Generation of an iPSC line from a Pontocerebellar Hypoplasia 1B patient harboring a homozygous c.395 A > C mutation in EXOSC3 along with a family matched control

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Abstract

Pontocerebellar Hypoplasia 1B (PCH1B) is a severe autosomal recessive neurological disorder that is associated with mutations in the exosome complex component RRP40 (EXOSC3) gene. We generated and characterized an iPSC line from an individual with PCH1B that harbors a recessive homozygous c.395 A > C mutation in EXOSC3 and a family matched control from the probands unaffected mother. Each iPSC line presents with normal morphology and karyotype and express high levels of pluripotent markers. UAZTi009-A and UAZTi011-A are capable of directed differentiation and can be used as a vital experimental tool to study the development of PCH1B.

1. Resource utility

Two iPSC lines generated allow for the generation of various cell types implemented in Pontocerebellar Hypoplasia 1B which will enable researchers to study the c.395 A > C mutation in EXOSC3 and the mechanisms by which it contributes to the disease phenotype (Table 1).

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2022.102944.
2. Resource details

Pontocerebellar Hypoplasia 1B is a severe autosomal recessive neurological disorder and is clinically characterized by large neuronal loss in the ventral pons and inferior olive, motor neuron degeneration in the spinal cord, and underdevelopment of the neocerebellum (Rudnik-Schoneborn et al., 2013). Exosome complex component RRP40 (EXOSC3) is a subunit of the RNA exosome complex and mutations in EXOSC3 are associated with Pontocerebellar Hypoplasia 1B and neuro degeneration (François-Moutal et al., 2018). Using Sendai virus reprogramming (Churko et al., 2013) we have generated one iPSC line from fibroblasts of a Pontocerebellar Hypoplasia 1B patient harboring the homozygous c.395 A > C, mutation in EXOSC3 (Fig. 1A), and an iPSC line from their unaffected mother. All cell lines possess normal morphology (Fig. 1B) and were negative for mycoplasma (Supplementary Fig. 1A) and Sendai virus (Supplementary Fig. 1B). All iPSC lines expressed the iPSC markers SOX2, NANOG and OCT4 at the mRNA level and OCT4, and SSEA4 at the protein level assessed by reverse transcription quantitative polymerase chain reaction (RT-qPCR) (Fig. 1C) and immunocytochemistry (Fig. 1D) respectively. iPSC lines were able to undergo directed differentiation into the three germ layers and were positive for mRNA markers of Endoderm, Ectoderm and Mesoderm when differentiated (Fig. 1E). Furthermore, no karyotypic abnormalities were observed (Fig. 1F).

3. Materials and methods

3.1. Reprogramming

Reprogramming of patient fibroblasts was performed at the University of Arizona iPSC core under IRB approval. Fibroblasts were isolated from skin puncture biopsies and 250,000 cells were incubated with Sendai virus to express Sox2, Oct3/4, klf4 and cMyc using the Cyto-Tune®-iPS v2.0 Reprogramming Kit (Thermo Fisher Scientific; A16517). After ~20 days, single iPSC colonies were picked and expanded in Essential 8 (E8) medium (Thermo Fisher Scientific).

3.2. Cell Culture

iPSC lines were cultured in E8 media on 6 well plates coated with growth factor reduced Matrigel (Corning™; Cat #CB356238) until 70% confluency under normoxic conditions (37°C, 5% CO2, 20% O2). iPSC lines were passaged in E8 and 10 μM ROCK inhibitor (Y27632, Tocris; #Cat1254) at a 1:6 ratio every 4–5 days.

3.3. Mutation analysis

DNA was extracted and purified from each iPSC line before PCR amplification using PrimeStar GXL polymerase (Takara; R050B) according to manufacturer’s instructions. Amplicons were sequenced for the c.395 A > C mutation in EXOSC3 (Eton Biosciences) to confirm the genotype of each individual cell line by sanger sequencing.

3.4. Immunocytochemistry

iPSC lines were seeded onto Matrigel coated coverslips and cultured in E8 medium until fixation. Cells were fixed in 3.7% formalin for 60 min at room temperature. Fixed cells
were permeabilized with 0.15% Triton X-100 in PBS prior to blocking in 1% BSA, 22.52 mg/ml glycine and 0.1% Tween 20 in PBS (PBST). Cells were incubated with primary antibodies (Table 2) in blocking solution (minus glycine) overnight in a humidified chamber at 4 °C. Following the primary antibody incubation, secondary antibodies (Table 2) were incubated at room temperature in the dark for 60 min. Following secondary antibody incubations, cells were washed 3 × 5 min in PBST prior to nuclear counter staining using DAPI (ThermoFisher; D1306).

