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SUPPLEMENTARY INFORMATION

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Organoid single-cell genomic atlas uncovers human-specific features of brain development

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Progression of major cell types during cerebral organoid culture

We used the time course single-cell RNA-seq data from cerebral organoids to track a progression through pluripotent, neuroectodermal, and neuroepithelial stem cell states during the first 15 days of differentiation. By 1 month, cells diversify into neural progenitors from multiple brain regions including the forebrain (dorsal and ventral telencephalon, diencephalon), midbrain (mesencephalon), and hindbrain (rhombencephalon). A small subpopulation resembling retinal progenitors of the developing eye field is also present, but these cells were only detected in an iPSC 409b2-derived organoid. In addition, a non-neuronal mesenchymal population appears from both cell lines early in the differentiation time course. By 2 months, excitatory and inhibitory neuronal fates have differentiated from progenitors of multiple brain regions, and by 4 months astrocytes have emerged.

Focusing on 2-month organoids, we observed neuronal differentiation trajectories representing ventral and dorsal telencephalon, as well as distinct populations of cortical excitatory (GLI3, EOMES, NEUROD6), ventral telencephalon inhibitory (DLX1, SOX6, GAD1/2), diencephalon excitatory, diencephalon inhibitory (with Cajal-Retzius cell signatures), mesen- (or midbrain) and rhombencephalon (hindbrain) excitatory, and mesen- and rhombencephalon inhibitory neurons. Each iPSC line contributed cells to multiple differentiation trajectories, however the proportions of cells in each trajectory varied across organoid and iPSC. For example, over 90% of cells from the line Kucg2 were on the cortical excitatory (dorsal) trajectory in each of the 3 organoids, whereas Hoik1-derived organoids predominantly contained cells from non-telencephalic regions.

In the single-cell RNA-seq data we generated from human, chimpanzee, and macaque 2 and 4 month organoids using 10X Genomics, we observed very strong signatures of deep and upper layer cortical neuron differentiation in chimpanzee and macaque organoids. This bifurcation of deep and upper layer cortical neurons was much less pronounced in human organoids from the same time point. We analyzed additional scRNA-seq data (Smart-seq2, Fluidigm C1) data from 52 human organoids from 15 individuals, 38 chimpanzee/bonobo organoids from 11 lines, and one macaque organoid from one line. Based on this data, we find that there is variation in the degree of specification of upper and deep layer cortical neurons, which makes it unclear whether this is a reliable measure of organoid maturation. It is unclear if this variation is due to batch, lines, organoid or scRNA-seq protocols. However, we found that neuron projection, synapse, and neurotransmitter related genes were consistently and significantly expressed higher at an earlier time point in the chimpanzee organoids compared to the human organoids, suggesting that human organoids mature slower than those of other primates.

Potential biological significance of chosen genes with differential properties between human and chimpanzee

We compared scATAC-seq data of human and chimpanzee cerebral organoids and identified 8,099 peaks (7.4% of all accessible peaks) that gained accessibility in humans relative to chimpanzee, whereas 9,836 peaks (9% of all accessible peaks) lost accessibility. We annotated peaks that are DA between humans and chimpanzees with various evolutionary signature. For instance, we identified 62 human accelerated regions that

overlap DA peaks (32 gain accessibility in human, 30 gain accessibility in chimpanzee), with one of these sites being nearby a gene with human-specific expression. In this case, the potential regulatory region is 244 Kb away from cadherin 7 (CDH7), a gene with higher expression specifically in human cortical neurons, and has increased accessibility in human neurons relative to chimpanzee and macaque. We also find DA regions nearby two genes, Ly6/PLAUR domain-containing protein 1 (LYPD1) and Ras-related C3 botulinum toxin substrate 1 (RAC1), that have human-specific expression in NPCs and neurons, respectively. LYPD1 is involved in neurotransmitter receptor-binding and anxiety-related behaviors[1] and RAC1 is a GTPase involved in diverse processes including glucose uptake and cytoskeletal reorganization and genetic variants in this gene can lead to micro- or macrocephaly[2]. In addition, we identify 22 regions that are accessible in chimpanzee NPCs or neurons that are highly conserved in mammals, but the DNA has been deleted in humans (so-called human conserved deletion, hCONDELs)[3] and 1 of these are located nearby a DE gene (FADS1). FADS1 encodes the fatty acyl desaturases (delta-5 desaturase) which catalyze key steps in the ω-3 and ω-6 lipid biosynthesis pathways[4], and has been reported to be abundantly expressed in the brain[5], and therefore could have contributed to the fast divergence of lipids between human and other primates like chimpanzee and macaque[6].

With the single-nucleus RNA-seq data of human, chimpanzee and macaque adult prefrontal cortex, we identified 479 DE genes in adult excitatory neurons between human and chimpanzee. Among them, 53 overlap with DE genes in dorsal telencephalon cells between human and chimpanzee organoid. They include COL6A1, the gene encoding alpha1-chain of collagen VI, the broadly distributed extracellular matrix protein. COL6A1 has been suggested to play roles in central nervous system development and diseases[7], and has been shown to have a protective role limiting autophagy and apoptosis in aging neurons[8]. Another example is RIC3, which shows not only human-specific DE in adult neurons, but also human-specific DE in organoids in a neuron-specific manner. RIC3 encodes a protein which functions as a chaperone influencing the folding, assembly of specific 5-hydroxytryptamine type 3 receptor and nicotinic acetylcholine receptor subtypes, and regulates the number and maturation of acetylcholine-gated ion channels in neurons[9].

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