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Disease-specific induced pluripotent stem (iPS) cells

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Abstract

Tissue culture of immortal cell strains from diseased patients is an invaluable resource for medical research, but is largely limited to tumor cell lines or transformed derivatives of native tissues. Here we describe the generation of induced pluripotent stem (iPS) cells from patients with a variety of genetic diseases with either Mendelian or complex inheritance that include: adenosine deaminase deficiency-related severe combined immunodeficiency (ADA-SCID), Shwachman-Bodian-Diamond syndrome (SBDS), Gaucher disease (GD) type III, Duchenne (DMD) and Becker muscular dystrophy (BMD), Parkinson disease (PD), Huntington disease (HD), juvenile-onset, type 1 diabetes mellitus (JDM), Down syndrome (DS)/trisomy 21 and the carrier state of Lesch-Nyhan syndrome. Such patient-specific stem cells offer an unprecedented opportunity to recapitulate both normal and pathologic human tissue formation *in vitro*, thereby enabling disease investigation and drug development.

Introduction

Cell culture has been the back-bone of basic biomedical research for many decades, and countless insights into both normal and pathologic cellular processes have been gleaned by studying human cells explanted *in vitro*. Most of the human cell lines in wide use today carry genetic and epigenetic artifacts of accommodation to tissue culture, and are derived either from malignant tissues or are genetically modified to drive immortal growth (Grimm, 2004). Primary human cells have a limited lifespan in culture, a constraint that thwarts inquiry into the regulation of tissue formation, regeneration, and repair. Indeed, many human cell types have never faithfully been adapted for growth *in vitro*, and the lack of accessible models of normal and pathologic tissue formation has rendered many important questions in human development and disease pathogenesis inaccessible.

Human embryonic stem cells isolated from excess embryos from *in vitro* fertilization clinics represent an immortal propagation of pluripotent cells that theoretically can generate any cell type within the human body (Lerou et al., 2008; Murry and Keller, 2008). Human embryonic stem cells allow investigators to explore early human development through *in vitro* differentiation, which recapitulates aspects of normal gastrulation and tissue formation. Embryos shown to carry genetic diseases by virtue of preimplantation genetic diagnosis (PGD; genetic analysis of single blastomeres obtained by embryo biopsy) can yield stem cell lines that model single gene disorders (Verlinsky et al., 2005), but the vast majority of diseases that show more complex genetic patterns of inheritance are not represented in this pool.

A tractable method for establishing immortal cultures of pluripotent stem cells from diseased individuals would not only facilitate disease research, but also lay a foundation for producing autologous cell therapies that would avoid immune rejection and enable correction of gene defects prior to tissue reconstitution. One strategy for producing autologous, patient-derived pluripotent stem cells is somatic cell nuclear transfer (NT). In a proof of principle experiment, NT-ES cells generated from mice with genetic immunodeficiency were used to combine gene and cell therapy to repair the genetic defect (Rideout et al., 2002). To date, NT has not proven successful in the human, and given the paucity of human oocytes, is destined to have limited utility. In contrast, introducing a set of transcription factors linked to pluripotency can directly reprogram human somatic cells to produce induced pluripotent stem (iPS) cells, a method that has been achieved by several groups worldwide (Lowry et al., 2008; Park et al., 2008b; Takahashi et al., 2007; Yu et al., 2007). Given the robustness of the approach, direct reprogramming promises to be a facile source of patient-derived cell lines. Such lines would be immediately valuable for medical research, but current methods for reprogramming require infecting the somatic cells with multiple viral vectors, thereby precluding consideration of their use in transplantation medicine at this time.

Human cell culture is an essential complement to research with animal models of disease. Murine models of human congenital and acquired diseases are invaluable but provide a limited representation of human pathophysiology. Murine models do not always faithfully mimic human diseases, especially for human contiguous gene syndromes such as trisomy 21 (Down syndrome or DS). A mouse model for the DS critical region on distal human chromosome 21 fails to recapitulate the human cranial abnormalities commonly associated with trisomy 21 (Olson et al., 2004). Orthologous segments to human chromosome 21 are present on mouse chromosomes 10 and 17 and distal human chromosome 21 corresponds to mouse chromosome 16 where trisomy 16 in the mouse is lethal (Nelson and Gibbs, 2004). Thus, a true murine equivalent of human trisomy 21 does not exist. Murine strains carrying the same genetic deficiencies as the human bone marrow failure disease Fanconi anemia demonstrate DNA repair defects consistent with the human condition (e.g. (Chen et al., 1996), yet none develop the spontaneous bone marrow failure that is the hallmark of the human disease.

For cases where murine and human physiology differ, disease-specific pluripotent cells capable of differentiation into the various tissues affected in each condition could undoubtedly provide new insights into disease pathophysiology by permitting analysis in a human system, under controlled conditions *in vitro*, using a large number of genetically-modifiable cells, and in a manner specific to the genetic lesions in each - whether known or unknown. Here, we report the derivation of human iPS cell lines from patients with a range of human genetic diseases.

Results and Discussion

Dermal fibroblasts or bone marrow-derived mesenchymal cells were obtained from patients with a prior diagnosis of a specific disease, and used to establish disease-specific lines of human iPS cells (Table 1). This initial cohort of cell lines was derived from patients with Mendelian or complex genetic disorders, including: Down syndrome (DS; trisomy 21); adenosine deaminase deficiency-related severe combined immunodeficiency (ADA-SCID); Shwachman-Bodian-Diamond syndrome (SBDS); Gaucher disease (GD) type III; Duchenne type (DMD) and Becker type (BMD) muscular dystrophy; Huntington chorea (Huntington disease; HD); Parkinson disease (PD); juvenile-onset, type 1 diabetes mellitus (JDM); and Lesch-Nyhan syndrome (LNSc; carrier state).

Patient-derived somatic cells were transduced with either four (*OCT4*, *SOX2*, *KLF4*, and c-*MYC*) or three reprogramming factors (lacking c-*MYC*). Following two to three weeks of culture in hES cell supporting conditions, compact refractile ES-like colonies emerged amongst a background of fibroblasts, as previously described (Park et al., 2008a; Park et al., 2008b). Although our previous report used additional factors (hTERT and SV40 LT) to achieve reprogramming of adult somatic cells, we have found the four-factor cocktail to be sufficient as long as we employ a higher multiplicity of retroviral infection. Additionally, we generated a single line from a carrier of Lesch-Nyhan Syndrome using five doxycycline-inducible lentiviral vectors (*OCT4*, *SOX2*, *KLF4*, c-*MYC*, and *NANOG*) a strategy that has been used to isolate murine iPS cells (Brambrink et al., 2008; Stadtfeld et al., 2008), but previously had not been attempted with human somatic cells. Characterization of the iPS lines is presented below.

