Healthcare, Gene therapy, Drug devlopment

A human isogenic iPSC-derived cell line panel identifies major regulators of aberrant astrocyte proliferation in Down syndrome

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Abstract

Astrocytes exert adverse effects on the brains of individuals with Down syndrome (DS). Although a neurogenic-to-gliogenic shift in the fate-specification step has been reported, the mechanisms and key regulators underlying the accelerated proliferation of astrocyte precursor cells (APCs) in DS remain elusive. Here, we established a human isogenic cell line panel based on DS-specific induced pluripotent stem cells, the XIST-mediated transcriptional silencing system in trisomic chromosome 21, and genome/chromosome-editing technologies to eliminate phenotypic fluctuations caused by genetic variation. The transcriptional responses of genes observed upon XIST induction and/or downregulation are not uniform, and only a small subset of genes show a characteristic expression pattern, which is consistent with the proliferative phenotypes of DS APCs. Comparative analysis and experimental verification using gene modification reveal dose-dependent proliferation-promoting activity of DYRK1A and PIGP on DS APCs. Our collection of human isogenic cell lines provides a comprehensive set of cellular models for further DS investigations.

Background & Results

Down syndrome (DS; trisomy 21) is the most common form of chromosomal aberration, which results from an extra copy of human chromosome 21. All individuals with DS exhibit various types of clinical features, including intellectual disability and cognitive deficits. Although the healthy human brain contains almost equal numbers of neuronal and glial cells, studies with DS brains showed a significantly reduced number of neurons and nearly twice as many astrocytes compared with that of age-matched controls.

To eliminate biological 'noise', which can result from genetic variability, we established an isogenic iPSC panel where all cell lines share a single genetic background by combining DS-specific iPSCs, *XIST*-induced chromosome silencing, and genome/chromosome-editing technologies. These cell lines were subjected to astrocytic differentiation, and comparative analysis between their gene-expression profiles and proliferative phenotypes (with a common genetic background) was performed. Once *XIST*-induced silencing was stabilized, the transcriptional levels of most genes were continuously suppressed after the removal of doxycycline (Dox). However, the enhanced proliferative phenotype of APCs in DS, which was suppressed by chromosome silencing, returned to aberrantly accelerated conditions by Dox removal. Careful analysis of this discrepancy between transcriptional and phenotypic responses enabled us to narrow down the causative genes responsible for astrocyte overproliferation. We further established various types of systematically designed partial trisomy 21 iPSCs (Partial-Tri21 iPSCs), leading to the identification of two responsible genes, namely dual-specificity tyrosine-phosphorylation-regulated kinase 1A (*DYRK1A*) and phosphatidylinositol glycan anchor biosynthesis, class P (*PIGP*).

Significance of the research and Future perspective

DS astrocytes exert toxic effects on the formation and maturation of neural networks and neuron survival by reducing neuronal activity, inducing morphological alterations, and promoting neuronal apoptosis. In the DS brain, astrocytes may act as important modulators in DS pathophysiology. Thus, identifying critical regulators for astrocyte overpopulation may be a critical first step for investigating disease mechanisms and developing new therapeutic strategies for DS.

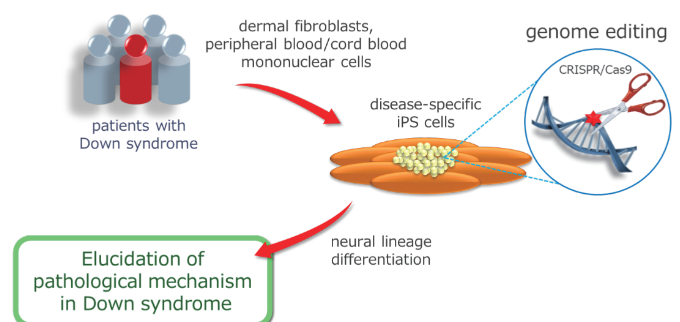

Fig1. Modeling Down syndrome with patient-specific iPS cells
Down syndrome-specific iPS cells were generated from dermal fibroblasts or peripheral/cord blood mononuclear cells. Genome-edited iPS cells using CRISPR/Cas9 were differentiated into the neural lineage for the elucidation of pathological mechanism in Down syndrome.

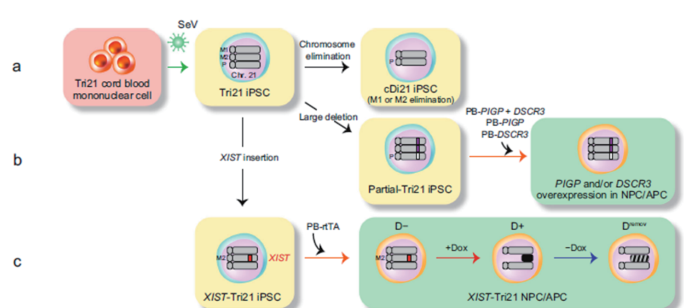

Fig. 2 Generation of an isogenic iPSC panel for disease modelling of DS.
a, DS-specific iPSC line was established using Sendai virus. A corrected-disomy cell line was generated by eliminating a single copy of chromosome 21 from a Tri21 iPSC line.
b, A 4-Mb region corresponding to a 'Down syndrome critical region' was selectively deleted only from the paternal chromosome 21 in Tri21 iPSCs to generate Partial-Tri21 iPSC.
c, Dox-inducible human XIST cDNA was inserted into one copy of chromosome 21 in Tri21 iPSCs (XIST-Tri21 iPSC) for chromosome silencing.

Patent	
Treatise	Kawatani, Keiji; Nambara, Toshihiko; Kitabatake Yasuji et al. A human isogenic iPSC-derived cell line panel identifies major regulators of aberrant astrocyte proliferation in Down syndrome. Commun Biol. 2021; 4(1): 730. doi: 10.1038/s42003-021-02242-7
URL	https://www.nature.com/articles/s42003-021-02242-7
Keyword	down syndrome, iPS cell, genome editing, astrocyte