



Toxicity of Notch 3 extracellular domain (N3ECD) on cellular function

Project description for master thesis (30-45 credits)

Background: Mutations in *Notch 3* gene are the cause of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), a hereditary angiopathy leading to strokes and dementia. N3ECD is cleaved after several proteolytic processing steps of the Notch 3 receptor and can appear as granular osmophilic material (GOM) which in conjunction with degeneration of vascular smooth muscle cells (VSMCs) showing the key features in CADASIL pathogenesis. Recent data suggest that mutated N3ECD aggregates and forms hetero-, homo-dimers and oligomers, which might be toxic and thereby have a role in CADASIL pathogenesis. A key question is: Do aggregation of N3ECD trigger apoptosis?

Aim: In this project we are interested in investigating the effect of N3EDC on cell viability and mitochondrial function in VSMCs. Furthermore, we want to study whether N3ECD will be internalized by the cells and degraded.

Materials and Methods:

To examine this, we will transfect either human HEK293 cell line or human T/G HA-VSMC with wt or mutant N3ECD conjugated with GFP. The transfection of the cells will be identified by confocal microscopy. Cell viability will be measured with alamar blue assay. Live-cell imaging will be performed to visualize MitoTracker labelled mitochondria (a specific probe to visualize mitochondria), or other organelles to see the internalization and co-localization of N3EDC in cells. Mitochondrial membrane potential TMRM labeled (a marker for measurement of mitochondrial membrane potential) cells and ROS production will be analyzed by flow cytometry.

- The student will get the practical supervision from the main supervisor and the student will get help from other people in the laboratory.
- It is not necessary to perform all suggested experiments (above), and suitable experiments will be selected based on the student's previous expertise.

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