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Lab resource: Stem cell line

Induced pluripotent stem cell line from an atopic dermatitis patient heterozygous for c.2282del4 mutation in filaggrin: KCLi001-A

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ABSTRACT

We have generated an induced pluripotent stem cell (iPSC) line KCLi001-A (iOP118) from a female atopic dermatitis (AD) patient, heterozygous for the loss-of-function mutation c.2282del4 in the filaggrin gene (FLG). Epidermal keratinocytes were reprogrammed using non-integrating Sendai virus vectors. The entire process of derivation and expansion of AD-iPSCs were performed under xeno-free culture conditions. Characterization of KCLi001-A line included molecular karyotyping, mutation screening using restriction enzyme digestion and Sanger sequencing, while pluripotency and differentiation potential were confirmed by expression of associated markers in vitro and by in vivo teratoma assay.

Resource table

| Unique stem cell line identifier | KCLi001-A |
| Alternative name(s) of stem cell line | iOP118 |
| Institution | King’s College London, London UK |
| Contact information of distributor | Dusko ILIC, dusko.ilic@kcl.ac.uk |
| Type of cell line | iPSC |
| Origin | Human |
| Sex: Female |
| Ethnicity: Caucasian |
| Cell source | Epidermal keratinocytes |
| Clonality | Clonal |
| Method of reprogramming | Non-integrating SeV-mediated delivery of OCT4, SOX2, c-MYC and KLF4 |
| Genetic modification | None |
| Type of modification | N/A |

Associated disease: Atopic dermatitis (AD) or eczema, OMIM #605803
Gene/locus: Filaggrin gene (FLG), loss-of-function mutation NM_002016.1:c.2282del4
Method of modification: N/A
Name of transgene or resistance: N/A
Inducible/constitutive system: N/A
Date archived/stock date: December 2017
Cell line repository/bank: N/A
Ethical approval: Ethics Committee of the Medical University of Innsbruck, Austria (AN2016-0260)

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Resource utility

Generation of a library of human iPSC lines with the most common variants in the FLG gene can be efficiently used to construct highly specific in vitro 3D skin models (Petrova et al., 2014) for drug discovery towards novel personalized therapies in AD.

Resource details

AD or eczema is an incurable, non-contagious, extensive inflammatory and extremely pruritic chronic cutaneous disorder. AD is one of the most common skin diseases which affects up to 20% of children and approximately 3% of adults worldwide, while its
prevalence is continuously increasing, particularly in underdeveloped countries (Asher et al., 2006). Several loss-of-function mutations within FLG exon 3, including c.2282del4 variant, are considered to be the most significant risk factors for atopic dermatitis in the European population (Palmer et al., 2006). The epidermal keratinocytes derived from a female AD patient who is heterozygous for c.2282del4, were programmed into iPSCs following previously established protocol with genome non-integrating Sendai virus (SeV) vectors (Miere et al., 2016a). Three weeks post-transduction colonies with a typical morphology of pluripotent stem cells appeared and were selected to establish feeder-free iPSC clones (Fig. 1A). After ten passages, the examination of the SeV vectors was confirmed in the KCLi001-A cell line by RT-PCR using specific primers (Fig. 1B). The clones were screened with restriction enzyme digestion and we have verified that the AD-related mutation (NM_002016.1:c.2282del4) was retained in the iPSCs. This finding was also confirmed independently by Sanger sequencing (Fig. 1C). Endogenous expression of pluripotency-related molecular markers (TRA-1-60, TRA-1-81, OCT4, NANOG) in the iPSCs was assessed by double immunofluorescence technique (Fig. 1D). Furthermore, undifferentiated colonies were also positive for alkaline phosphatase (AP) (Fig. 1D). Differentiation capacity of the KCLi001-A cells into three germ layers was determined by specific immunofluorescence staining of AFP (liver, endoderm), ACTA2 (cardiac muscle, mesoderm), and TUBB3 (neurons, ectoderm) in vitro (Fig. 1E), as well as in vivo through a teratoma formation assay. All three germ layers, ectoderm, mesoderm, and endoderm, were present in the teratoma, as demonstrated by immunohistochemical analysis (Fig. 1F).

