Investigating Human Brain Expansion Using Pluripotent Stem Cell-Derived Neural Cultures and Comparative Genomics

Abstract

The evolution of the human brain underlies our higher-order cognitive functions. Particularly, the cerebral cortex, the outermost layer of the brain, has rapidly expanded in human compared to other species. Much of this expansion is attributed to the increase of the pool of neural precursors (NPs), which proliferate and differentiate into the neurons and glia of the brain. Relative to other primate species, such as macaque and marmoset, the human cortex contains approximately 10-fold and 67-fold more neurons, respectively. How the human cerebral cortex has evolved continues to puzzle many researchers today. My thesis work addresses this standing question through comparative genomics of primate pluripotent stem cell (PSC)-derived neural cultures. I first compared the acute response of human and macaque cortical NPs to a differentiation cue, such as growth factor (GF) reduction. Human NPs proliferate for longer compared to macaque NPs, which differentiate and reduce their proliferation shortly after GF reduction, recapitulating *in vivo* developmental pacing where human NPs expand for longer compared to their macaque counterparts. Through transcriptomic analysis, I identified E2F target genes to be upregulated after GF reduction in human but not in macaque, pointing to a potential mechanism underlying species-specific differences in NP proliferative capacity. Pharmacological and genetic inhibition of E2F signaling promoted neuronal differentiation and decreased proliferation in human NPs. Furthermore, outer radial glia (oRG) are an NP subtype presumed to contribute to cortical expansion as it is largely abundant in human and mammals with folded brains. Intriguingly, marmosets are an exception to this observation as they contain a significant oRG population yet a small and smooth brain. I pioneered a PSC platform to compare oRG across species. By leveraging HOPX as a specific oRG marker, I generated HOPX-tdTomato (HOPX-tdT) PSC lines in human, macaque, and marmoset using CRISPR-Cas9 technology. By generating human and macaque HOPX-tdT NPs, I showed that oRG emerge later compared to other NPs and become the predominant NP subtype, as they do *in vivo*. I also identified oRG-specific accessible

chromatin regions by epigenetic profiling of sorted HOPX-tdT populations. Overall, my thesis project furthers our understanding of human cortical expansion.