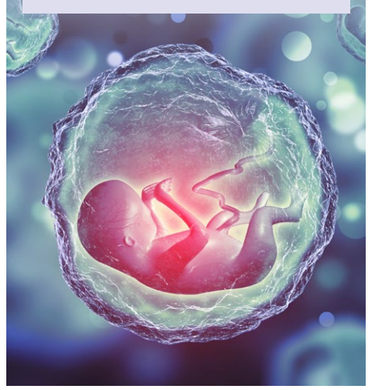
HIF-2α is implicated in embryogenesis and neuroblastoma

Dr Sofie Mohlin and her research group at Lund University in Sweden study the function of a protein, HIF-2α, in foetal development and cancer, mainly childhood cancer form neuroblastoma. Until recently, little was known about the function of HIF-2α in embryogenesis. However, the group's latest research found that HIF-2α is an important protein for normal neural crest development, as dysregulated levels severely delay embryogenesis. The cells also show characteristics of tumour cells, as they have enhanced self-renewal capacity and are more proliferative. These findings can hopefully elucidate the connection between changes in normal embryogenesis and tumorigenesis by HIF-2α.



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Hypoxia-inducible factors (HIFs) are transcription factors or proteins that allow cells to respond and adapt to hypoxia, which is a low-oxygen cellular environment. The discovery of the HIF system was awarded the Nobel Prize in Physiology or Medicine in 2019, and the importance of HIFs and other oxygen-sensing systems across eukaryotic kingdoms for evolution, development and tumorigenesis was recently extensively reviewed by Dr Mohlin and colleagues (Hammarlund *et al.*, Science 2020). When activated, there is an induction of HIF transcriptional activity for tissue protection, such as adapting the hypoxic tissue to oxygen deprivation and anaerobic ATP synthesis (cell respiration in the absence of oxygen). One of the transcriptional factors is HIF-2α, which is encoded by the EPAS1 gene. HIF-2α induces, amongst many other genes, vascular endothelial growth factor (VEGF) and erythropoietin (EPO), which stimulates the formation of blood vessels and the production of red blood cells, respectively, to improve oxygen supply to hypoxic cells.

It has been known that HIF-2α plays an important role in tumour progression and metastasis. Cancer is caused by ten rate-limiting traits (the hallmarks of cancer), as described by Hanahan and Weinberg (Cell, 2011), which include the induction of new blood vessel formation. This vascularisation is especially important for cancerous cells to grow and invade surrounding healthy tissues. Transcription factors, such as VEGF, are switched on in the cancerous cells to ensure they obtain enough oxygen and nutrients.

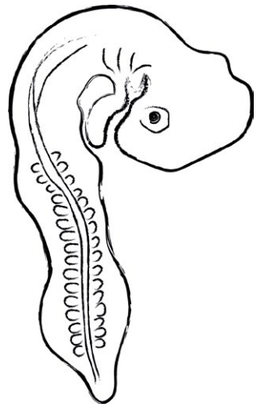
As tumours continue to grow intensively, more oxygen supply is needed. However, there is an imbalance between oxygen demand and supply. This is due to increased distance between existing vasculature and cells, which impedes on oxygen diffusion and creates more hypoxic tumour environments. These hypoxic environments activate HIF-2α, which switches on VEGF activity and ensures increased vascularisation and sufficient oxygen supply in tumours. To increase complexity, some tumour cells have overcome the setbacks of the hypoxic environment to produce an aggressive trait.

Previous studies have shown that inhibiting HIF-2α prevents *in vivo* growth and tumorigenesis in various human cancers. When HIF-2α is inhibited, the activity of receptor tyrosine kinases and downstream signalling pathways that usually promotes tumorigenesis are also inhibited. This indicates that HIF-2α might at least partly exert its proliferative and tumorigenic effects by activating such signalling pathways. Previous studies also demonstrated a strong correlation between HIF-2α expression levels and poor patient outcome in various tumour forms, including neuroblastoma (Holmquist-Mengelbier *et al.*, Cancer Cell, 2006).

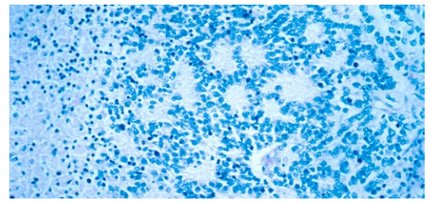
WHAT IS NEUROBLASTOMA?

Neuroblastoma is one of the most common types of solid cancers that affects children. As it is an embryonal tumour, it is most common in children under the age of 5 years old. It develops from immature neuronal cells, likely from trunk neural crest. Trunk neural crest stem cells make up a transient structure during embryonic development and are one of four derivatives of the neural crest. After neural crest cells have migrated following neural tube closure, they give rise to a diverse range of cells, including pigment cells (called melanocytes), adrenal medulla (the innermost part of the adrenal gland), and parts of the nervous system, which includes the dorsal root ganglia and sympathetic ganglia (clusters of neurons that connect to other neurological structures in the body). Neuroblastoma commonly arises in the adrenal glands and is believed to arise due to abnormalities during embryonic development.

