Quantitative characterization of variability in cortical neurons between healthy human induced pluripotent stem cell lines

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Human iPSC lines used to derive cortical neurons

Cell Line	Cell Type	Age of Donor	Sex	Ethnicity	Source of Cells	Reprogramming method	Media
NCRM1	cord blood	NA	male	NA	NIH	episomal	mTeSR1
KYOU	fibroblast	36	female	caucasian	ATCC	retrovirus	mTeSR1
AIW002-02	PBMC	37	male	caucasian	MNI	retrovirus	mTeSR1
AJC001-5	fibroblast	37	male	caucasian	MNI	retrovirus	mTeSR1
AJG001-C4	PBMC	37	male	caucasian	MNI	episomal	mTeSR1
TD02	PBMC	48	female	caucasian	MNI	episomal	mTeSR1
3448	PBMC	48	male	caucasian	MNI	episomal	E8
3450	PBMC	37	male	caucasian	MNI	episomal	E8
AJD002-3	PBMC	44	male	caucasian	MNI	retrovirus	E8
TD03	PBMC	44	male	caucasian	MNI	episomal	E8
TD10	PBMC	64	female	south asian	MNI	episomal	E8

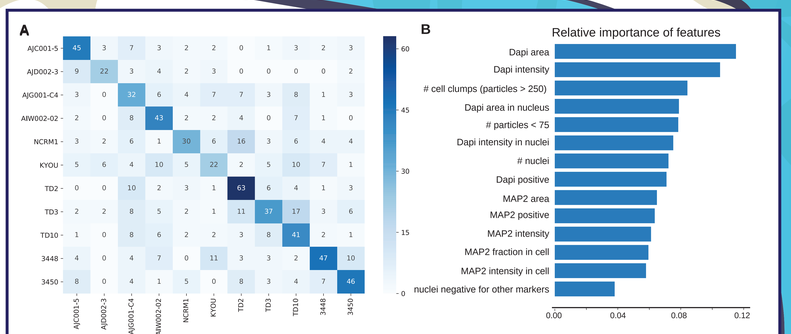


Figure 1: Cortical neurons from different cell lines can be distinguished by cytoskeletal (MAP2) and nuclear (Dapi) staining. Random Forest predictions of cell lines from a test set of immunofluorescence images trained with a separate set of images from 4 week old iPSC derived cortical cultures. A) Confusion matrix of the cell line prediciton acurracy. B) Relative improtance of the features used by the classifier

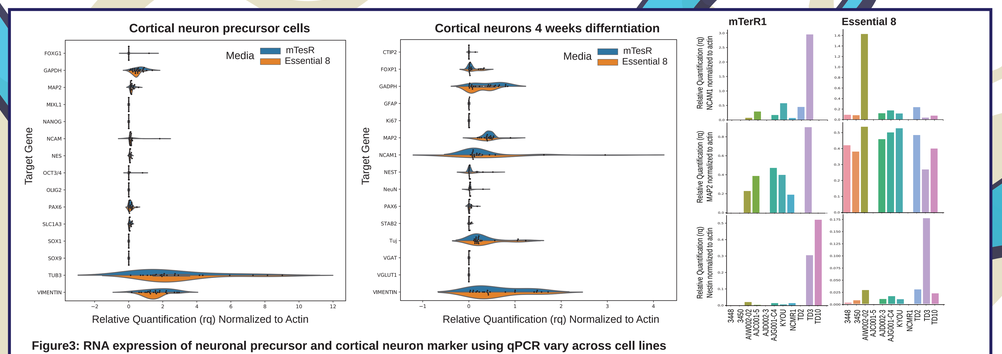


Figure3: RNA expression of neuronal precursor and cortical neuron marker using qPCR vary across cell lines

RNA expression of the indicated markers quantified by qPCR using relative expression to actin. A) cortical neuronal precursors samples across 11 cell lines. Violin plots showing distribution of gene expression in two different iPSC growth medias before differntiation. B) RNA expression of the indicated markers in 11 cell lines differentiated into cortical neurons 4 weeks post NPC culture. C) RNA expression of NCAM1, MAP2 and nestin in RNA from 4 week old cortical neuron cultures derived from iPSCs grown in the indicated media.

