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Differentiation of Human iPSCs Into Telencephalic Neurons Using 3D Organoids and Monolayer Culture

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Human induced pluripotent stem cells (hiPSCs) are emerging as a useful tool for modelling *in vitro* early brain development and neurological disorders. Molecular mechanisms and cell interactions that regulate the neurodevelopment at early stages remain unclear because of human brain's complexity and limitations of functional studies. Two major culture methodologies are used to differentiate *in vitro* hiPSCs into neurons: monolayer (2D) and organoid (3D) cultures. Here we investigate the effect of cell dissociation and the loss of 3D organization during the early differentiation process of neuronal progenitors. Using the same culture media, we first differentiated hiPSCs into neural progenitor cells (NPCs) and then induced their differentiation into neurons in 3 different modalities: 3D undissociated organoids, dissociated NPCs followed by immediate re-aggregation into an organoid, and dissociated NPCs cultured as monolayer. We assessed neuronal differentiation efficiency of each method by immunocytochemistry, qPCR, western blot, and RNA-Seq analysis over a time course. Our data revealed substantial differences in gene and protein expression among the three systems, including genes of the Notch pathway (e.g. NEUROD1, NEUROG2), earliest determinants of cortical region differentiation (e.g. SOX1, FEZF1) as well as later transcriptional regulators that specify cortical neuron subtypes (e.g. TBR1, CTIP2), which were all downregulated in monolayer. Moreover, we found that genes and pathways mediating cell-to-cell interactions (e.g. CNTNs, CAMs) were mostly upregulated in the 3D culture systems, whereas cell-extracellular matrix interaction molecules (e.g. ITG, LAM) were mostly upregulated in 2D, indicating that cell surface molecules may be involved in specification of neuronal cell types. Our results address the methodological question of the appropriateness of a differentiation method for a particular experimental goal, and, beyond that, reveal important early determinants that exert a decisive influence on neuronal differentiation and regional specification of human neural stem cells.

Keywords
hiPSCs, organoid, monolayer, cell dissociation, cortical development, RNA-seq