Photo Credit: Sofie Mohlin

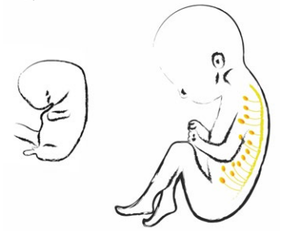


The chick embryo is used as a model organism.



Microscopic view of typical neuroblastoma.

Human embryos at different development stages.



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Previous studies have shown that there exists a subpopulation of cells in neuroblastoma that are immature neural crest-like and express HIF-2α despite locating near vessels, hence residing in an oxygenated surrounding (Pietras *et al.*, J Pathol, 2008; Persson *et al.*, Exp Cell Res, 2020). Moreover, deletion of HIF-2α in mice models resulted in defects in the sympathetic nervous system, a tissue that arises from trunk neural crest and is implicated as the origin of neuroblastoma. Hence, there is a clear link between HIF-2α and neuroblastoma. However, there has been a lack of data to elucidate the expression and function of HIF-2α during embryogenesis, specifically during normal trunk neural crest development and its potential relationship with neuroblastoma.

CONVERGENCE BETWEEN NORMAL AND TUMOUR DEVELOPMENT

Dr Sofie Mohlin and her team at Lund University, Sweden, aim to discover the link between embryogenesis and tumour development. One such project aims to investigate the underlying causes of neuroblastoma during embryonic development by studying the function of HIF-2α. As mentioned previously, HIF-2α is known to promote tumorigenesis. By utilising concepts from both developmental and tumour biology, Dr Mohlin transfers the knowledge of normal cellular growth and differentiation during embryogenesis to the abnormal development of cancerous cells. Through this convergence, Dr Mohlin aims to elucidate how cells with the same genetic information adopt different fates and change their behaviours and identities to a cancerous one.

Dr Sofie Mohlin and her team from Lund University aim to discover the link between embryogenesis and tumour development by studying the function of HIF-2α.

THE ROLE OF HIF-2α IN EMBRYONIC DEVELOPMENT

In a recent study published in *Developmental Dynamics*, Dr Mohlin used the chick embryo, a model organism mostly used in developmental biology and immunocytochemistry, to show over a developmental time course that HIF-2α protein was detected at lower levels in pre-migratory neural crest cells in the developing neural tube and at substantial levels in trunk neural crest cells that had delaminated from the tube and started to migrate throughout the embryo at later time points. This was confirmed in two additional species, human and mouse, importantly demonstrating the feasibility to use other vertebrate organisms to study functional mechanisms corresponding to human development.

Furthermore, by knocking down the expression of HIF-2α using morpholinos, a common method to block the translation of a targeted protein, the chick embryos were developmentally delayed. A second widely used genetic engineering tool, CRISPR-Cas9, which has won its developers the Nobel Prize in Chemistry 2020, was used to confirm results from the previous method. Both sets of experiments resulted in a decreased expression of genes associated with early and migrating neural crest, specifically from the trunk derivative. These experiments also showed a reduced number of trunk neural crest cells that migrated to form the sympathetic ganglia. However, the cells became highly proliferative and showed enhanced self-renewal capacity, a known stem cell trait. The same effects were observed when HIF-2α levels were manually increased in chick embryos, suggesting that HIF-2α levels must be strictly controlled

Photo Credit: Sofie Mohlin

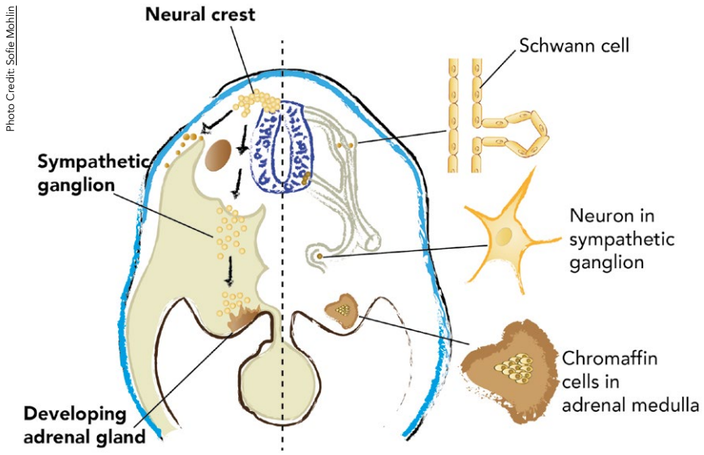

Illustration of neural crest development. Neuroblastoma commonly arises in the adrenal glands.

during embryogenesis, so that neural crest cell proliferation, migration, and development proceed normally.

It can be inferred that HIF-2α is an important protein for embryos to develop normally, specifically for the sympathetic ganglia. An increased or decreased level of HIF-2α during embryonic development may cause the neural crest to develop abnormally, which consequently may make it more susceptible for neuroblastoma to form.