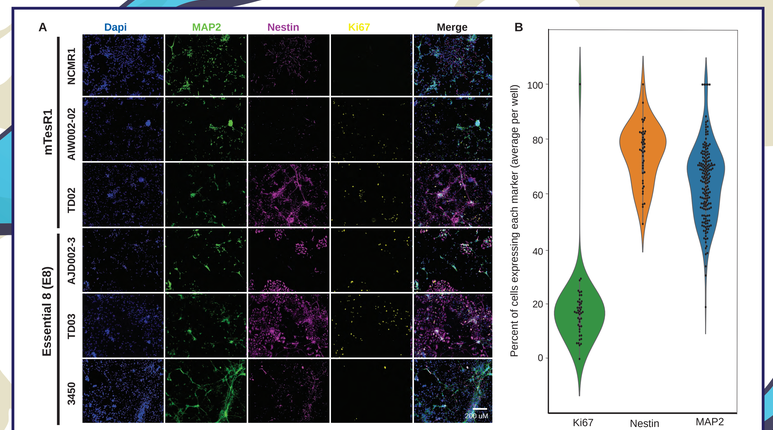


Figure 4: Cortical neural precursor cells (CNPCs) differentiated from iPSCs vary in marker expression across cell lines. All cell lines express Nestin and MAP2. A) Example images of CNPCs from 6 different cell lines growing in different media. B) Quantification of the percentage of cells positive for the indicated markers. Values are from 11 CNPC lines from iPSC neurons proliferated in either E8 or mTesR1.

Summary

When studying developmental neurological diseases such as autism spectrum disorder, human brain tissue samples are only available postmortem. The breakthrough advances allowing reprogramming of human blood or skin cells into pluripotent stem cells (iPSCs), which can be differentiated into numerous cell types including neurons and astrocytes, allow for human cell models of neurodevelopmental disorders. How different control iPSCs behave under different culturing conditions has not been compared in parallel. Here, we characterize 11 healthy control human iPSC lines differentiated into cortical neurons, 2 commercial and 9 in house lines using a combination qPCR and immunofluorescence (IF). We first tested if cell lines could be distinguished based on IF in microscopy images using a Random Forest classifier. We find that cell lines can be clearly distinguished. We next used unsupervised machine learning to cluster neurons into groups. We measure a panel of genes using qPCR across 11 cell lines from NPC and 4 weeks cortical neurons derived from iPSC grown in two types of media. We further characterize the cell lines by quantifying the proportion of cells expressing neuronal precursor and cortical neuron markers using an automated image analysis macro.

Conclusions

- Gene expression varies more across cortical neurons from iPSCs in Essential 8 media than mTesR
- While all iPSC lines form neurons that express cortical neuron markers, expression levels vary widely across cell lines
- When using iPSCs to study development and disease, multiple control lines should be included in experiments
- The iPSC preferred growth media should be determined for each line and will effect the expression profiles of cotrical neurons

Caveats

- Here we have examined cultures of mostly cortical neuron and the characteristics of the cultures will likely be altered if other differ-entiation protocols or co-cultures with astrocytes were tested
- This data is presented as experiment replicates from two batches of iPSC passaging and differentiation, we did not have enough batch replicates to quantify the variation between iPSC passages and replicate experiments within each cell line

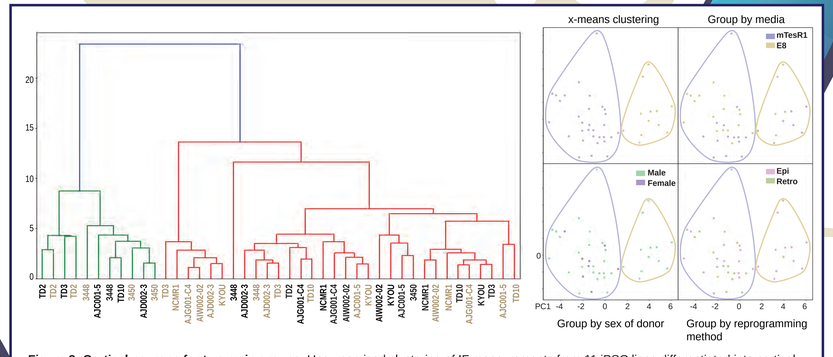
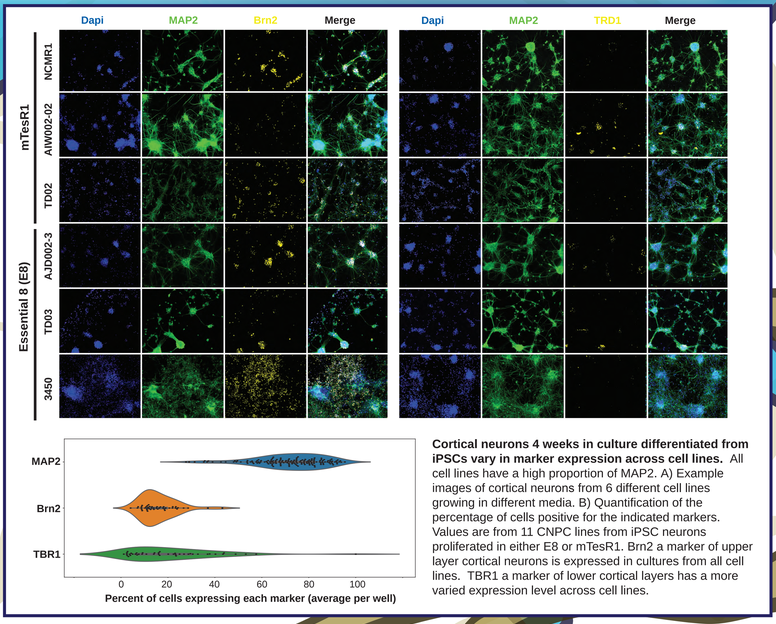


