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Lab Resource: Stem Cell Line

Generation of an induced pluripotent stem cell line that mimics the disease phenotypes from a patient with Fanconi anemia by conditional complementation

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ABSTRACT

Generation of Fanconi anemia (FA) patient-specific induced pluripotent stem cells (iPSCs) has been reported to be technically challenging due to the defects in the FA-pathway in the patients' somatic cells. By inducible complementation of FA-pathway, we successfully reprogrammed the fibroblasts of an FA patient to iPSCs. CSCR19i-indCFANCA, one of the iPSC lines generated by the inducible complementation of FA-pathway, was extensively characterized for its pluripotency and karyotype. In the absence of doxycycline (DOX) and FANCA expression, this line showed the cellular phenotypes of FA, suggesting it is an excellent tool for FA disease modeling and drug screening.

Resource table.

Name of stem cell line	CSCR19i-indCFANCA
Institution	Christian Medical College and Centre for Stem Cell Research
Person who created resource	Shaji R Velayudhan
Contact person and email	Shaji R Velayudhan rvshaji@cmcvellore.ac.in
Date archived/stock date	22.10.2014
Origin	Human skin fibroblasts
Type of resource	Fanconi anemia induced pluripotent stem cell (iPSC) line that mimics the disease phenotype
Sub-type	Induced pluripotent stem cell line
Key transcription factors	OCT4, SOX2, KLF4 and cMYC
Authentication	Identity and purity of the cell line was confirmed by analysis of expression of pluripotency markers and *in vivo* differentiation potential (Fig. 1) and DNA fingerprinting (Supplementary Fig. 1)
Link to related literature	https://www.ncbi.nlm.nih.gov/pubmed/18035408 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2720823/ https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3369681/ https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4719133/
Information in public databases	https://rarediseases.org/rare-diseases/fanconi-anemia/ https://ghr.nlm.nih.gov/condition/fanconi-anemia
Ethics	Informed consent from the patient and Institution Review Board approval were obtained

1. Resource details

Fanconi anemia (FA) is caused by genetic defects in the Fanconi anemia pathway (FA-pathway), which is involved in DNA inter-strand cross-link repair. The disease is characterized by progressive bone marrow failure with a significant number of patients developing additional complications including leukemia, myelodysplastic syndrome and solid tumours. Somatic cells from FA patients are highly refractory to reprogramming, and restoration of the functional FA-pathway was found to be essential for the generation of FA-iPSC lines with normal karyotypes (Chlon et al., 2016; Müller et al., 2012; Raya et al., 2009; Yung et al., 2013). For the derivation of an FA patient-specific hiPSC line capable of mimicking the disease phenotypes *in vitro*, we used inducible complementation strategy. Dermal fibroblasts were obtained from an individual diagnosed to have FA based on clinical features and a very high number of chromosome breakages in the peripheral blood lymphocytes after treatment with Mitomycin C. Complementation analysis (Casado et al., 2007; Chandra et al., 2005; Pinto et al., 2009) was performed by transducing the fibroblasts with a DOX-inducible lentiviral vector encoding FANCA protein (pINDUCER20-FANCA) (Fig.

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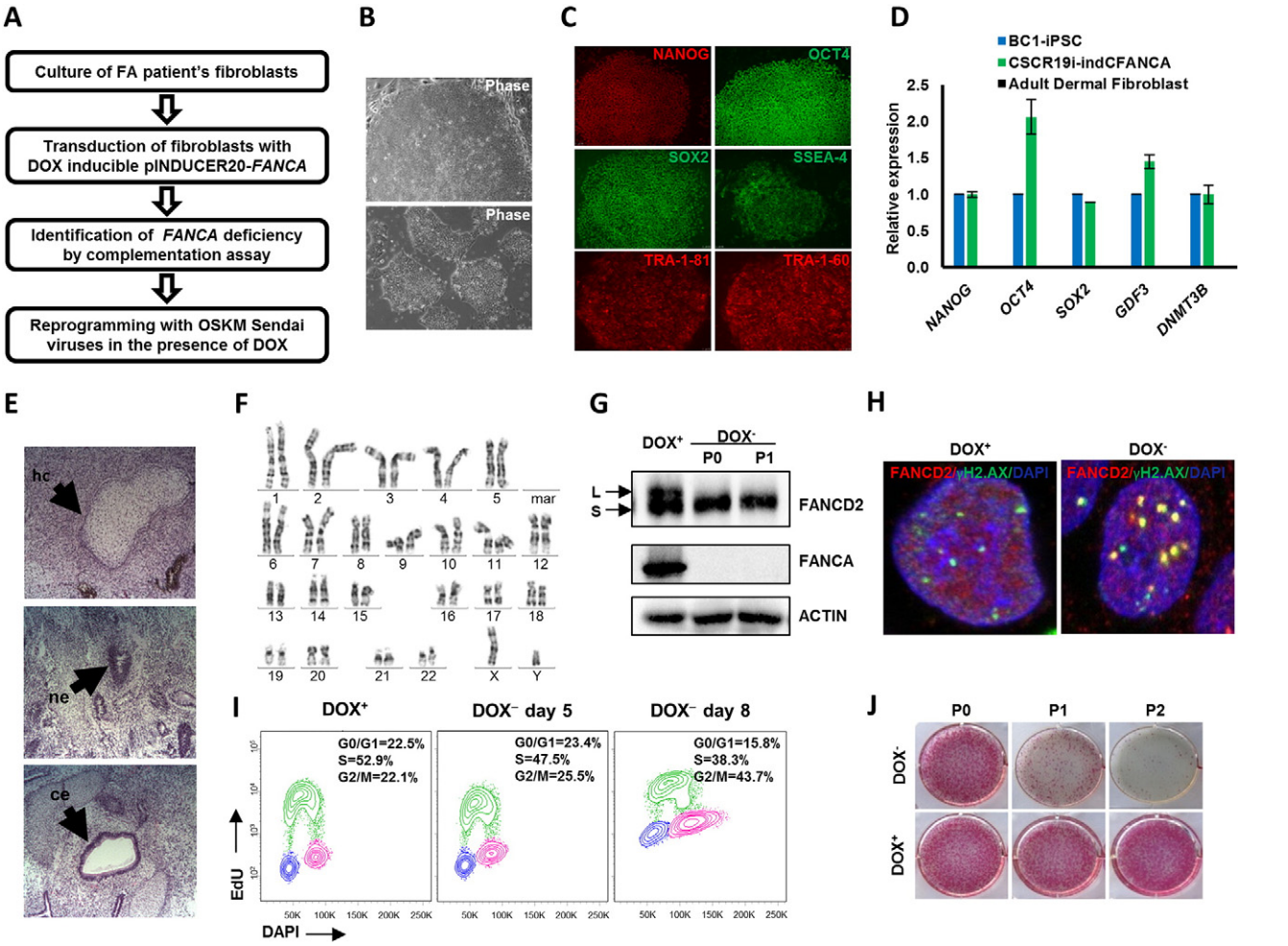