Table 1
Characterization and validation.

| Classification                        | Test                                                                 | Result                                                                 | Data               |
|---------------------------------------|----------------------------------------------------------------------|------------------------------------------------------------------------|--------------------|
| **Morphology**                        | Light microscopy                                                     | hESC-like morphology (compact, dense, roundly shaped colonies with sharp edges, high nucleus to cytoplasm ratio) | Fig. 1 panel A     |
| **Phenotype**                         | Qualitative analysis (Immunofluorescence staining and AP activity)   | Expression of pluripotency- markers TRA-1-60, TRA-1-81, OCT4, NANOG; AP-positive | Fig. 1 panel D     |
| Quantitative analysis (Immunofluorescence counting) | Percentage of cells positive for pluripotent markers: OCT4-94%, NANOG - 95, TRA-1-60: 95%, TRA-1-81: 93% | Submitted in archive with journal | Fig. 1 panel D     |
| **Genotype**                          | Array CGH                                                           | Not tested                                                             | N/A                |
| **Identity**                          | STR analysis                                                        | DNA fingerprinting PCR, 17 specific markers tested                     | Submitted in archive with journal | Fig. 1 panel C     |
| **Mutation analysis**                 | Sequencing                                                          | Heterozygous, c.2282del4 in exon 3 of FLG                             | Fig. 1 panel C     |
| Restriction enzyme digestion          | Mutation 2282del4 creates a new DraIII site, which was used to screen short, highly specific PCR fragments for this variant. | Supplementary Fig. 1 |                    |
| **Microbiology and virology**         | Mycoplasma                                                          | LookOut Mycoplasma PCR Detection Kit: negative (Supplementary file 1) | Fig. 1 panel E     |
| **Differentiation potential**         | Embryoid body formation                                             | Expression of smooth muscle actin (ACTA2), a-fetoprotein (AFP) and JilII-tubulin (TUBB3) | Fig. 1 panel F     |
| Teratoma formation                    | Alcian blue/periodic acid Schiff (PAS)-stained cartilage and desmin for mesoderm, TUBB3 and glial fibrillar acidic protein (GFAP) for ectoderm, and GATA4 and AFP for endoderm, while mitochondrially encoded cytochrome C oxidase II (MTCO2) only immunostains human mitochondria in the cells of the teratoma | Not tested | N/A                |
| Directed differentiation into keratinocytes | The iPSC-derived keratinocytes expressed the epithelial cell markers: KRT14, KRT18, and isoform of TP63 (ΔNp63) |Fig. 1 panel G          |
| **Donor screening**                   | HIV 1 + 2 Hepatitis B, Hepatitis C                                  | Not tested                                                             | N/A                |
| Genotype additional info              | Blood group genotyping                                              | Not tested                                                             | N/A                |
|                                      | HLA tissue typing                                                   | Not tested                                                             | N/A                |

Since our aim is to use the line for modeling AD in vitro, we tested differentiation of the KCLi001-A iPSCs into epidermal keratinocytes (Petrova et al., 2014). The cells expressed keratinocyte-specific markers - keratins 14 and 18 (KRT14, KRT18), and isoform of TP63 known as ΔNp63 within three weeks in culture as expected (Fig. 1G). Examination of the genomic integrity of our AD-iPSC line using array CGH after more than twenty passages showed a normal female karyotype (46, XX), whereas smaller imbalances have not been excluded (submitted in archive with journal). Taken together, these results prove that we have successfully produced a stable AD patient specific iPSC line which can provide a powerful tool for: 1) developing the first iPSC-derived 3D in vitro AD-human skin equivalents (HSE); 2) deciphering the molecular mechanisms of the disease; 3) innovative drug screening platform in atopic dermatitis. (Table 1).
Materials and methods

Epidermal keratinocytes reprogramming

Patient keratinocytes of passage 3 were transduced with genome integration-free SeV virus kit (CytoTune 2.0, Life Technologies) as described (Miere et al., 2016a). Clonal selection of fully reprogrammed cells was performed manually by picking individual clones with hESC-like appearance (Table 1). The iPSCs under feeder-free culture conditions were assessed (Table 1), as previously described (Petrova et al., 2014).

Molecular karyotyping

Array comparative genomic hybridization (aCGH) and short tandem repeat (STR) analysis of 17 STR loci were conducted at Viapath Genetics Centre.

Pluripotency markers

The pluripotency status of KCLi001-A line was evaluated by immunostaining for three germ layer markers in spontaneously differentiated cells (Table 1) as previously described (Petrova et al., 2014).
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