Figure 2: Cortical neurons for two main groups. Unsurpervised clustering of IF measurements from 11 iPSC lines differentiated into cortical neurons for 2 weeks from two separate batches of iPSC grown in two medias. cortical cultures. A) Dendrogram using the Ward algorithm creates two main groups. B) x-means clustering also results in two main groups (upper left) with groups outlined. iPSC growth media, gender of the donor and the method of reprogramming are all partially separated into the the x-means clusters.



Cortical neurons 4 weeks in culture differentiated from iPSCs vary in marker expression across cell lines. All cell lines have a high proportion of MAP2. A) Example images of cortical neurons from 6 different cell lines growing in different media. B) Quantification of the percentage of cells positive for the indicated markers. Values are from 11 CNPC lines from iPSC neurons proliferated in either E8 or mTesR1. Brn2 a marker of upper layer cortical neurons is expressed in cultures from all cell lines. TBR1 a marker of lower cortical layers has a more varied expression level across cell lines.

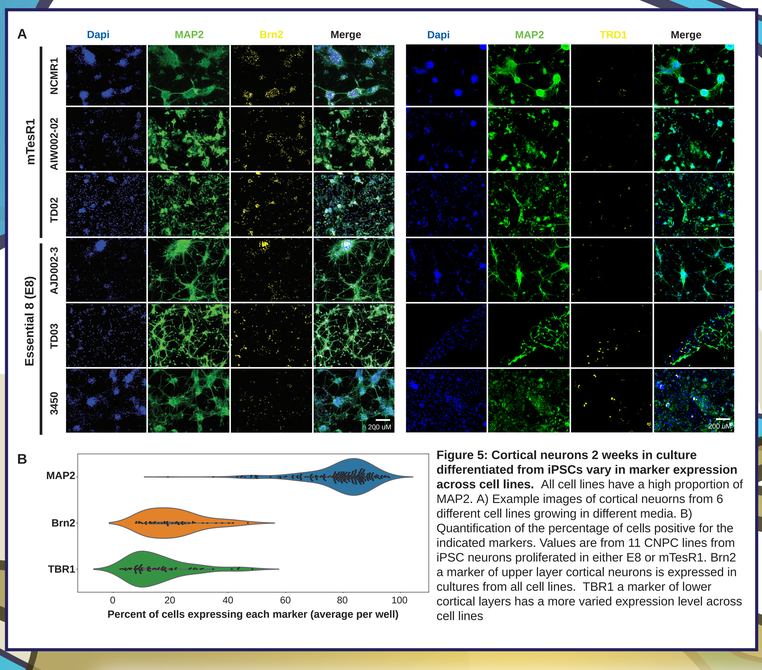


Figure 5: Cortical neurons 2 weeks in culture differentiated from iPSCs vary in marker expression across cell lines. All cell lines have a high proportion of MAP2. A) Example images of cortical neurons from 6 different cell lines growing in different media. B) Quantification of the percentage of cells positive for the indicated markers. Values are from 11 CNPC lines from iPSC neurons proliferated in either E8 or mTesR1. Brn2 a marker of upper layer cortical neurons is expressed in cultures from all cell lines. TBR1 a marker of lower cortical layers has a more varied expression level across cell lines

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