Advancing Knowledge of Down Syndrome Brain Development and Function with Human Stem Cells

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Background: Human stem cells to model Down syndrome neural development

Our bodies are made up of over 250 specific cell types, and all initially arise from stem cells during embryonic development. Stem cells have two characteristics that make them unique: 1) they are pluripotent, meaning that they can differentiate into all cell types of the body, and 2) they are capable of self-renewal to generate more of themselves and are thus able to populate an organism. Human pluripotent stem cells were first isolated from human embryos twenty years ago [1], and more recently, technology to reprogram somatic cells, such as skin and blood, to induced pluripotent stem cells has emerged [2-4].Induced pluripotent stem cells, or iPSCs, are particularly valuable as disease specific iPSCs can be generated from individuals with specific genetic mutations diseases.

Researchers have harnessed the power of stem cells to understand many aspects of developmental biology in model organisms (e.g. worms, mice) and more recently, in humans. Human stem cells in culture recapitulate development. For example, formation of the brain occurs prenatally and follows a specific pattern of timing and cell generation. Human stem cells in the culture dish follow a similar pattern when exposed to developmental cues and can thus be used to understand aspects of prenatal human brain development that are not accessible by other means.

Disease-specific iPSCs are a valuable tool to model neural development in specific neurodevelopmental disorders like Down syndrome. Down syndrome is classic developmental disorder; mistakes that are made during development of a particular organ system result in the characteristics of the disorder. In the brain, mistakes during prenatal brain development lead to intellectual disability. Trisomy 21 (Ts21) iPSCs generated from somatic cells of Down syndrome individuals may enable us to understand the mistakes made during Down syndrome brain development.

Advances: Can Ts21 iPSCs recapitulate neuropathology?

We know from histopathological analyses over the last 50 years that the structure of the Down syndrome brain is different than normal [5, 6]. The most striking neuropathologies are the smaller cortical lobes and smaller cerebellum that are due to reduced number of neurons, synaptic deficits, oxidative stress and Alzheimer's pathology.

To determine if Ts21 iPSCs can recapitulate these neuropathologies, we can differentiate them into neurons that resemble those of the cerebral cortex using established protocols [7, 8]. The resulting neurons have characteristics of cortical neurons [9-13] and transcriptional analysis indicates that there is a general overrepresentation of chromosome 21 genes, thus validating that gene expression is based largely on gene dosage. In addition, the gene expression patterns recapitulate gene expression patterns from fetal Down syndrome tissue [11, 14], confirming that these neuron in culture are similar to prenatal brain. The Ts21 neurons in culture have high oxidative stress [9, 11, 13] and lower electrical activity [11], reproducing the oxidative stress and synaptic deficits characteristics of Down syndrome. Thus, Ts21 iPSCs can mimic features of Down syndrome neuropathology.

Future Outlook: Insight into brain development and function

While neurons have been the predominant focus of studies using Ts21 iPSCs to study Down syndrome neural development, additional cell types contribute to intellectual disability and their development and function can be studied using Ts21 iPSCs. For example, recent studies indicate that reduced myelination is a characteristic of Down syndrome. Oligodendrocytes are the myelin forming cells and can be differentiated from iPSCs to study their dysfunction in Down syndrome.

Neurodegeneration associated with Alzheimer's disease in Down syndrome is a particular area of focus and Ts21 iPSCs are being used to investigate the link between chromosome 21 genes (e.g. APP) and neurodegeneration [15]. These types of studies will not only provide establishment of phenotypes, but will enable screening of potential therapies. In addition to the new areas of application of Ts21 iPSCs, new technologies will likely move the field forward. Advances in cell culture (e.g. 3 dimensional cultures), gene manipulation (e.g. CRISPR Cas9) and the ability to manipulate intracellular signaling pathways (e.g. DREADDs, optogenetics) will undoubtedly improve our ability to use Ts21 iPSCs to better understand the consequences of trisomy 21 and how they lead to the characteristics of Down syndrome.

References

1. Thomson, J.A., et al., *Embryonic stem cell lines derived from human blastocysts.* Science, 1998. **282**(5391): p. 1145-7.
2. Takahashi, K., et al., *Induction of pluripotent stem cells from adult human fibroblasts by defined factors.* Cell, 2007. **131**(5): p. 861-872.
3. Yu, J., et al., *Induced pluripotent stem cell lines derived from human somatic cells.* Science, 2007. **318**(5858): p. 1917-1920.
4. Park, I.H., et al., *Reprogramming of human somatic cells to pluripotency with defined factors.* Nature, 2008. **451**(7175): p. 141-146.
5. Lott, I.T., *Neurological phenotypes for Down syndrome across the life span.* Prog.Brain Res., 2012. **197**: p. 101-121.
6. Lott, I.T. and M. Dierssen, *Cognitive deficits and associated neurological complications in individuals with Down's syndrome.* Lancet Neurol., 2010. **9**(6): p. 623-633.
7. Zhang, S.C., et al., *In vitro differentiation of transplantable neural precursors from human embryonic stem cells.* Nat Biotechnol, 2001. **19**(12): p. 1129-33.
8. Chambers, S.M., et al., *Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling.* Nat Biotechnol, 2009. **27**(3): p. 275-80.
9. Briggs, J.A., et al., *Integration-Free Induced Pluripotent Stem Cells Model Genetic and Neural Developmental Features of Down Syndrome Etiology.* Stem Cells, 2012.
10. Lu, H.E., et al., *Modeling neurogenesis impairment in down syndrome with induced pluripotent stem cells from Trisomy 21 amniotic fluid cells.* Exp.Cell Res., 2012.
11. Weick, J.P., et al., *Deficits in human trisomy 21 iPSCs and neurons.* Proc Natl Acad Sci U S A, 2013. **110**(24): p. 9962-7.
12. Hibaoui, Y., et al., *Modelling and rescuing neurodevelopmental defect of Down syndrome using induced pluripotent stem cells from monozygotic twins discordant for trisomy 21.* EMBO Mol Med, 2014. **6**(2): p. 259-77.

13. Murray, A., et al., *Brief report: isogenic induced pluripotent stem cell lines from an adult with mosaic down syndrome model accelerated neuronal ageing and neurodegeneration.* Stem Cells, 2015. **33**(6): p. 2077-84.

14. Olmos-Serrano, J.L., et al., *Down Syndrome Developmental Brain Transcriptome Reveals Defective Oligodendrocyte Differentiation and Myelination.* Neuron, 2016. **89**(6): p. 1208-1222.

15. Shi, Y., et al., *A human stem cell model of early Alzheimer's disease pathology in Down syndrome.* Sci.Transl.Med., 2012. **4**(124): p. 124ra29.