The developmental delay might be explained because HIF-2α is an important oxygen regulator in the cell; previous papers have indeed shown that alteration in oxygen levels during neural crest development. Moreover, increased cell proliferation may delay cell migration, as cells during their active migratory phase do not proliferate much.

The capacity for self-renewal is an important feature in stem-like cells and tumours. Previous studies showed that neuroblastoma cells with dysregulated HIF-2α expression are more stem-like, indicating that HIF-2α is important for cells to mature and develop, like seen here where manipulating HIF-2α expression in trunk neural crest cells leads to enhanced self-renewal capacity.

Even though HIF-2α affected the development of trunk neural crest cells, with early and migrating neural crest cells; it also affected other downstream genes of HIF-2α expression. After conducting gene set enrichment analysis (GSEA), a method to show association with a disease phenotype, Dr Mohlin discovered the connection between HIF-2α and tumorigenesis. Genes involved in invasion of tumour cells (for example, genes involved in epithelial-to-mesenchymal transition required for cells to move from their primary to secondary sites in the body) and growth of tumour in size and volume were enriched. There is also enrichment of genes associated with embryonic development and growth arrest, in concordance with the biological phenotype observed in the experimental chick embryos. These are all hallmarks of cancer cell function. Signalling pathways, such as phosphatidylinositol 3-kinase (PI3K), which promotes cell proliferation, survival, and growth and has been shown to be upregulated in cancer, was also enriched when HIF-2α levels were dysregulated. Previous studies have demonstrated a link between PI3K and HIF-2α and neuroblastoma growth (Mohlin *et al.*, Cancer Res, 2015), and together, these results further suggest a possible connection of HIF-2α between normal and tumour development.

HIF-2α levels must be strictly controlled during embryogenesis so neural crest proliferation, migration, and development are normal.

IMPLICATION OF THE STUDY

Neuroblastoma has one of the lowest survival rates of all childhood cancers, at 67% in the U.K. Therefore, research that elucidates any connection between normal embryonic development, specifically the trunk neural crest cells (the cells that eventually will differentiate into the cells that are affected in neuroblastoma), and neuroblastoma by HIF-2α is important. This will be the future outlook for Dr Mohlin's research group.

There are broad applications because if the HIF-2α protein is found to play a role in neuroblastoma, it could be used as a marker for early detection of the tumour, and elucidating by which roads such effects are mediated, novel therapeutic targets could be identified. This would reduce the need for invasive treatments and improve prognosis and quality of life for patients living with neuroblastoma.

critically affects embryonic growth. And although the classical mode of HIF-2α is to stabilise at hypoxia to initiate a cellular adaptation process, it has in recent studies become evident that HIF-2α can exert other functions also under oxygenated conditions (termed pseudohypoxia). This is in concordance with the study by Dr Mohlin where HIF-2α seems to be expressed and function partly independent of surrounding oxygen concentrations its deletion in the embryo did not to an obvious degree affect cell lineage specification, which is the process by which cells decide what kind of cells to become next. This is evidenced by the presence of SOX9 in the embryos, which is an important marker expressed in pre-migratory neural crest cells that determines their lineage.

Moreover, dysregulated levels of HIF-2α not only affected genes associated

Behind the Research

Dr Sofie Mohlin

E: sofie.mohlin@med.lu.se **W:** https://www.tcr.lu.se/research-groups/molecular-physiology
@mohlinlab

Research Objectives

Dr Mohlin studies protein HIF-2α and its function during the development of embryos and childhood cancer.

Detail

Sofie Mohlin
Lund University
Translational Cancer Research
Medicon Village 404:C3
Scheelevagen 8
223 63 Lund
Sweden

Bio

Dr Sofie Mohlin received an MSc in Engineering (Biotechnology) from Lund Faculty of Technology, Sweden, and obtained a PhD in Molecular Medicine, Lund University. Between 2015 and 2017 she worked as a post-doc with Prof Marianne Bronner at the California Institute of Technology, US. After a position as Assistant Professor (2017-2019), Dr Mohlin is currently Associate Professor of Molecular Physiology at Lund University Sweden.

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Personal Response

What inspired you to conduct this research?

"Following my PhD in cancer biology I became interested in understanding how even infants could develop tumours. I decided to study the functional and mechanistic reasons behind how a normal cell can transform into a cancer cell and was lucky to find a model organism (chick embryo) to do so and obtain sufficient funding to establish my own group. Science and especially basic molecular research really excite me, and I love to work in a team where we share both failed experiments as well as those fantastic moments when everything just falls into place."

What other experiments have been planned to build a stronger link between embryogenesis and neuroblastoma through HIF-2α?

"We are further elucidating the mechanisms by which HIF-2α affects embryogenesis from a more long-term developmental perspective to increase our knowledge on the connection between trunk neural crest and cancer. We focus on the migratory effects considering the importance of EMT and migration for proper cell specification and organ formation as well as cancer metastasis. We use chick embryos to investigate these processes at single cell level by tissue explants, stem cell cultures and single cell RNA sequencing. We allow the embryos to develop terminally to be able to determine the full effects of HIF-2α abnormalities."

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