Mutation analysis in iPS lines

The iPS lines were evaluated to confirm, where possible, the disease-specific genotype of their parental somatic cells. Analysis of the karyotype of iPS lines derived from two individuals with Down syndrome showed the characteristic trisomy 21 anomaly (Figure 1A). Aneuploidies such as that occurring in DS are unambiguously associated with advanced maternal age (reviewed in Antonarakis et al., 2004) and as such, are occasionally detected in the preimplantation embryo when IVF is coupled with PGD. While it is possible that a discarded IVF embryo found to have trisomy 21 could be donated to attempt hES cell derivation, it is important to point out that many gestating DS embryos do not survive the prenatal period. Some studies place the frequency of spontaneous fetal demise (miscarriage) in DS to be above 40% (Bittles et al., 2007). Thus, the derivation of a human iPS line with trisomy 21 from an existing individual may be preferable, as such a line is most likely to harbor the complex genetic and epigenetic modifiers that favor full term gestation, and by virtue of the often lengthy medical history, will be a more informative resource for correlative clinical research.

Creation of iPS lines from patients with single-gene disorders allows experiments on disease phenotypes *in vitro*, and an opportunity to repair gene defects *ex vivo*. The resulting cells, by virtue of their immortal growth in culture, can be extensively characterized to ensure that gene repair is precise and specific, thereby reducing the safety concerns of random, viral-mediated gene therapy. Repair of gene defects in pluripotent cells provides a common platform for combined gene repair and cell replacement therapy for a variety of genetic disorders, as long as the pluripotent cells can be differentiated into relevant somatic stem cell or tissue populations.

Three diseases in our cohort of iPS cells are inherited in a classical Mendelian manner as autosomal recessive congenital disorders, and are caused by point mutations in genes essential for normal immunologic and hematopoietic function: adenosine deaminase deficiency, which causes severe combined immune deficiency (ADA-SCID) due to the absence of T-cells, B-cells, and NK-cells; Shwachman-Bodian-Diamond syndrome, a congenital disorder characterized by exocrine pancreas insufficiency, skeletal abnormalities, and bone marrow failure; and Gaucher disease type III, an autosomal recessive lysosomal storage disease characterized by pancytopenia and progressive neurological deterioration due to mutations in the acid beta-glucosidase (GBA) gene. Sequence analysis of the ADA gene in the disease-associated ADA-iPS2 line revealed a compound heterozygote: a GGG to GAA transition mutation at exon 7, causing a G216R amino acid substitution (Figure 1B); the other allele is known to have a frame-shift deletion (-GAAGA) in exon 10 (Hirschhorn et al., 1993). The SBDS-iPS8 line harbors point mutations at the IV2+2T>C intron 2 splice donor site (Figure 1B) and IVS3-1G>A mutation (Austin et al., 2005). Molecular analysis of the GBA gene in the Gaucher disease line revealed a 1226A>G point mutation, causing a N370S amino acid substitution (Figure 1B); the second allele is known to have a frame-shifting insertion of a single guanine at cDNA nucleotide 84 (84GG) (Beutler et al., 1991). The Lesch-Nyhan syndrome carrier line harbors heterozygous deficiency of the HPRT gene (Nussbaum et al., 1983).

Two lines were derived from dermal fibroblasts cultured from patients with muscular dystrophy. Multiplex PCR analysis with primer sets amplifying several (but not all) intragenic intervals of the dystrophin gene (Beggs et al., 1990; Chamberlain et al., 1988) revealed the deletion of exons 45–52 in the iPS cells derived from a patient with Duchenne muscular dystrophy (DMD; Figure 1C). Despite analysis for gross genomic defects by multiplex PCR, a deletion was not detected in iPS cells derived from a patient with Becker type muscular dystrophy (BMD; Figure 1C). As BMD is a milder form of disease, and the dystrophin gene one of the largest in the human genome, definition of the genetic lesion responsible for this condition is sometimes elusive (Prior and Bridgeman, 2005).

Given that numerous groups have pioneered the directed differentiation of neuronal subtypes, and that genetically defined ES cells from animal models of amyotrophic lateral sclerosis have revealed important insights into the pathophysiology of motor neuron deterioration (Di Giorgio et al., 2007), there is considerable interest in generating iPS lines from patients afflicted with neurodegenerative disease. We generated iPS lines from a patient with Huntington chorea (Huntington disease; HD), and verified the presence of expanded (CAG)n polyglutamine triplet repeat sequences (72) in the proximal portion of the huntingtin gene (Figure 1C; (Riess et al., 1993) in one allele and 19 repeats in the other (where the normal range is 35 or less (Chong et al., 1997).

Pluripotent cell lines will likewise be valuable for studying neurodegenerative conditions with more complex genetic predisposition, as well as metabolic diseases known to have familial predispositions but for which the genetic contribution remains unexplained. We have generated lines from a patient diagnosed with Parkinson disease and another from a patient with juvenile onset (Type I) diabetes mellitus (Table 1). Given that these conditions lack a defined genetic basis, genotypic verification is impossible at this time.

The Lesch-Nyhan syndrome is caused by mutations in Hypoxanthine-guanine phosphoribosyltransferase (HPRT), an X-linked enzyme in purine metabolism that when deficient leads to abnormal accumulation of uric acid and a neurologic disorder characterized by cognitive deficits and self-mutilating behavior. Cells carrying either intact or deficient HPRT enzyme function can be selectively cultured in media containing Hypoxanthine-Aminopterin-Thymidine (HAT) or 6-thioguanine (6-TG), respectively. Strategies for inducing specific mutation or gene repair by homologous recombination were first established for the HPRT locus (Doetschman et al., 1987; Doetschman et al., 1988; Thomas and Capecchi, 1987). We have generated an iPS line from a female carrier (LNSc-iPS2) that will be a valuable resource for studies of homologous recombination in iPS cells, and for analysis of X chromosome reactivation during reprogramming and random inactivation with differentiation.

Characterization of disease-related iPS lines

All iPS colonies, which were selected based on their morphologic resemblance to colonies of ES cells, demonstrated compact colony morphology and markers of pluripotent cells, including alkaline phosphatase (AP), Tra-1-81, Tra-1-60, OCT4, NANOG, SSEA3 and SSEA4 (Figure 2). Quantitative RT-PCR indicated the expression of pluripotency-related genes including *OCT4*, *SOX2*, *NANOG*, *REX1*, *GDF3*, and *hTERT* regardless of the genetic condition represented within the parental somatic cells (Figure 3; control lines are shown in panel l). Retroviral transgenes were largely silenced in the iPS lines, with expression of the relevant reprogramming factors assumed by endogenous loci (Figure 4), as described (Park et al., 2008b). PCR-based DNA fingerprint analysis using highly-variable number of tandem repeats (VNTR) confirmed that the iPS lines were genetically matched to their parental somatic lines, ruling out the possibility of cross-contamination from existing cultures of human pluripotent cells (Supplemental Figure 1). Also, iPS cells showed normal 46 XX, or 46 XY karyotypes (Supplementary Figure 2).

