Rapid Generation of Motor Neurons using modified Ngn2 induced iPSC's to study ALS Disease

Erika Norabuena[1], James Chow[1], Sandy Hinckley[1]

[1]QurAlis, Cambridge Massachusetts, United States

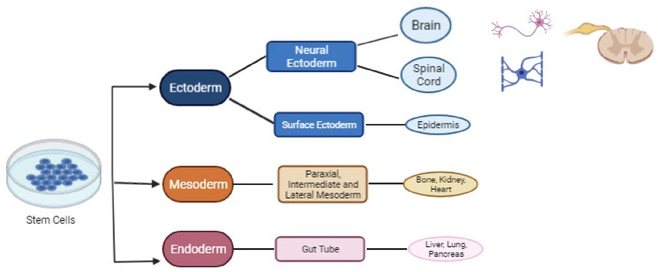
Introduction to Stem Cells, Motor Neuron and Disease relevance

- **Stem Cells**

➢ Pluripotent Stem Cells, either embryonic or induced pluripotent stem cells can be used to model human diseases.

➢ Human disease modeling is advantageous because it can overcome the limitations of animal models for certain disorders, and it removes the developmental, genetic and physiological differences between the different mammalian species commonly used for research (i.e. mice, pig, dogs, cats, nonhuman primates).

➢ Pluripotent stem cells can give rise to the three germ layers that will generate almost all the cell types of the human body (ectoderm, mesoderm and endoderm) mimicking gastrulation during human development.



- **Motor Neurons**

➢ Motor neurons (MN) are a type of neuronal subtype that exhibits vulnerability in neurodegenerative diseases such as Amyotrophic Lateral Sclerosis (ALS) and Spinal Muscular Atrophy (SMA).

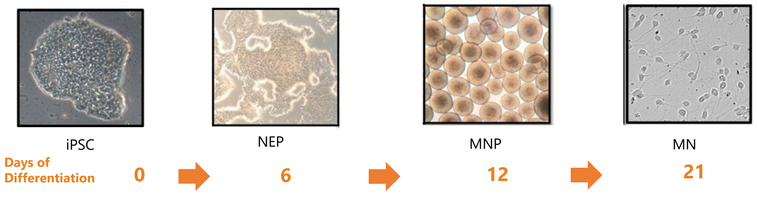
➢ Motor Neuron generation from pluripotent stem cells are a powerful model system that could allow us to gain insight into the mechanism of motor neuron diseases.

➢ During embryonic development, after the neural tube is formed, precursors cells default to a rostral and dorsal positional identity through the combined actions of the BMP, WNT, and FGF signaling pathways. Signaling pathways that operate along the rostrocaudal and dorsoventral neuraxes first establish a matrix of positional cues, that influence precursor cell fate specification by regulating the identities and concentrations of morphogenetic signals to which they are subjected.

➢ In vitro, motor neuron generation from stem cells can be achieved using two differentiation methods:

- **Direct Differentiation:**

Cells are exposed to specific signaling pathways modulators to mimic the natural sequence of development in vivo. This process is lengthy. Depending on the cell type of interest, it could take 21+ days. This method also goes through progenitor stages that could generate a variety of other cell types decreasing the purity of the desired cell population.



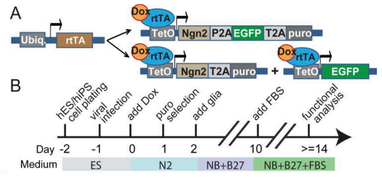
- **Induced Differentiation**

➢ Different published studies have shown that somatic cells can be directly reprogrammed to induce neurons by the overexpression of transcription factors such as the neurogenin 2 (NGN2) [1,2]

➢ NGN2 lentivirus design contains a Dox-inducible promoter that mediates its expression

➢ NGN2 overexpression converts pluripotent stem cells into neurons with high efficiency and reproducibility.

➢ The lentiviral vector that we used to induce the motor neuron differentiation contains a Dox-inducible promoter.



