditionally, the latter was considered a passive drainage system, but recent studies have uncovered its active role in tumor development and metastasis, and it depends on interaction between and with tumor cells. Cancer cells exploit this system by stimulating lymphatic endothelial cells (LECs) within preexisting lymphatic vessels to form new vessels, initiating a sprouting process known as lymphangiogenesis.

NOTCH, a transmembrane receptor expressed in a wide range of cell types including endothelial cells, plays a pivotal role in regulating cell fate decisions, particularly by orchestrating vascular patterning. Depending on the cellular context and interactions with other signaling pathways, NOTCH can either promote or inhibit lymphatic sprouting. In the nervous system, NOTCH signaling has been shown to interact with Reelin (RELN), a secreted glycoprotein, which enhances NOTCH activity.

To date, the crosstalk between NOTCH and Reelin in a lymphatic system has not been documented. This master's thesis addresses this gap by first characterizing the NOTCH-Reelin interaction within a broader biological framework through literature review and database curation, and subsequently refining it to a lymphatic-specific context using both literature and RNA-sequencing data. In parallel, a comprehensive, knowledge-based of the NOTCH-Reelin interaction map was constructed in *CellDesigner* and made available for interactive exploration on the *MINERVA* platform.

Then, to enable dynamic analysis of the NOTCH–Reelin interplay, a Boolean model was derived from the interaction map using the *CaSQ* conversion tool. This model was simulated with *pyMaBoSS* under three distinct scenarios: a non-parameterized model, an arbitrarily parameterized model, and a semi-quantitatively parameterized model. The latter incorporates biological insights specific to LECs, derived from publicly available transcriptomics datasets, under the assumption that external components to the cells, such as ligands, ubiquitin ligases, and so on, are non-limiting and may vary across biological contexts.

The model simulations ultimately suggest that Reelin enhances the activity of the NOTCH intracellular domain (NICD), the cytoplasmic effector of NOTCH signaling, by downregulating its proteasomal degradation. Additionally, Reelin signaling provides an alternative mechanism for NICD nuclear import, facilitating its translocation into the nucleus. While Reelin is not

essential for the activation of NOTCH signaling, its absence results in a reduction of pathway activity rather than complete inhibition. The model also indicates that the system is more sensitive to perturbations in γ-secretase activity than to ADAM proteases, two proteolytic complex and protein required for NOTCH cleavage. This is evidenced by *in silico* drug stimulation using Semagacestat and DAPT, which both target γ-secretase, compared to BB94, which targets ADAMs. This heightened sensitivity persists even though multiple human γ-secretase variants (CPX-2176, CPX-4231, CPX-4232, and CPX-4233) are capable of substituting for one another, as they still rely on the coordinated presence of multiple subunits to form an active complex.

To experimentally assess the influence of the *in silico*-selected compounds (BB94, DAPT, Semagacestat, PP2, and lauric acid) on the NOTCH/Reelin axis, human primary LECs were first treated with each drug, followed by wound healing assays to assess migration. Although the *in vitro* results showed variability across conditions, one consistent observation was the downregulation of *HES1*, a canonical NOTCH target gene, in almost all treatments. Additionally, *HEY2* exhibited pronounced overexpression across almost all treatments compared to *HEY1*, suggesting potential compensatory mechanisms or pathway divergence in response to pathway inhibition. Decreased migration was unaffected by drug treatments except for the one targeting γ-secretase and ADAMs, where, for the latter, a tip cell phenotype was observed.

This work therefore presents a dual *in vitro–in silico* approach to investigate the crosstalk between Reelin and NOTCH in a lymphatic-specific context, and underscores the relevance of such integrative strategies, still too often underestimated, for uncovering novel insights into complex biological processes. Furthermore, developing tools and pipelines to reinterpret existing biological data in a targeted context represents an essential step toward translating established knowledge into new biological systems.

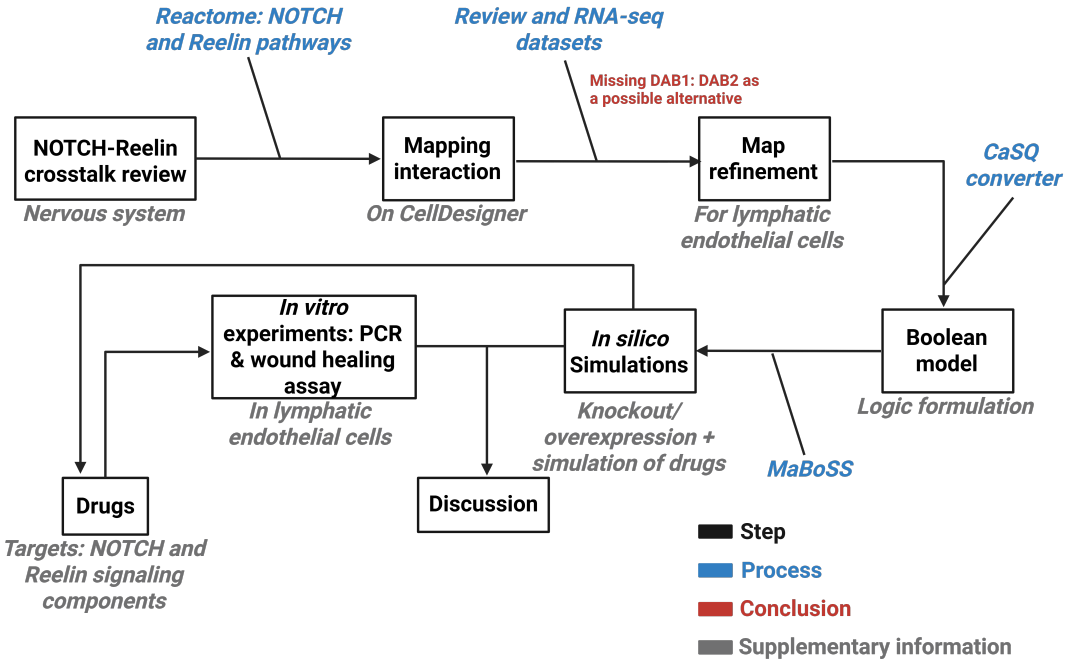

Figure 1: **Workflow of the Master Thesis.**