Modulation of activity of human-specific enhancers in iPSC-derived astrocytes

Humans possess unique intellectual abilities which distinguish us from our closest relatives – apes and monkeys. Human brains are much bigger than primate brains, but the real source of their uniqueness lies in features of cells of which they are composed.

Human brain is composed of multiple types of cells. The best known, and most studied types are neurons, which are the source of ability of brain to "think" through transmission of electrical signals. However, neurons would not be able to fulfill their functions without support and protection of other cell type, glia. Glia are supporting cells in the brain, the "glue" that holds the brain together and provides neurons with protection and nutrition. Astrocytes which are studied in our laboratory, are named for their star-like shape (from Ancient Greek – *ástron*, a star), and are the most common and important type of glia.

Human astrocytes are especially unique – they are bigger and more "star-like" than astrocytes in other species. This observation has led to the proposal that astrocytes might be an important element contributing to qualities of human brain. Our laboratory is trying to uncover mechanisms which are behind uniquely human characteristics of astrocytes.

Differences between species that arise during evolution ultimately come from alterations in sequence of DNA in cells of those organisms. Those changes can come from two different sources – changes in instructions of how to make protein (called "protein coding sequence") or instructions on when and where to produce a given protein (called "gene regulatory program"). Changes in protein coding sequences are rare in recent human brain evolution. Therefore, according to current knowledge, changes in gene regulatory programs might the source of specifically human brain features.

Gene regulatory programs are encoded in regions of DNA that do not code proteins, called regulatory elements. Regulatory elements that cause increased "production" ("expression") of genes are called enhancers. In our project, we are investigating which enhancers increase expression of genes in human astrocytes.

Because astrocytes cannot be studied directly in w the human brain, we are using *in vitro* models of cells, which were derived from induced pluripotent stem cells. Induced pluripotent stem cells can be obtained from skin cells, and they have the ability to change ("differentiate") into any cell type, including astrocytes. By using this model, we can apply the most modern methods of molecular biology, including modified CRISPR-Cas9, to astrocyte genetics. This technique allows us to precisely "switch-off" enhancers that have changed during evolution, draw conclusions on their importance in brain evolution.

While the main motivation of our work is to understand how human astrocytes evolved, it also has a wider context – correct functions of astrocytes, including features that were shaped by the recent evolutionary process, are important for healthy brain function. Exploring them might contribute to a better understanding of serious diseases which involve changes in astrocytes, such as autism, schizophrenia and dementia.