Human disease-associated iPS lines were characterized by a standard set of assays to confirm pluripotency and multi-lineage differentiation. iPS lines (n=7) were allowed to differentiate *in vitro* into embryoid bodies as described (Park et al., 2008b), and their potential to develop along specific lineages was confirmed by PCR for markers of all three embryonic germ layers (ectoderm, mesoderm, and endoderm; Supplemental Figure 6a). Hematopoietic differentiation of disease-specific iPS lines (n=2) produced myeloid and erythroid colony types (Figure 5). The ultimate standard of pluripotency for human cells is teratoma formation in immunodeficient murine hosts (Lensch et al., 2007). When injected sub-cutaneously into immunodeficient Rag2$^{-/-}\gamma$C$^{-/-}$ mice, disease-specific iPS lines (n=7) produced mature, cystic masses representing all three embryonic germ layers (Figure 6).

The technique of factor-based reprogramming of somatic cells generates pluripotent stem cell lines that are effectively immortal in culture and can be differentiated into any of a multitude of human tissues. By comparison of normal and pathologic tissue formation, and by assessment of the reparative effects of drug treatment *in vitro*, cell lines generated from patients offer an unprecedented opportunity to recapitulate pathologic human tissue formation *in vitro*, and a new technology platform for drug screening. The Harvard Stem Cell Institute has committed resources to establish a Core Facility for the production of disease-specific iPS lines, with the goal of making each of these lines available to the biomedical research community.

Experimental Procedures

Somatic cell culture, isolation and culture of iPS cells

Fibroblasts from patients with ADA-SCID (ADA, GM01390), Gaucher disease (GD, GM00852), Duchenne type muscular dystrophy (DMD, GM04981; DMD2, GM05089), Becker type muscular dystrophy (BMD, GM04569), Down syndrome (DS1, AG0539A), Parkinson disease (PD, AG20446), juvenile (Type I) diabetes mellitus (JDM, GM02416), Huntington disease (HD, GM04281; HD2, GM01187), and Lesch-Nyhan syndrome carrier

(LNSc, GM00013) were obtained from Coriell. Fibroblasts from patients with Down syndrome (DS2, DLL54) and normal fetal skin fibroblasts (Detroit 551) were purchased from ATCC. Bone marrow mesenchymal cells from SBDS patient (SBDS, DF250) has been described (Austin et al., 2005). Cells were grown in alpha-MEM containing 10 % inactivated fetal serum (IFS), 50 U/ml penicillin, 50 mg/ml streptomycin, and 1 mM L-glutamine. Retroviruses expressing *OCT4*, *SOX2*, *KLF4*, and *MYC* were pseudotyped in VSVg and used to infect 1×10^5 cells in one well of a six-well dish. iPS cells were isolated as described previously (Park et al., 2008b). iPS cells from LNSc fibroblasts were isolated using an inducible lentiviral system as previously described (Stadtfeld et al, 2008). cDNAs encoding human OCT4, SOX2, cMYC, KLF4, and NANOG were cloned into doxycycline inducible vectors and were co-infected with a lentivirus harboring a constitutively expressed reverse tetracycline transactivator (rtTA). Infected fibroblasts were split to feeders under hES culture conditions. Doxycycline was added to the culture for 30 days, and then withdrawn. Colonies that appeared were picked and expanded into lines in the absence of doxycycline. iPS colonies were maintained in hES medium (80% DMEM/F12, 20% KO Serum Replacement,10 ng/ml bFGF, 1 mM L-glutamine, 100 μM nonessential amino acids, 100 μM 2-mercaptoethanol, 50 U/ml penicillin, and 50 mg/ml streptomycin).

Characterization of genetic defects in iPS cells

Genomic DNA was isolated from cells using DNeasy kit (Qiagen). PCR reactions were performed using 50 ng of genomic DNA with primers corresponding to the mutated regions of genes responsible for each condition (ADA-SCID, Gaucher disease, SBDS (Calado et al., 2007), and Huntington disease). Primers sequences are provided in Supplementary Table 1. PCR products were resolved via agarose gels, purified and sequenced, or cloned into the TOPO vector (Invitrogen) for sequencing. The number of CAG repeats in the HD gene was determined by amplifying the 5− end of the huntingtin gene by PCR and sequencing. The deletion of exons within the dystrophin gene in DMD-iPS cells and BMD-iPS cells was determined by PCR using Chamberlain or Beggs' multiplex primer sets (Beggs et al., 1990; Chamberlain et al., 1988).

Karyotype analysis

Chromosomal studies including karyotype of trisomy 21 in DS1-iPS and DS2-iPS10 cells were performed at the Cytogenetics Core of the Dana-Farber/Harvard Cancer Center or Cell Line Genetics using standard protocols for high-resolution G-banding.

Fingerprinting analysis

50 ng of genomic DNA was used to amplify across discrete genomic intervals containing highly variable numbers of tandem repeats (VNTR). PCR products were resolved in 3 % agarose gels to examine the differential amplicon mobility for each primer set: D10S1214, repeat (GGAA)n, average heterozygosity 0.97; D17S1290, repeat (GATA)n, average heterozygosity 0.84; D7S796, repeat (GATA)n, average heterozygosity 0.95; and D21S2055, repeat (GATA)n, average heterozygosity 0.88 (Invitrogen).

Immunohistochemistry and AP staining of iPS cells

iPS cells grown on feeder cells were fixed in 4% paraformaldehyde for 20 min, permeabilized with 0.2% Triton X-100 for 30 minutes, and blocked in 3% BSA in PBS for 2 hours. Cells were incubated with primary antibody overnight at 4 °C, washed, and incubated with Alexa Fluor (Invitrogen) secondary antibody for 3 hours. SSEA-3, SSEA-4, TRA 1–60, TRA 1–81 antibodies were obtained from Millipore. OCT3/4 and NANOG antibodies were obtained from Abcam. Alkaline phosphatase staining was done per the manufacturer's recommendations (Millipore).

Analysis of gene expression

Total RNA was isolated from iPS cells using an RNeasy kit (Qiagen) according to the manufacturer's protocol. 0.5 μg of RNA was subjected to the RT reaction using Superscript II (Invitrogen). Quantitative PCR was performed with Brilliant SYBR Green Master MiX in Stratagene MX3000P machine using previously described primers (Park et al., 2008b). Semi-quantitative PCR was performed to look at the expression of total, endogenous and recombinant pluripotency genes, and genes representing the three embryonic germ layers using primers described previously and in Supplementary Table 1.

Differentiation of iPS cells

iPS cells were washed with DMEM/F12, treated with collagenase for 10 min, and collected by scraping. Colonies were washed once with DMEM/F12, and gently resuspended in EB differentiation medium. EBs were differentiated with low-speed shaking and the medium was changed every three days. After two weeks of differentiation, EBs were dissociated and plated in MethoCult (Stem Cell Technologies).

