From Neanderthal to Homo sapiens: Experimental evaluation of genetic shifts implicated in brain growth

- Project background

During the course of evolution, the human brain has undergone tremendous expansion. This has led to the development of complex neurocircuits of unmatched complexity, highly social behaviour, expanded language use and cognitive superiority. A small number of infants, however, are born with significantly smaller brains, a condition called primary microcephaly (PM). Infants with PM suffer from a number of symptoms including cognitive and motor impairments (i.e. babies are unable to sit before the age of 2 years), epilepsy, cerebral palsy, a delay in speaking and defects of the eyes, including retinal degeneration [1]. Some PM babies also show overall growth reduction, as is the case in Seckel or Meier-Gorlin Syndrome, which is then called microcephalic primordial dwarfism [2]. Such inborn cases of microcephaly are believed to result from defects in neural progenitor proliferation, differentiation or apoptosis during early development. Molecularly, microcephaly arises often from defects in centrosome biogenesis or DNA replication. Recently, we have shown that defects in ciliogenesis can similarly impact on brain development [3]. In order to identify new factors involved in brain expansion, Dr Travis Stracker (guest scientist of the graduate school in 2017) has mined the Neanderthal genome for genes, which have been adapted in the transition to the brain of homo sapiens. This collection of genes that show fixed changes in homo sapiens is rich with genes known to cause PM, suggesting that others may be candidates for neurodevelopmental disorders. In the proposed collaborative project we will investigate the molecular function of candidate genes on cell cycle progression, centrosomes and cilia in zebrafish and cell culture.

- Preparatory work

a) of the Stracker lab

Travis Stracker is one of this years’ guest scientists of the grad school and an expert in molecular defects leading to cilia defects and microcephaly [4, 5]. The Stracker lab has identified 7 genes, which have fixed mutations from Homo neanderthalensis to Homo sapiens and which are candidate genes for brain growth based on domain composition or limited functional analysis. Initial knockdown experiments in retinal pigment epithelial (RPE) cells demonstrated a function in both mitotic division and ciliogenesis for all 7 genes. In addition, supernumerary centrosomes could be observed for 5 of them and increased levels of p53 were observed in all cases. These data suggest that these genes are indispensable for normal cell division and their mutation provokes cellular signalling that would lead to cell death and attrition in the organism.

b) of the Philipp lab

The Philipp lab has by now a strong track record in cilia biology, signal transduction and microcephaly using zebrafish as model organism [3, 6-10]. We have studied cilia and related processes for more than five years and identified a cilia defect as the underlying cause of Seckel Syndrome, a PM disorder very recently [3]. All genes identified by Dr. Stracker are highly conserved in zebrafish, allowing their study in the development of key organ systems.

- Working programme

Aim 1: Candidate gene during zebrafish brain development (Philipp lab)

In a first step, expression of the candidate genes at different stages during zebrafish development will be analyzed. Only genes, which show enrichment in ciliated structures and most importantly the brain or neural progenitors will be chosen for further analysis. The student will then be trained in manipulating zebrafish eggs to generate loss-of-function (LOF) embryos using traditional MO-based knockdown and Crispr/Cas9 mediated knockout approaches. We will then score the LOF embryos for smaller head size as a measure of microcephaly and cilia dysfunction phenotypes such as body curvature, otolith defects and generalized edema. In situ hybridization analysis, as well as immunofluorescence experiments of the injected embryos, will reveal cilia and neural progenitor defects.

Aim 2: Investigation of molecular mechanism of selected genes (Philipp and Stracker lab)

Secondly, one or two lead genes according to the results from aim 1 will be selected to uncover the molecular mechanism by which these genes facilitate ciliogenesis and thus brain growth. To do so we
will apply BioID, a proximity-dependent biotin identification assay [11]. Here, the gene of interest will be fused to the engineered biotin ligase BirA* and expressed in HEK293 cells. All proteins, which come in close proximity (i.e. direct interactors or components of protein complexes interacting with the protein of interest) will be biotinylated and can be isolated with the help of Streptavidin-coated beads. Subsequent mass spectrometry analysis will reveal interacting proteins. Since the Stracker lab has successfully applied BioID for several other projects (in press in Cell Death Diff. and revision at Nat. Cell Bio.) the PhD student will visit the Stracker lab and receive training in this technology. Interacting proteins will be analyzed in zebrafish for a potential function in ciliogenesis and brain expansion (Philipp lab) and for their impact on cilia, centrosomes and mitotic spindles (Stracker lab).

Overall, microcephaly, the subject of this study is currently of high interest for the scientific community and the general public. Because of the combined effort of both our labs and our proven expertise in this field the project is very likely to succeed. We anticipate for the newly hired student to publish at least one paper as first author and to graduate within the time requirements of the grad school.

- Cooperation partner
Dr. Travis Stracker, Oncology Programme, IRB Barcelona, guest scientist of the IGradU.

References