Supplementary Methods and Results

Isolation of clonal amniotic stem cell lines with potential for therapy

Paolo De Coppi[1], Georg Bartsch, Jr.[1], M. Minhaj Siddiqui[1], Tao Xu[1], Cesar C. Santos[1], Laura Perin[1], Gustavo Mostoslavsky[2], Angéline C. Serre[2], Evan Y. Snyder[2], James J. Yoo[1], Mark E. Furth[1], Shay Soker[1] & Anthony Atala[1]

[1]*Wake Forest Institute for Regenerative Medicine, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC, 27157-1094*

[2]*Children's Hospital and Harvard Medical School, 300 Longwood Avenue, Boston, MA, 02115*

Supplementary Online Material

Supplementary Methods and Results

Table S1

Supplementary Figures S1, S2, S3

Supplementary Video Clip S4

Supplementary Methods and Results

Flow cytometry. To determine the antigenic profile of AFS cells, staining and analysis with monoclonal antibodies was performed according to standard protocols using a FACSCalibur analyzer (BD Biosciences, San Jose, CA) and FlowJo software (Tree Star, Inc., Palo Alto, CA) or an Epics analyzer and CELLQuest software (Beckman Coulter). Assays for surface antigens were carried out with live cells. The cells were grown to approximately 50% of confluence, detached from culture dishes by mild trypsinization, and washed with phosphate buffered saline (PBS). For experiments the cells were detached from culture dishes by incubation with sodium citrate. For assay of Oct4, the cells were fixed for 10 minutes with 4% paraformaldehyde in PBS and then made permeable by incubation with cold ethanol for 1 minute. Monoclonal antibodies were obtained from the sources indicated: CD29, CD44, CD45, CD34, CD45, CD73, CD90, CD105, HLA-DR (Immunotech, Beckman Coulter, Marseille, France); HLA-ABC (BD Biosciences Pharmingen, Europe); Oct-4, SSEA-1, SSEA-3, SSEA-4, Tra-1-60, Tra-1-81 (Santa Cruz Biotechnology, Santa Cruz, CA); CD133 (Miltenyi Biotech, Auburn, CA). Primary antibodies were diluted to the concentration recommended by the supplier (generally 1 μg/ml). Secondary antibodies were FITC-goat anti-mouse IgG (Beckmann Coulter, Fullerton, CA) or AlexaFluor488-goat anti-mouse IgG (Molecular Probes / Invitrogen, Eugene, OR), diluted 1:100. Results are shown in Supplementary Fig. S1.

Experiments with mouse AFS cells were carried out similarly to those with human AFS cells. Primary antibodies were diluted to a final concentration of 3.3 μg/ml in PBS with 3% (v/v) goat serum and incubated with up to 5 x 10^5 cells in a volume of 0.3 ml for 1 hour at 4º C. Unbound antibody was removed by three cycles of washing in PBS-3% goat serum. The cells then were incubated with phycoerythrin (PE) labelled goat anti-mouse IgG and IgM or PE-goat anti-rat IgG secondary antibody (both from Jackson ImmunoResearch Laboratories, West Grove, PA), diluted 1:75, for 30 min at 4º C and washed three times prior to analysis. Rat monoclonal

antibodies against mouse antigens were obtained from the sources indicated: CD34 (clone RAM34), CD44 (IMG7), CD45 (30-F11), CD90 (G7), CD105 (MJ7/18), CD117 (ACK45), Sca-1 (D7) (BD Biosciences Pharmingen, San Jose, CA); MHC Class II (ER-TR2) (Abcam, Cambridge, UK), MHC Class I (ER-HR52) (Bachem AG, Bubendorf, Switzerland). Normal rat IgG (Jacson ImmunoResearch) was used as a control for rat monoclonal antibodies. Monoclonal mouse IgM to SSEA-1 (clone 480) was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). The negative control for SSEA-1 was a mouse IgM (clone PGM1- 146.1, BD Pharmingen). Results obtained with mouse AFS cells are shown in Supplementary Fig. S2. Expression of Oct4 is documented here by RT-PCR (Supplementary Fig. S2a) and was confirmed by flow cytometry (not shown). The murine AFS cells express the surface antigens SSEA-1 [1], Sca-1 (Ly-6A) [2], CD90 (Thy-1), and CD44, but not CD105. They express neither CD34 nor CD45, lack detectable MHC Class II antigens, and express low levels of MHC Class I (Supplementary Fig. S2b).

1. Solter, D. & Knowles, B.B. Monoclonal antibody defining a stage-specific mouse embryonic antigen (SSEA-1). *Proc Natl Acad Sci U S A* 75, 5565-5569 (1978).
2. van de Rijn, M., Heimfeld, S., Spangrude, G.J. & Weissman, I.L. Mouse hematopoietic stem-cell antigen Sca-1 is a member of the Ly-6 antigen family. *Proc Natl Acad Sci U S A* 86, 4634-4638 (1989).