Teratoma formation from iPS cells

iPS cells were washed with DMEM/F12, treated with collagenase for 10 min at room temperature, scraped using glass pipette, and collected by centrifugation. Cells were washed once with DMEM/F12, and mixed with Matrigel (BD Biosciences) and collagen (Sigma). 2×10^6 cells were intramuscularly injected into immune deficient $Rag2^{-/-}\gamma C^{-/-}$ mice. After 6 weeks of injection, teratomas were dissected, rinsed once with PBS, and fixed in 10% formalin. Embedding in paraffin, sectioning of tissue, and Hematoxylin/Eosin staining were performed by the Rodent Histopathology service of the Dana Farber Cancer Institute.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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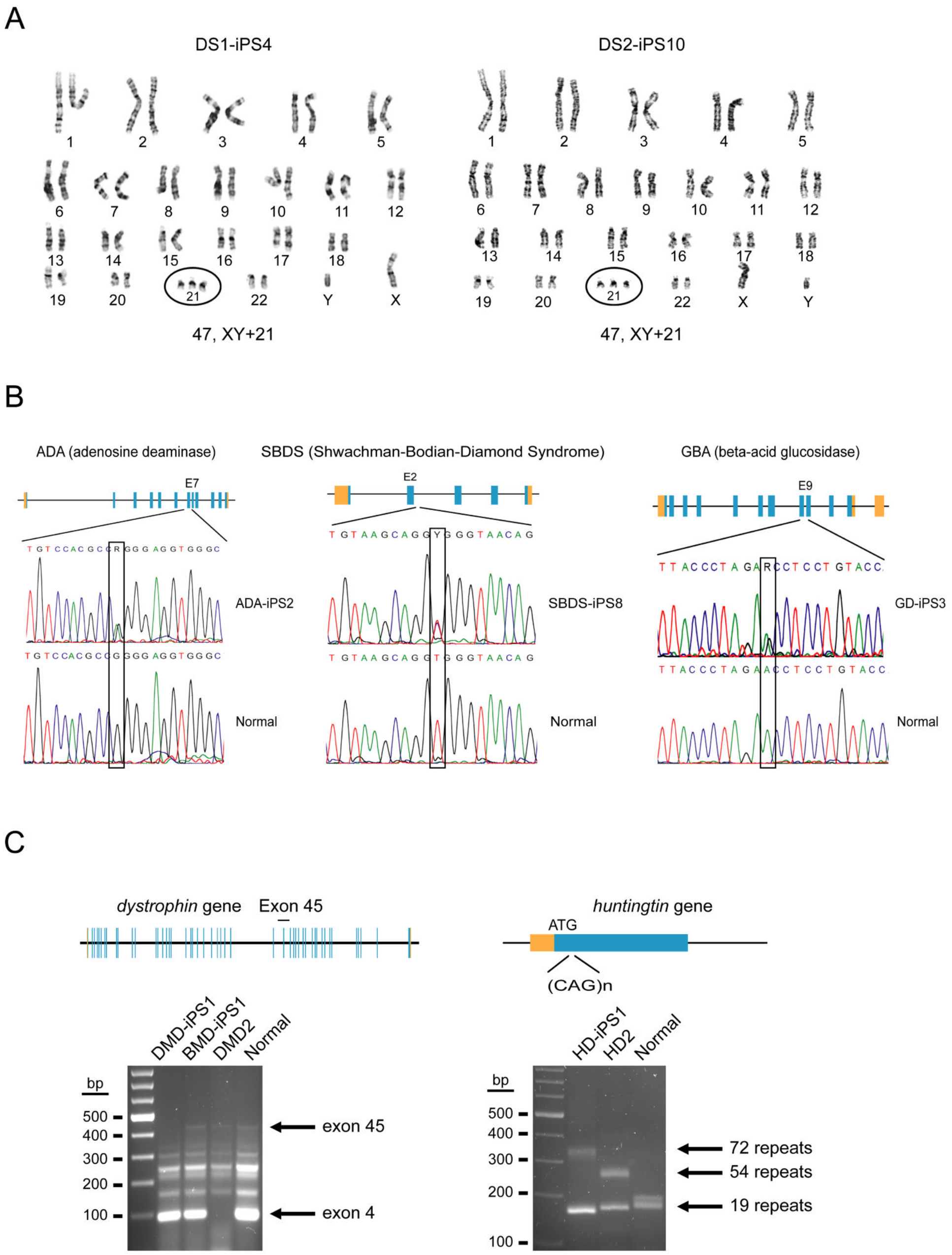
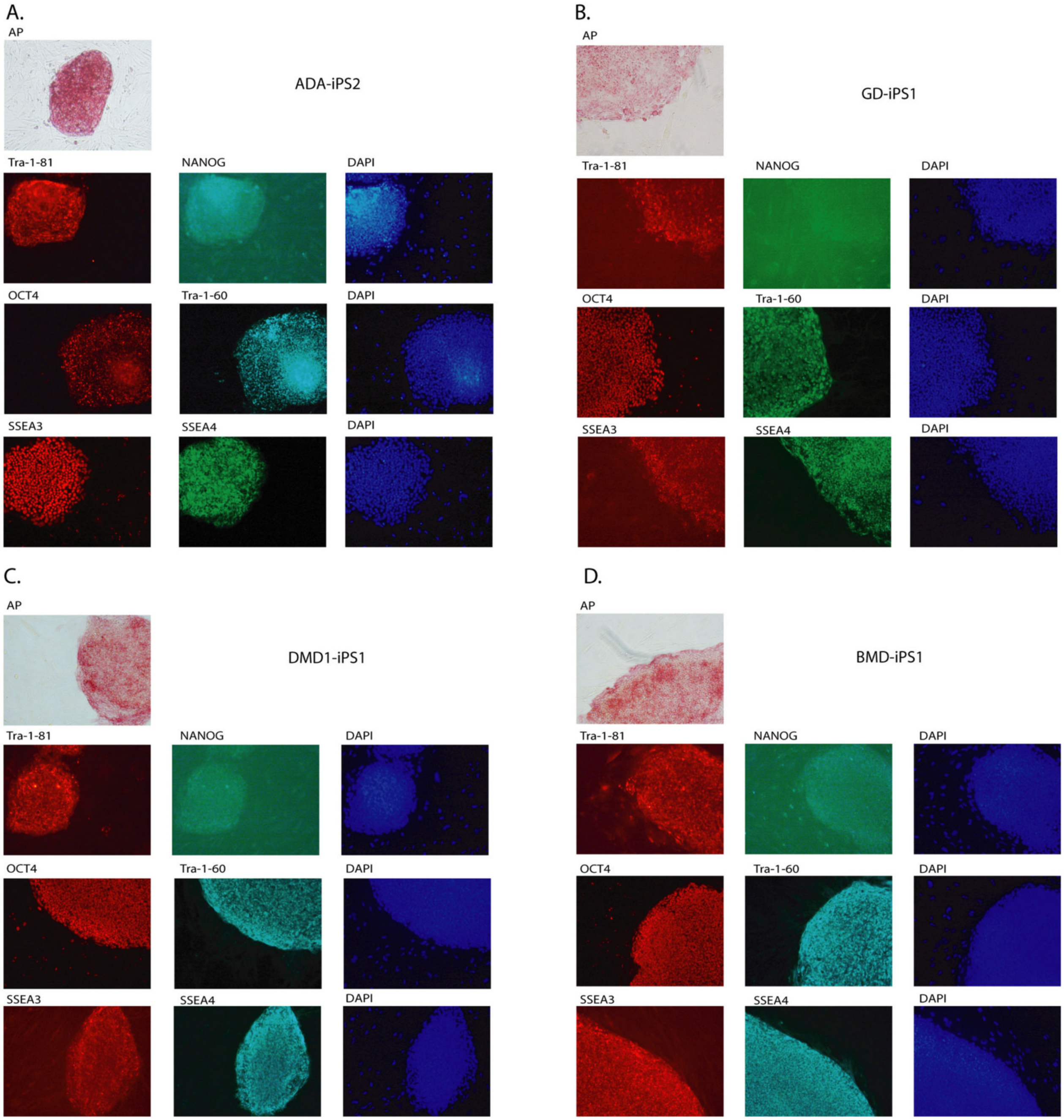