3.5. Trilineage Differentiation

All iPSC lines were differentiated into Endoderm, Ectoderm and Mesoderm using the StemMACSTM Trilineage Differentiation Kit (Miltenyi Biotech; 130-115-660) according to manufacturer instructions.

3.6. RT-qPCR

RNA was extracted and isolated from iPSCs at passage 15–18 using the Direct-zol RNA Miniprep kit (Zymo Research; R2052). Isolated RNA was reverse transcribed into cDNA using the SuperScript VILO master mix (ThermoFisher; 11755050). qPCR was performed using the power track SYBR Green Master Mix (ThermoFisher; A46012). Samples were normalized to the H7 hESC line (WiCell).

3.7. Mycoplasma testing

Mycoplasma analysis was conducted using the PCR Mycoplasma Test Kit I/C (PromoKine, PK-CA91-1024) using the high sensitivity method, according to manufacturer instructions.

3.8. Karyotyping and STR analysis

2 × 10^6 cells were collected from each line at passage 15–17 and karyotyping analysis was conducted by Life Technologies using the KaryoStat™ assay (ThermoFisher). Primary human fibroblast samples were used for STR analysis to confirm genetic similarity between patient fibroblasts and iPSC lines.

3.9. Sendai virus Clearance

RNA was collected from cells at passage 15–16 using the the Directzol RNA Miniprep kit (Zymo Research; R2052). RNA was amplified by PCR using Taq DNA polymerase (GoldBio, T-514). Cells at passage 1 were used as a positive control.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations:

EXOSC3 Exosome Component 3
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Fig. 1.
Characterization of UAZTi009-A and UAZTi011-A iPSC lines.
Characterization and validation.

| Classification          | Test                                      | Result                                      | Data                        |
|------------------------|-------------------------------------------|---------------------------------------------|-----------------------------|
| **Morphology**         | Bright field                              | Normal                                      | Fig. 1Panel B               |
| **Phenotype**          | Qualitative analysis; Immunocytochemistry | Assess staining of pluripotency markers; OCT4, SSEA-4 | Fig. 1Panel D               |
|                        | Quantitative analysis; RT-qPCR            | Assessed the Pluripotency markers OCT4, NANOG, SOX2 | Fig. 1Panel C               |
| **Genotype**           | Karyotype (KaryoStat) 150k SNPs analysed >2 Mb (Chromosomal gains) >1 Mb (Chromosomal losses) | Normal                                      | Fig. 1 Panel F              |
| **Identity**           | Microsatellite PCR (mPCR) OR STR analysis | Not performed Normal                        | NA                          |
| **Mutation analysis (IF APPLICABLE)** | Sanger Sequencing                        | UAZTi009-A; Heterozygous Mutation UAZTi011-A; Homozygous Mutation | Fig. 1 Panel A              |
|                        | Southern Blot OR WGS                      | NA                                          | NA                          |
| **Microbiology and virology** | Mycoplasma                               | Mycoplasma Negative; Tested by PCR          | Supplementary Fig. 1A       |
| **Differentiation potential** | Directed differentiation                  | Directed Differentiation into Ectoderm, Endoderm and Mesoderm germ layers | Fig. 1 Panel E              |
| **List of recommended germ layer markers** | RT-qPCR                                   | Markers Assessed via RT-qPCR                | Fig. 1 Panel E              |
|                        |                                          | Ectoderm: PAX6, SOX1                        |                             |
|                        |                                          | Endoderm: SOX17, FOXA2                      |                             |
|                        |                                          | Mesoderm: TBXT, TBX6                        |                             |
| **Donor screening (OPTIONAL)** | HIV 1 + 2 Hepatitis B, Hepatitis C        | N/A                                         | N/A                         |
| **Genotype additional info (OPTIONAL)** | Blood group genotyping                   | N/A                                         | N/A                         |
|                        |                                          | HLA tissue typing                           | N/A                         |
## Table 2

