SUPPLEMENTAL MATERIAL AND METHODS:

Cell culture

Primary fibroblast lines GM01660 and MRC-5 described and reprogrammed in this paper are from the Corriell Cell Repository. MRC-5 cells are primary embryonic lung fibroblasts and GM01660 cells were isolated from an unaffected heterozygous female carrier of an HPRT mutation causing in homozygous males the Lesh-Nyhan syndrome. Primary and secondary fibroblasts were cultured in fibroblast medium [DMEM supplemented with 15% FBS (Hyclone), 1 mM glutamine (Invitrogen), 1% nonessential amino acids (Invitrogen) and Penicillin/Streptomycin (Invitrogen)]. Epithilial cells (EpRas cells) were coultured as described previously (Oft et al., 1996).

Human embryonic stem cells BGO1 and BGO2 (NIH Code: BGO1 and BGO2, BresaGen, Inc., Athens, GA) were maintained on mitomycin C inactivated mouse embryonic fibroblast (MEF) feeder layers in human ES cell medium [DMEM/F12 (Invitrogen) supplemented with 15 % FBS (Hyclone), 5% KnockOut^{TM} Serum Replacement (Invitrogen), 1 mM glutamine (Invitrogen), 1% nonessential amino acids (Invitrogen), 0.1 mM β-mercaptoethanol (Sigma) and 4 ng/ml FGF2 (R&D systems)]. Cultures were passaged manually every 5 to 7 days. Human embryonic stem cells H9 (NIH Code:WA09, Wisconsin Alumni Research Foundation, Madison, WI) were maintained and passaged according to the provider's protocol.

Viral constructs

Lentiviral vectors containing the mouse c-DNAs for KLF4, OCT4, SOX2 and C-MYC under the control of the tetracycline operator and a minimal CMV promoter have been described previously (Brambrink et al., 2008). The human c-DNAs for KLF4, OCT4, SOX2 and C-MYC were cloned into the EcoRI sites of the same vector backbone (FUW-tetO). The reverse tetracycline transactivator M2rtTA (Urlinger et al., 2000) was subcloned blunt-ended into the EcoRI sites of the

FUW lentiviral backbone (Lois et al., 2002) containing a constitutively active human ubiquitin C promoter (FUW-M2rtTA).

Reverse transcription of total RNA and real-time PCR

RNA was isolated from hES and iPS cells, which were mechanically separated from feeder cells, using Trizol extraction and subsequent precipitation. Reverse transcription was perfromed on 1µg of total RNA using oligo dT priming and thermoscript reverse transcriptase at 50°C (Invitrogen). Real-time PCR was performed in an ABI Prism 7000 (Applied Biosystems) with Platinum SYBR green pPCR SuperMIX-UDG with ROX (Invitrogen).

Primers for the analysis of endogenous gene expressions were:

hNANOG 5'-GCAGAAGGCCTCAGCACCTA-3'
hNANOG 5'-AGGTTCCCAGTCGGGTTCA-3'

hOCT4 5'-GCTCGAGAAGGATGTGGTCC-3'
hOCT4 5'-CGTTGTGCATAGTCGCTGCT-3'

hSOX2 5'-CACTGCCCCTCTCACACATG-3'
hSOX2 5'-TCCCATTTCCCTCGTTTTTCT-3'

Primers for exogenous gene expression were:

RT-FUW-hOCT4-for: 5'-CCCCTGTCTCTGTCACCACT-3'
RT-FUW-hKLF4-for: 5'-GACCACCTCGCCTTACACAT-3'
RT-FUW-hc-Myc-for: 5'-CAGCTACGGAACTCTTGTGC-3'
RT-FUW-hKLF4/OCT4/MYC-rev: 5'-CCACATAGCGTAAAAGGAGCA-3'
RT-FUW-hSOX2-for: 5'-ACACTGCCCCTCTCACACAT-3'
RT-FUW-hSOX2-rev: 5'-CATAGCGTAAAAGGAGCAACA-3'

Gene expression was normalized using GAPDH primers:

RT-hGAPDH-for: 5'-CGTGGAAGGACTCATGACCAQ-3'
RT-hGAPDH-rev: 5'-CAGTCTTCTGGGTGGCAGTGA-3'

Teratoma formation and analysis:

IPS cells were collected by collagenase treatment (1.5mg/ml) and separated from feeder cells by subsequent washes with medium and sedimentation of iPS cell colonies. iPS cell aggregates were collected by centrifugation and resuspended in 250μl of phosphate buffered saline. iPS cell were injected subcutaneously in the back of SCID mice (Taconic). Tumors generally developed within 6-8 weeks and animals were sacrificed before tumor size exceeded 1.5 cm in diameter. Teratomas were isolated after sacrificing the mice and fixed in formalin. After sectioning, teratomas were diagnosed base on hematoxylin and eosin staining.

Methylation analysis

Genomic DNA was collected from hES and iPS cells by mechanical separation from feeder cells. 1μg of Proteinase K treated and phenol chloroform extracted DNA was subjected to conversion using the Qiagen EpiTect Bisulfite Kit. Promoter regions of OCT4 and NANOG were amplified using previously described primers (Takahashi et al., 2007; Yu et al., 2007):

OCT4 Forward: ATTTGTTTTTTGGGTAGTTAAAGGT
OCT4 Reverse: CCAACTATCTTCATCTTAATAACATCC

NANOG S: TGGTTAGGTTGGTTTTAAATTTTTG
NANOG AS: AACCCACCCTTATAAATTCTCAATTA

PCR products were cloned using the pCR2.1-TOPO vector and sequenced using M13 forward and reverse primers.

Immunostaining

Cells were fixed in 4% paraformaldehyde in PBS and immunostained according to standard protocols using the following primary antibodies: SSEA4 (mouse monoclonal, Developmental Studies Hybridoma Bank); Tra 1-60, (mouse monoclonal, Chemicon International); hKLF (goat polyclonal, R&D Systems); hSOX2 (goat polyclonal, R&D Systems); Oct-3/4 (mouse monoclonal, Santa Cruz Biotechnology); hNANOG (goat polyclonal R&D Systems); antiProly1-4 hydroxylase beta (mouse monoclonal clone #3-2B12A, Acris Antibodies). Appropriate Molecular Probes Alexa Fluor® dye conjugated secondary antibodies (Invitrogen) were used.

Southern blotting

XbaI digested genomic DNA was separated on a 0.7% agarose gel, transferred to a nylon membrane (Amersham) and hybridized with ^{32}P random primer (Stratagene) labeled probes for OCT4 (EcoRI-PstI fragment of pFUW-tetO-hOCT4 plasmid), KLF4 (full length hKLF4 cDNA), C-MYC (full length C-MYC cDNA) and SOX2 (FspI-EcoRI fragment of pFUW-tetO-hSOX2 plasmid).

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