Figure 1. Genotypic analysis of disease-specific iPS cell lines

A) Two different, primary fibroblast specimens, DS1 and DS2 from male patients with Down syndrome (trisomy 21) were used to derive DS1-iPS4 and DS2-iPS10. Each has a 47, XY+21 karyotype over several passages (G-banding analysis). **B)** Fibroblast (ADA and GBA) or bone marrow mesenchymal cells (SBDS) were used to generate iPS lines. Mutated alleles identical to the original specimens were verified by DNA sequencing. Adenosine deaminase deficiency line ADA-iPS2, a compound heterozygote: GGG to GAA double transition in exon 7 of one allele (G216R substitution); the second allele is an exon 10 frame-shift deletion (-GAAGA) (Hirschhorn et al., 1993). Shwachman-Bodian-Diamond syndrome line SBDS-iPS8 is also a compound heterozygote: point mutations at the IV2+2T>C intron 2 splice donor site and an IVS3-1G>A mutation of the SBDS gene (Austin et al., 2005). GD-iPS3 (Gaucher disease type III); a 1226A>G point mutation (N370S substitution) and a guanine insertion at nucleotide 84 of the cDNA (84GG) (Beutler et al., 1991). **C)** Fibroblasts from patients diagnosed with either Duchenne (DMD) or Becker type muscular dystrophy (BMD): DMD-iPS1 has a deletion over exons 45–52 (multiplex PCR for the dystrophin gene). We could