

# Abstract

## Background

Extracellular vesicles (EVs) are nano-sized, cell-derived phospholipid membrane enclosed vesicles that constitute important cell-to-cell messengers, regulating diverse cellular functions of recipient cells. Up to date, there is scarce information on the impact of EVs on the brain development of the extremely preterm infant. The choroid plexus epithelium (CPE) has been shown to secrete EVs, which can be transported to the brain parenchyma via the cerebrospinal fluid (CSF). Insulin-like growth factor 1 (IGF-1) is a fetal growth regulator for the central nervous system with abruptly decreased serum levels following preterm birth. We hypothesize that IGF-1 regulates EV secretion from the CPE, through interaction with the IGF-1 receptor (IGF-1R), and thereby acts as paracrine signaling, via the CSF, to the brain parenchyma.

One hurdle in current EV research is isolating EVs. AcouTrap uses acoustic trapping for purifying EVs from plasma and urine. Although the technique has been shown to work well, for refined proteomic analyses of plasma-derived EVs, the method requires optimization in order to reduce plasma protein background.

## Research questions/Methods

In project I-IV, we aim to build a better understanding of the effects of IGF-1 on the immature brain by investigating how IGF-1 **1**) activates the IGF-1R of CPE cells (Project I-II), **2**) affects EV secretion from CPE cells (Project III), and **3**) affects CPE cell metabolism (Project IV).

In project I and II we characterized IGF-1R activation and downstream signaling in the CPE of the immature preterm rabbit pup brain following systemic injection of IGF-1 in complex with its binding protein IGFBP-3.

In project III-IV we investigated EV secretion from CPE as well as CPE metabolism *in vitro* and *in vivo* following exposure to IGF-1. CPE cells were exposed to free IGF-1 in a transwell system and cells/apical supernatant vesicle content were/will be analyzed using nano-tracking analysis, microscopy, metabolomics and transcriptomics. EVs derived from preterm piglet CSF, following exposure to systemic IGF-1 (in complex with IGFBP-3), were/will be analyzed with quantitative PCR, microscopy and metabolomics.

In project V our aim is to evaluate the affinity of highly abundant plasma proteins to hydrophobic polystyrene and hydrophilic silica beads respectively during acoustic trapping of EVs, utilizing the technique AcouTrap. Proteome and quantity of plasma EVs after AcouTrap purification will be analyzed using mass spectrometry, nano-tracking analysis and microscopy.

In project VI-VII, our aim is to characterize plasma and urine EVs in the term and preterm infants.

## Results/Preliminary results

IGF-1R is abundantly expressed on the CPE in the preterm rabbit (Project I). Circulating IGF-1 interacts with the cells of CPE, affecting its mRNA content, increasing expression of genes involved in vascular maturation and structure (Project I and II). IGF-1 increases the secretion of EVs from the CPE. These EVs contain IGF-1 and are readily taken up by hippocampal neurons (Project III). Systemically administered IGF-1 alters the composition of metabolites in the piglet CSF, such as increasing the rate of saccharides (Project IV).

## Significance

Understanding the role of growth factors, in particular IGF-1 mechanisms, in development of the immature brain is of great importance in order to develop new treatments. Furthermore, more research on the communication of CPE with other areas of the brain, as well as understanding the involvement of EVs is needed. Here, we present data showing that IGF-1 affects the CPE, the EV secretion from CPE cells, and a possibility to mediate signaling to hippocampus derived neurons.

## List of publications

Gram, M., Ekström, C., Holmqvist, B., Carey, G., Wang, X., Vallius, S., Hellström, W., **Ortenlöf, N.**, Agyemang, A.A., Smith, L.E.H., Hellström, A., Mangili, A., Barton, N., Ley, D. (2021). Insulin-like growth factor 1 in the preterm rabbit pup: Characterization of cerebrovascular maturation following administration of recombinant human insulin-like growth factor 1/insulin-like growth factor 1-binding protein 3. *Dev Neurosci* 43, 281-295.

**Ortenlöf, N.**, Vallius, S., Pankratova, S., Ley, D., Gram, M. Insulin-like growth factor 1 stimulated release of extracellular vesicles from the choroid plexus. A novel mode of transport from the circulation to the developing brain? Manuscript.