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## Creating a unique cell model for pheochromocytoma

### Clinical relevance

Patients with pheochromocytoma have tumors that originated from chromaffin cells in the adrenal gland. Chromaffin cells are neuro-endocrine cells that produce epinephrine (adrenaline) and norepinephrine (noradrenalin). Therefore, besides having cancer, patients suffer from periods of extreme and life-threatening hypertension. Every year, between 100 and 150 new pheochromocytoma cases are reported in The Netherlands. Unfortunately, there currently is no effective treatment for patients with pheochromocytoma other than removal of a tumor when operable.

### Background

The strongest indicator of malignancy is a mutation in succinate dehydrogenase B (SDHB). The SDHB protein is part of a four subunit (A-D) protein complex, the succinate dehydrogenase (SDH) complex. The SDH complex functions as part of the TCA cycle, converting succinate to fumarate. When this complex is dysfunctional there is a build-up of succinate in the cell. Succinate stabilizes hypoxia-inducible transcription factors (HIFs). HIF-targets are subsequently transcribed and in turn boost proliferation and angiogenesis, two essential processes for tumor formation.

Insight in the mechanisms causing pheochromocytoma is urgently needed to develop therapeutics to cure this disease. Gaining this insight is difficult to achieve, because there are no proper cell models for pheochromocytoma. Therefore, we are working on the generation of a cell line to study the underlying mechanisms of pheochromocytoma by modifying tsAM5NE cells, a mouse nor-adrenergic chromaffin adrenal cell line. These cells divide at the permissive temperature of 33°C, but differentiate to adrenal cells when grown at the non-permissive temperature of 39°C.

We will first optimize the culture conditions of these cells and then generate SDHB mutant tsAM5NE cells with CRISPR/Cas9. To visualize HIF activation, permissive tsAM5NE cells will be stably-transfected with a HIF-responsive-element-GFP reporter construct. The best clones with homozygous SDHB deletions will subsequently be characterized at different levels: using untargeted metabolomics and proteomics analysis, concentrations of energy pathway metabolites and protein expression patterns will be obtained, which will be compared with already established expression databases for human SDHB-PPGLs. Expression of selected genes will be confirmed at mRNA (QRT-PCR) or protein (immunoblotting) levels. Cell lines showing the closest similarities to human pheochromocytoma data will be selected for metabolic flux studies. In these studies, flux rates and rate constants of energy pathway reactions using stable isotope precursors of pyruvate, lactate, glucose and glutamine will be determined and will give insight which (parts of) metabolic pathways may be therapeutic targets for treatment of pheochromocytoma. To test whether our obtained SDHB-tsAM5NE cells recapitulate human pheochromocytoma, the best two tsAM5NE SDHB mutant clones will be injected in mice. During 2 months, 3D tumor growth and metastasis and angiogenesis will be non-invasively monitored. Blood pressure, blood and urine output of catecholamines will be determined.

### Goals

We want to generate a cell model for pheochromocytoma and examine its phenotype in detail. After careful characterization, we will inject these cells in mice to study tumour growth and metastasis.

### We offer:

The possibility to perform and present exciting high-quality research in a professional, multi-cultural and highly-motivating working environment with about 35 colleagues in a well-equipped department. You will have the opportunity to learn a broad range of techniques and skills, such as cell culture, RT-qPCR, immunoblotting, RNA isolation, planning, scientific writing and presenting. This will all take place under the supervision of an excellent PhD student.

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