not determine a deletion in BMD-iPS1 using two different multiplex PCR sets though these assays do not cover the entire coding region. DMD2 is a patient control (exon 4 deletion). The control is genomic DNA from a healthy volunteer. Huntington disease (HD) is caused by a tri-nucleotide repeat expansion within the huntingtin locus. DNA sequencing shows that HD-iPS has one normal (<35 repeats) and one expanded allele (72 repeats). HD2 is a positive control from a second Huntington patient with one normal and one expanded allele (54 repeats). The control is genomic DNA from a healthy volunteer.


A.
AP
ADA-iPS2
Tra-1-81
NANOG
DAPI
OCT4
Tra-1-60
DAPI
SSEA3
SSEA4
DAPI
B.
AP
GD-iPS1
Tra-1-81
NANOG
DAPI
OCT4
Tra-1-60
DAPI
SSEA3
SSEA4
DAPI
C.
AP
DMD1-iPS1
Tra-1-81
NANOG
DAPI
OCT4
Tra-1-60
DAPI
SSEA3
SSEA4
DAPI
D.
AP
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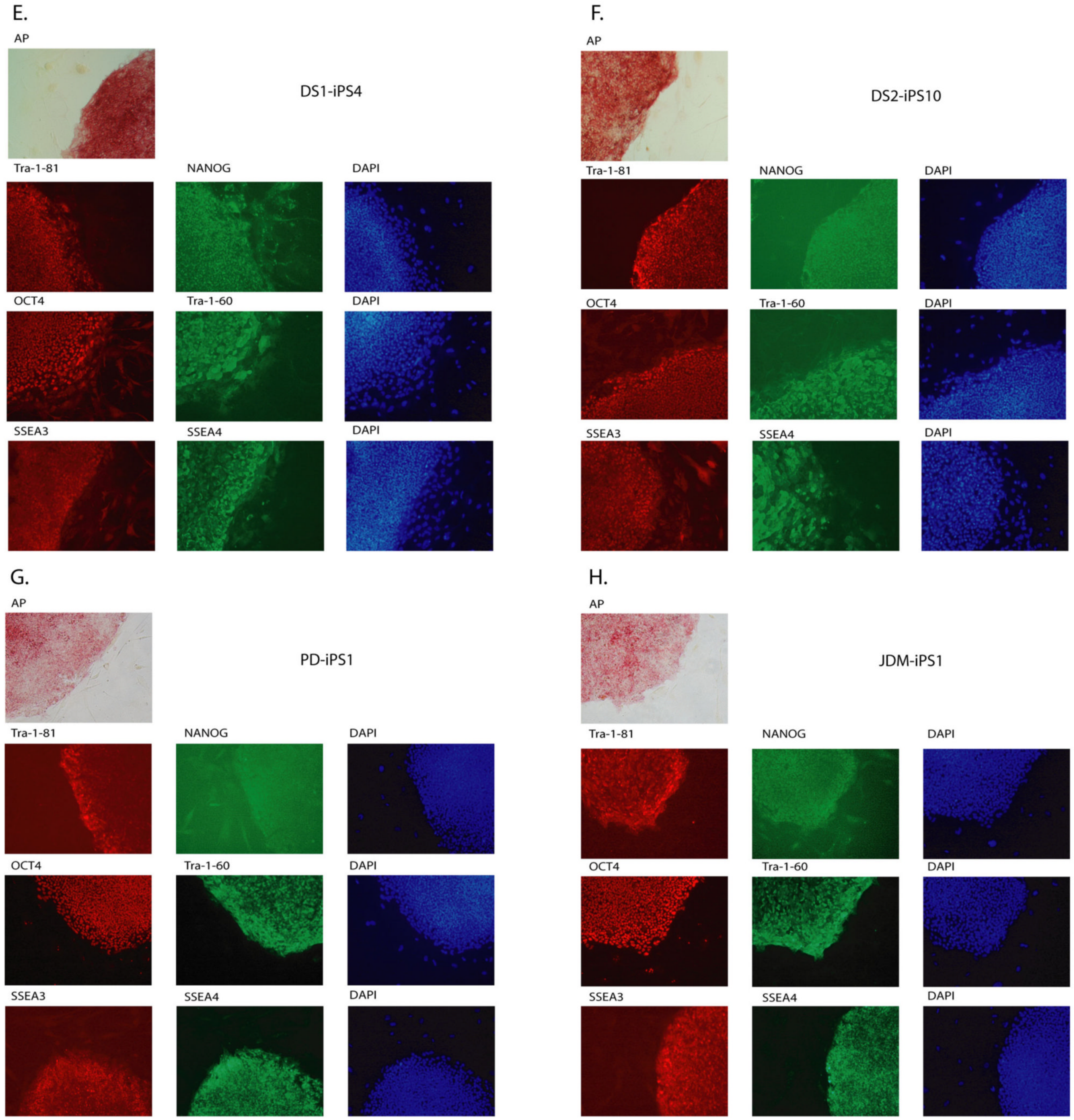
E.
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F.
AP
DS2-iPS10
Tra-1-81
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G.
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PD-iPS1
Tra-1-81
NANOG
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DAPI
SSEA3
SSEA4
DAPI
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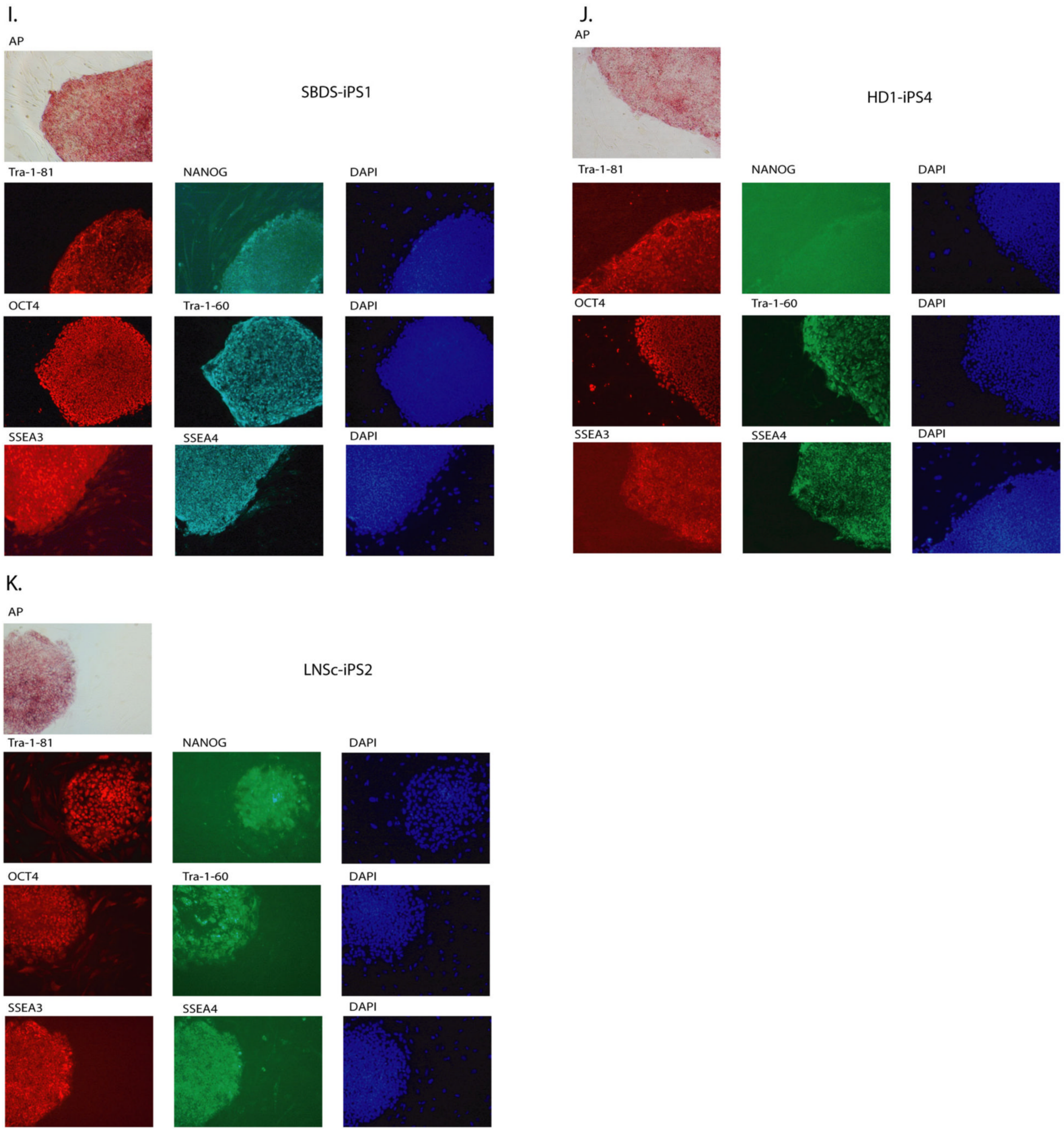