### Table 2: Reagents details.

| Antibodies used for immunocytochemistry/flow-cytometry | Antibody | Dilution | Company Cat # | RRID |
|--------------------------------------------------------|----------|----------|---------------|------|
| **Pluripotency Markers (ICC)**                         | Mouse anti-OCT4 | 1:100 | #60093 | AB_2801346 |
|                                                        | Mouse Anti SSEA4 | 1:100 | #560308 | AB_1645371 |
| **Secondary antibodies**                               | F(ab')2-Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 | 1:1000 | #A11017 | AB_2534084 |

| | Primers Target | Size of Band | Forward/Reverse primer (5′–3′) |
|-----------------|---------------|-----------------|-----------------------------|
| **Pluripotency Markers (qPCR)** | NANOg | 116 | TTTGTGGGCTGAAGAAAAC/CTCTTGCTCCTGAATAGCAG |
| | OCT4 | 106 | CCTGAAAGCAAGAGGATCACC/AAAGCGGCAGAGTGTCGTTGG |
| | SOX2 | 150 | AGAGAAGGAGGAGAAGAAAGGAGAGA/GAGAGAGGCAACTGGAATCAGATCAG |
| **Differentiation Markers; Ectoderm (qPCR)** | PAX6 | 131 | CTGAGGAAATCAGAGAAGAGAGG/ATGGAGCCAGATGTAAGGAGG |
| | SOX17 | 136 | GAGTGGGAAGGCTATGGTGG/GCTCTTTAAGGTCAGCCCTTCAGCATG |
| | FOXA2 | 134 | GAGAACACACCCTACTAGGCTCTCAAC/AGTTGACTAACCTTGCTTGG |
| **Differentiation Markers; Endoderm (qPCR)** | TBX6 | 153 | CTTCTGCTCAAACCTACTAGGCTCCTC/TCAAGGGAAGAGGAGG |
| | SOX17 | 112 | AGCCTTCTATGGTGGTGG/GCTTAAGGTAGCCCTTCACAGCAT |
| **Differentiation Markers; Mesoderm (qPCR)** | TBX6 | 136 | TCTCTTCTGCAGGCT/ACGCTTCTCCTCATAGAAGC |
| | FOXA2 | 134 | GGAACACACCCTACTAGGCTCTCAAC/AGTTGACTAACCTTGCTTGG |
| | TBX6 | 153 | CTTCTGCTCAAACCTACTAGGCTCCTC/TCAAGGGAAGAGGAGG |
| **House Keeping Gene(s)** | 18 s rRNA | 159 | ACCCGTGTGACGCGCTAGG/ATTTCCTTCTACCAATCAG |
| **Targeted mutation** | EXOSC3 | 941 | CCTCTGCTCAAACCTACTAGGCTCCTC/TCAAGGGAAGAGGAGG |
| **Sendai Virus Clearance** | SeV | 941 | CAGAGGACAGTCTACAGGAG/CCGAGGAGGAGGAGG |

RRID Requirement for antibodies: use [https://antibodyregistry.org/](https://antibodyregistry.org/) to retrieve RRID for antibodies and include ID in table as shown in examples.
### Resource Table:

| Unique stem cell lines identifier | 1) UAZTi009-A  
2) UAZTi011-A |
|----------------------------------|-----------------|
| Alternative name(s) of stem cell lines | UAZTi009-A; MKAZ1  
UAZTi011-A; MKAZ3 |
| Institution | University of Arizona |
| Contact information of distributor | Dr Jared Churko PhD  
jchurko@arizona.edu |
| Type of cell lines | iPSC |
| Origin | Human |
| Additional origin info required for human ESC or iPSC | UAZTi009-A; Female  
UAZTi011-A; Male |
| Cell Source | Fibroblast |
| Clonality | Clonal |
| Method of Reprogramming | Sendai Virus |
| Sendai Virus Clearance | PCR |
| Type of Genetic Modification | NA |
| Cell Culture System | Matrigel |
| Associated disease | Pontocerebellar Hypoplasia 1B |
| Gene/locus | EXOSC3 c.395 A > C |
| Date archived/stock date | May 31, 2022 |
| Cell line repository/bank | [https://hpscreg.eu/cell-line/UAZTi009-A](https://hpscreg.eu/cell-line/UAZTi009-A)  
[https://hpscreg.eu/cell-line/UAZTi011-A](https://hpscreg.eu/cell-line/UAZTi011-A) |
| Ethical approval | WIRB® Protocol #20120789, and University of Arizona #1808846797 |