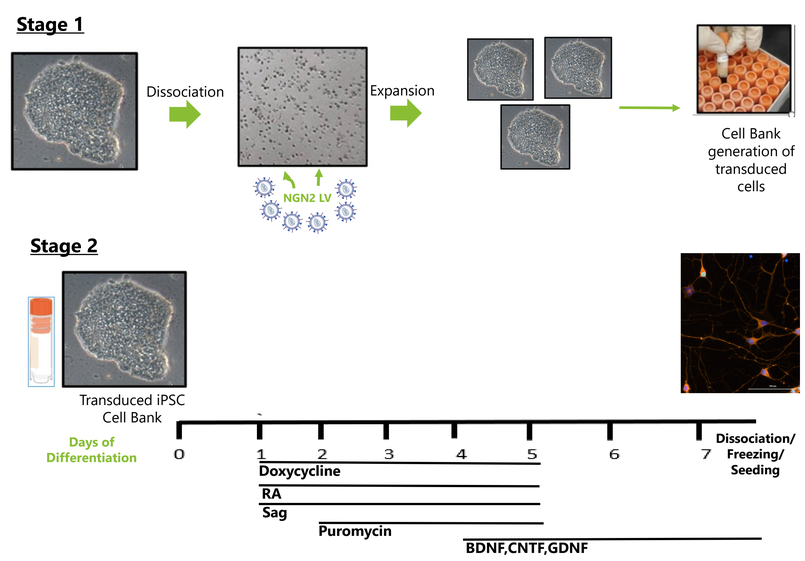
Zhang et al, Neuron 2013

Timeline to generate Motor Neurons derived from ALS Patient iPSCs

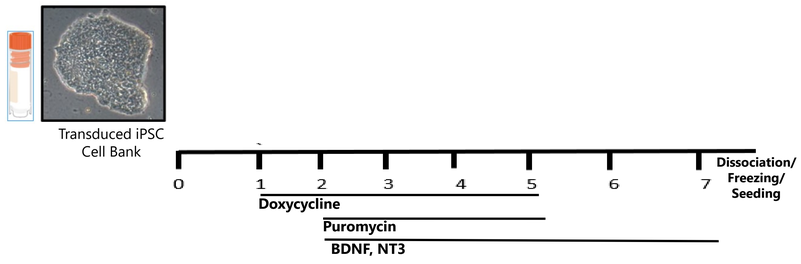
➢ Recent stem cell technology offers a new way to obtain neuronal cells by direct reprograming of iPSC's into neurons by overexpressing the transcription factor Ngn2. The field has been using this technology to generate cortical excitatory neurons in approximately 7 days.

➢ QurAlis has developed a protocol that can modified these Ngn2 excitatory neurons and drive their differentiation toward motor neurons, achieving a highly pure population of motor neurons identified by common motor neuron markers

Induced Differentiation for Motor Neurons



Induced Differentiation for excitatory neurons (Cortical neurons)

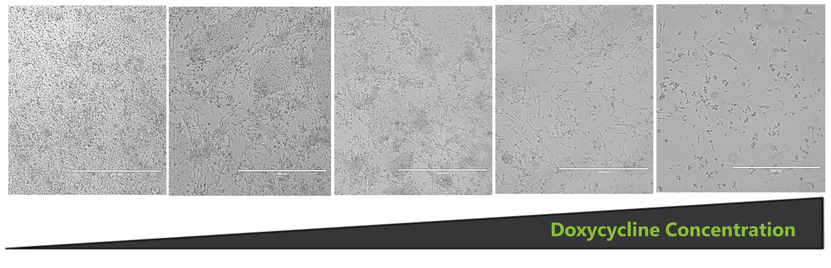


Results

Doxycycline response will change depending on the cell type used

➢ Not all cell types respond the same to pre established doxycycline treatments (i.e. recommended is 2ug/mL)

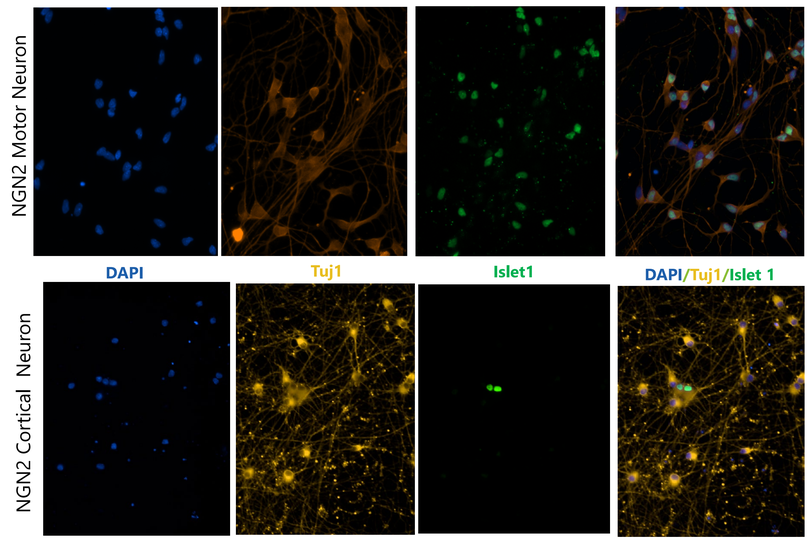
➢ There is a maximum tolerance to Dox, after that threshold is passed, the amount of cell death will be significant

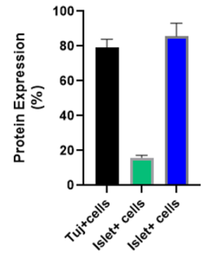


Results

➢ To assess the efficiency of motor neuron differentiation we performed immunocytochemistry to quantify the percentage of Islet1+ cells, a common motor neuron marker.

➢ Our results indicated that we achieved between 88-98% Islet1 + cells and 88-98% neuronal purity validated by quantification of Tuj1+ cells.





NGN2 Motor Neuron Cell Viability	
Cell Viability after dissociation	89%
Cell Viability after thawing	75%
% Motor Neurons (Islet1+/Tuj1+)	88.7%

Conclusions

➢ Stem Cell technology offer new tools to generate the cell types of interest to model disease and establish preclinical screening platforms.

➢ Our results shows that NGN2 excitatory neurons can be converted into NGN2 motor neurons with high purity yield by modifying the current protocols.

➢ Direct reprogramming such as Ngn2 differentiation is a tool that could be implemented in ALS research to decrease the experimental assay timelines and increase uniformity and reproducibility in downstream studies.

References

1. Zhang Y, Pak CH, Sudhof T. et al. Rapid Single-Step Induction of Functional Neurons from Human Pluripotent Stem Cells. Neuron. Jun 5;78(5):785-798. (2013)
2. Vierbuchen T,Wernig M, et al. Direct Conversion of fibroblast to functional neurons by defined factors. Nature Feb 25; 463 (7284) (2010).
3. Du, ZW., Chen, H., Liu, H. *et al.* Generation and expansion of highly pure motor neuron progenitors from human pluripotent stem cells. *Nat Commun* **6,** 6626 (2015)