Figure 2. Patient-derived iPS lines exhibit markers of pluripotency

ADA-iPS2, GD-iPS1, DMD-iPS1, BMD-iPS1, DS1-iPS4, DS2-iPS10, PD-iPS1, JDM-iPS1, SBDS-iPS1, HD-iPS4, LNSc-iPS2, JDM-iPS2 were established from a fibroblast or mesenchymal cells (Table 1). Disease specific iPS cell lines maintain a morphology similar to hES cells when grown in co-culture with mouse embryonic feeder fibroblasts (MEFs). Patient-specific iPS cells express alkaline phosphatase (AP). Also, as shown here via immunohistochemistry, patient-specific cells express pluripotency markers including Tra-1-81, NANOG, OCT4, Tra-1-60, SSEA3 and SSEA4. 4,6-Diamidino-2-phenylindole (DAPI) staining is shown at right and indicates the total cell content per image.

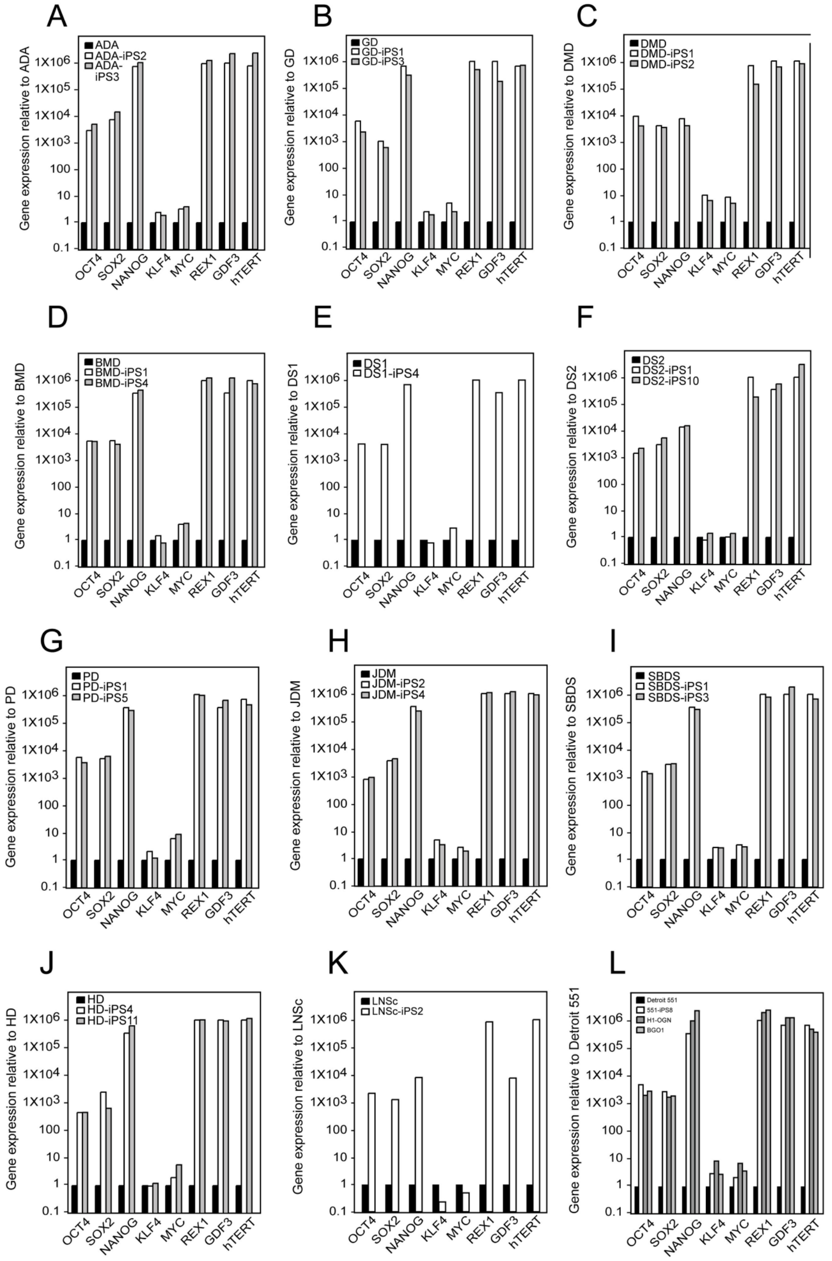


Figure 3. Expression of pluripotency-associate genes is elevated in patient-specific iPS lines relative to their somatic cell controls

In each panel, quantitative real-time PCR (QRT-PCR) assays for *OCT4*, *SOX2*, *NANOG*, *REX1*, *GDF3*, and *hTERT* indicates increased expression in patient-specific iPS cells relative to parent cell lines while expression of *KLF4* and *cMYC* remains largely unchanged. PCR reactions were normalized against internal controls (β-actin) and plotted relative to expression levels in their individual parent fibroblast cell lines. (A) the human iPS lines ADA-iPS2 and –iPS3 are derived from the adenosine deaminase deficiency-severe combined immunodeficiency fibroblast line ADA. (B) GD-iPS1 and –iPS3 are derived from the Gaucher disease type III fibroblast line GD. (C) DMD-iPS1 and –iPS2 are derived from the Duchenne muscular dystrophy fibroblast line DMD. (D) BMD-iPS1 and –iPS4 are derived from the Becker muscular dystrophy line BMD. (E) DS1-iPS4 is derived from the Down syndrome fibroblast line DS1. (F) DS2-iPS1 and –iPS10 are derived from the Down syndrome fibroblast line DS2. (G) PD-iPS1 and –iPS5 are derived from the Parkinson disease fibroblast line PD. (H) JDM-iPS2 and –iPS4 are derived from the juvenile-onset, type 1 diabetes mellitus line JDM. (I) SBDS-iPS1 and –iPS3 are derived from the Shwachman-Bodian-Diamond syndrome bone marrow mesenchymal fibroblast line SBDS. (J) HD-iPS4 and –iPS11 are derived from the Huntington disease fibroblast line HD. (K) LNSc-iPS1 and –iPS2 are derived from the Lesch-Nyhan syndrome carrier fibroblast line LNSc. (L) Detroit 551 human fibroblasts are used as the standard here in order to demonstrate the previously described expression pattern in Detroit 551 derived iPS cells (551-iPS8) relative to two *bona fide* hES cell lines: H1-OGN and BG01.

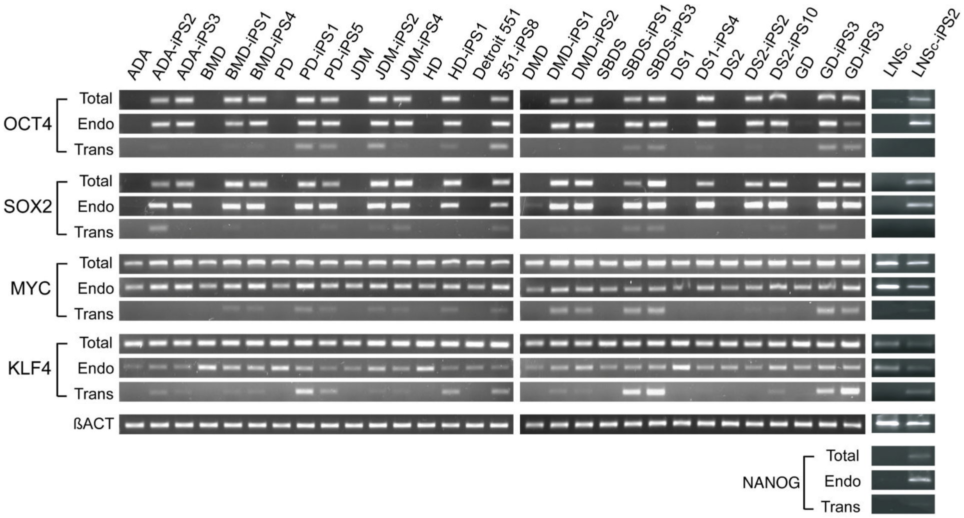
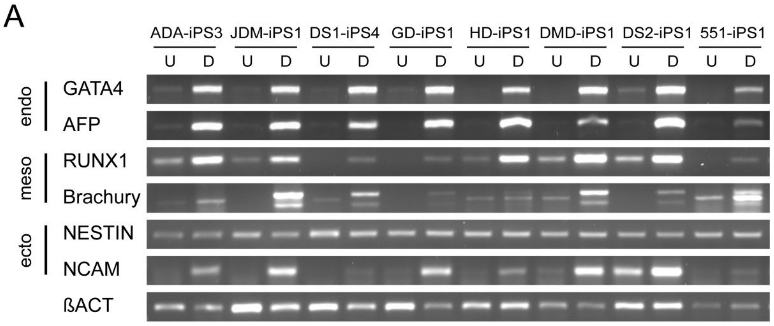


Figure 4. Pluripotency-promoting genes are chiefly expressed from the endogenous loci in patient-specific iPS lines, while the virally-delivered transgene is predominantly silenced

The patient-specific iPS cell lines shown here are preceded by their parental fibroblast controls (from left to right at top): adenosine deaminase deficiency-associate severe combined immunodeficiency (ADA), Becker muscular dystrophy (BMD), Parkinson disease (PD), juvenile type one diabetes mellitus (JDM), Huntington disease (HD), Detroit 551 control cells, Duchenne muscular dystrophy (DMD), Shwachman-Bodian-Diamond syndrome (SBDS), Down syndrome (DS), Gaucher disease type III (GD), and Lesch-Nyhan syndrome carrier (LNSc). The semi-quantitative expression (RT-PCR) of the four pluripotency-promoting genes used in the reprogramming process, *OCT4, SOX2, cMYC, KLF4* and *NANOG* is shown for each line using amplification conditions specific to the endogenous (Endo) or virally-delivered transgene (Trans) as well as the total expression for each (Total). Beta-actin is shown at the bottom as a loading control for each lane.