Fig. 1. Establishment of CSCR19i-indCFANCA line that exhibits the FA cellular phenotypes. A. Schematic of molecular diagnosis of FANCA deficiency and iPSC generation. B. Morphology of iPSCs grown on feeder layers (upper panel) and feeder-free vitronectin coated plates (lower panel). C. Immunofluorescence analysis of NANOG, OCT4, SOX2, SSEA-4, TRA-1-81 and TRA-1-60. D. Real-time PCR analysis of mRNA levels of NANOG, OCT4, SOX2, GDF3 AND DNMT3B, relative to the BC1 control iPSC line (data represented as mean ± SD). E. H&E-staining of teratoma sections showing trilineage differentiation; hc-hyaline cartilage (mesoderm), ne-neuroepithelium (ectoderm) and ce-columnar epithelium (endoderm). F. Chromosome analysis showing normal male karyotype. G. Western blot analysis of the cells treated with Hydroxyurea showing the lack of FANCD2-monoubiquitination in the absence of DOX and FANCA expression. L and S indicate the larger monoubiquitinated and the smaller unmodified FANCD2 bands, respectively. H. Immunofluorescence analysis showing nuclear foci formed by γH2A.X and FANCD2. The nuclei are stained with DAPI (violet colour). In DOX$^+$ cells, colocalization of the foci formed by γH2A.X and FANCD2 are seen as yellow spots due to the merging of green spots of γH2A.X foci and red spots of FANCD2 foci, and in DOX-cells, only green spots of γH2A.X foci are seen. I. EdU incorporation based cell cycle analysis showing a spontaneous progressive G2/M arrest in the absence of DOX and FANCA expression for two passages. J. Alkaline phosphatase staining showing spontaneous progressive exhaustion of iPSCs in the absence of DOX and FANCA expression for two passages.

1A), followed by western blot analysis for FANCD2 ubiquitination. Restoration of FANCD2 ubiquitination in these fibroblasts cultured in the presence of DOX, suggested that *FANCA* gene was defective in this patient. We reprogrammed the pINDUCER20-FANCA transduced fibroblasts using Sendai Viruses to express the reprogramming factors along with FANCA complementation in the presence of DOX (Fig. 1A). Six iPSC colonies were isolated based on the morphology and cultured in the presence of DOX. One of the iPSC lines that was extensively cultured for more than 40 passages to establish the inducible complementation iPSC line, CSCR19i-indCFANCA, is described in this article (Fig. 1B). This iPSC line expressed pluripotency markers at levels comparable to a control iPSC line, BC1-hiPSC line (Fig. 1C–D), could form teratomas with three germ layers (Fig. 1E) and had a normal karyotype (Fig. 1F). On DOX withdrawal, this cell line showed the absence of FANCA expression (Fig. 1G) and the features of FA cells, *i.e.* the lack of FANCD2 ubiquitination (Fig. 1G), lack of γ H2A.X FANCD2 colocalization on DNA damage sites (Fig. 1H), and cell cycle arrest at G2/M phase (Fig. 1I) leading to cell death and progressive exhaustion in culture (Fig. 1J). DNA fingerprinting analysis confirmed the genetic identify of this iPSC line and donor fibroblasts (Supplementary Fig. 1A). Cellular phenotype analysis of all the six clones showed the phenotypes depicted in Fig. 1G–J. Our results showed that the CSCR19i-indCFANCA line and the haematopoietic cells derived from this line, in the presence and the absence of DOX, can be used for understanding FA disease mechanisms.

2. Materials and methods

2.1. Fibroblast culture

Dermal fibroblasts were generated from a 10 mm^2 skin biopsy sample of an FA patient, after obtaining informed consent. The sample was cut into small pieces and was cultured in a six-well tissue culture plate containing Amniomax medium. The fibroblasts were passaged using 0.05% trypsin and maintained in a culture medium containing α-MEM with 20% FBS.

2.2. Preparation of pINDUCER20-FANCA lentiviruses

The DOX-inducible pINDUCER20-FANCA expression vector was constructed by Gateway cloning with pINDUCER20 destination vector (a