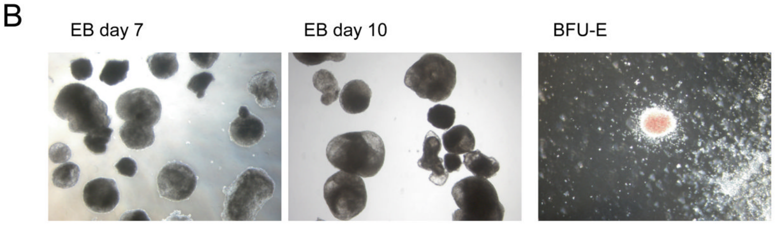


Figure 5. Differentiation of patient-specific iPS lines reveals lineage-specific gene expression and mature cell formation

A) At top (from left to right) are nine iPS cell lines in their undifferentiated (U) or differentiated (D) state. The lines are: adenosine deaminase deficiency-associated severe combined immunodeficiency (ADA), juvenile-onset type one diabetes mellitus (JDM), Down syndrome 1 (DS1), Gaucher disease type III (GD), Huntington disease (HD), Duchenne muscular dystrophy (DMD), Down syndrome 2 (DS2), and normal control Detroit 551 (551) cells. Differentiation (D) of these patient-specific iPS cells as embryoid bodies (EB) followed by RT-PCR analysis shows upregulated expression of lineage markers from the three embryonic germ layers relative to their undifferentiated controls (U) including: *GATA4* and *AFP* (endoderm), *RUNX1* and *Brachyury* (mesoderm), and *Nestin* and *NCAM* (ectoderm). Beta-actin serves as a positive amplification control for each. B) Differentiation of ADA-iPS2, a representative patient-specific iPS cell line, as embryoid bodies (EB) is highly reminiscent of that using hES cells where tight clusters of differentiating cells are well-formed by day 7 which will cavitate, becoming cystic, by day 10. Hematopoietic differentiation of patient-specific iPS cells yields various blood cell types in semi-solid methylcellulose colony-forming assays including burst-forming unit-erythroid (BFU-E) which are derivative of red blood cell progenitor cells.

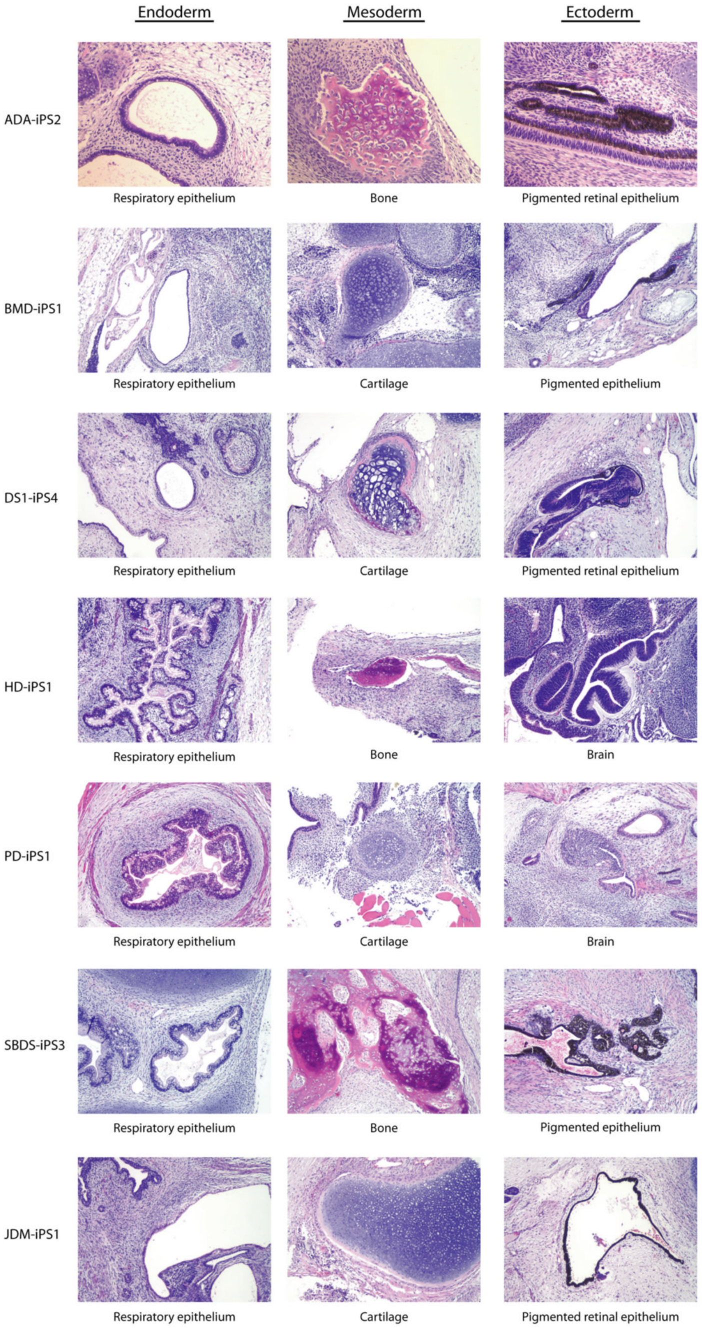


Figure 6. Patient-specific iPS lines form teratomas in immunodeficient mice

Shown here are the representative series of hematoxylin-eosin (H/E) stained sections from a formalin fixed teratoma produced from ADA-iPS2, BMD-iPS1, DS1-iPS4, HD-iPS1, PD-iPS1, SBDS-iPS3, and JDM-iPS1 cell lines. They formed mature, cystic teratomas with tissues representing all three embryonic germ layers including: respiratory epithelium (endoderm), bone and cartilage (mesoderm), and pigmented retinal epithelium and immature neural tissue (ectoderm).

Table 1

iPS cells derived from somatic cells of patients with genetic disease

Name	**Disease**	**Molecular Defect**	**Donor cell**	**Age**	**Sex**
ADA	ADA-SCID	GGG->AGG, exon 7 and Del(GAAGA) exon 10, *ADA gene*	fibroblast	3 M	Male
GD	Gaucher disease type III	AAC->AGC, exon 9, and G-insertion, nucleotide 84 of cDNA, *GBA* gene	fibroblast	20 Y	Male
DMD	Duchenne muscular dystrophy	Deletion of exon 45–52, *dystrophin* gene	fibroblast	6 Y	Male
BMD	Becker muscular dystrophy	Unidentified mutation in *dystrophin*	fibroblast	38 Y	Male
DS1, DS2	Down Syndrome	Trisomy 21	fibroblast	1 Y, 1 M	Male
PD	Parkinson disease	Multifactorial	fibroblast	57 Y	Male
JDM	Juvenile diabetes mellitus	Multifactorial	fibroblast	42 Y	Female
SBDS	Shwachman-Bodian-Diamond syndrome	IV2+2T->C and IVS3-1G>A, *SBDS* gene	bone marrow mesenchymal cells	4 M	Male
HD	Huntington disease	72 CAG repeats, *huntingtin* gene	fibroblast	20 Y	Female
LNSc	Lesch-Nyhan Syndrome (carrier)	Heterozygosity of *HPRT1*	fibroblast	34 Y	Female