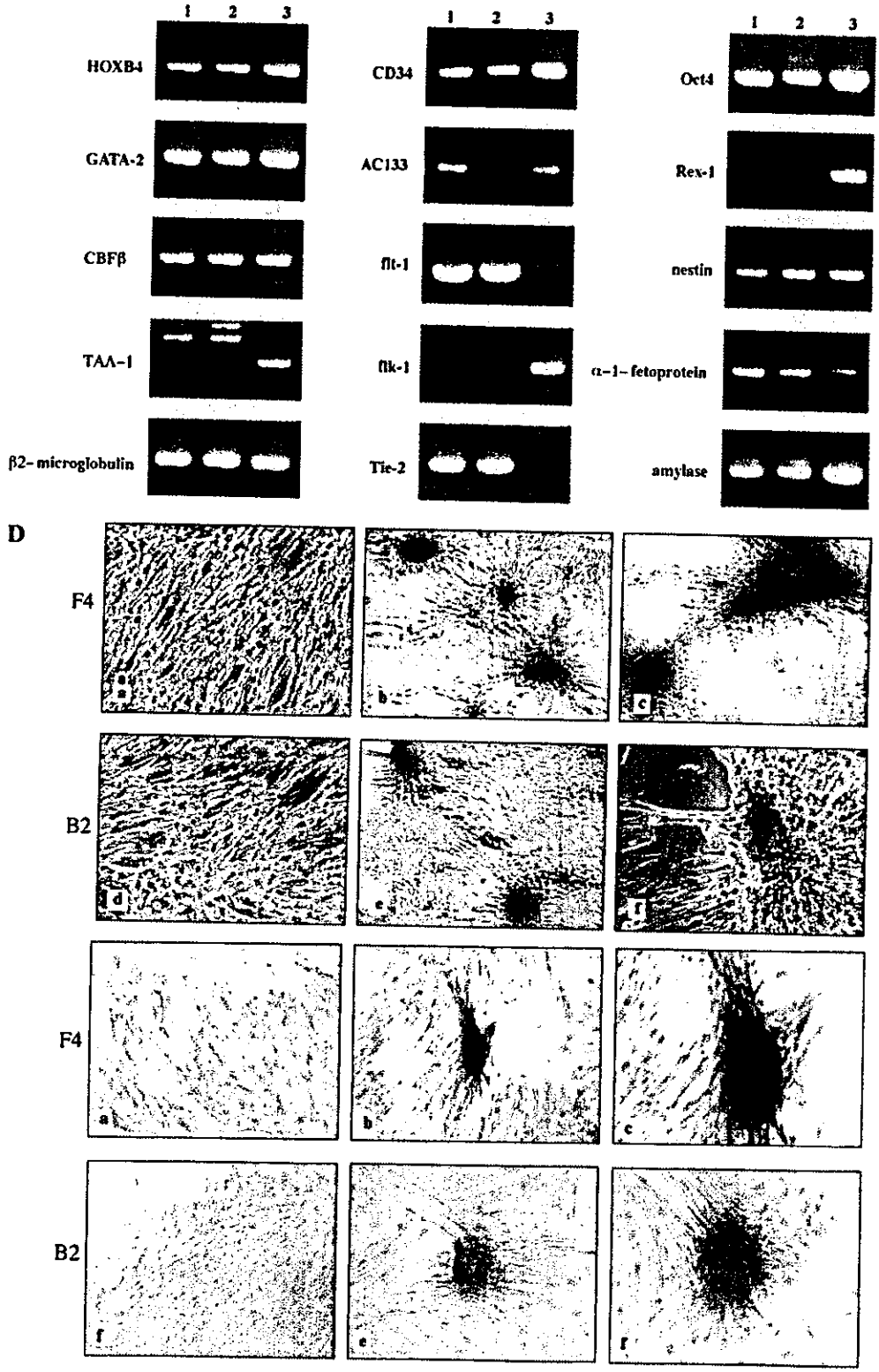

Figure 4 (continued). These were obtained from subcloning of 10 cells per well. Subclones B2 (lane 1) and F4 (lane 9) had a single retroviral insert. **(B):** Immunophenotype of clones. Clones F4 and B2 were stained with phycoerythrin-conjugated antibodies against CD45, CD31, AC133, CD54, CD29, and CD44 or immunoglobulin isotype control antibodies then analyzed by fluorescence-activated cell sorter Calibur. **(C):** Gene expression patterns of clones. Clones F4 and B2 were characterized by reverse transcription–polymerase chain reaction analysis. Samples are as follows: lane 1, clone F4; lane 2, clone B2; lane 3, positive control (same positive controls were used in Fig. 2). **(D):** Differentiation potential of clones. Clones F4 and B2 were cultured in osteogenic (b, c, e, f), adipogenic (h, i, k, l), or regular medium (a, d, g, j) for 2 weeks. After the culture periods, each of the clones was evaluated for osteogenic or adipogenic differentiation using specific staining and hematoxylin counterstaining. Magnification: a, b, d, e, g, h, j, k, ×40; c, f, i, l, ×100.

References

1 Liechty KW, MacKenzie TC, Shaaban AF et al. Human mesenchymal stem cells engraft and demonstrate site-specific differentiation after in utero transplantation in sheep. Nat Med 2000;6:1282–1286.

2 Reyes M, Lund T, Lenvik T et al. Purification and ex vivo expansion of postnatal human marrow mesodermal progenitor cells. Blood 2001;98:2615–2625.

3 Jiang Y, Jahagirdar BN, Reinhardt RL et al. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002;20:1–9.

4 Bartholomew A, Sturgen C, Siatskas M et al. Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. Exp Hematol 2002;30:42–48.

5 Gerson SL. Mesenchymal stem cells: no longer second class marrow citizens. Nat Med 1999;5:262–264.

6 Deans RJ, Moseley AB. Mesenchymal stem cells: biology and potential clinical uses. Exp Hematol 2000;28:875–884.

7 Pittenger MF, Mackay AM, Beck SC et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143–147.

8 Rao MS, Mattson MP. Stem cells and aging: expanding the possibilities. Mech Aging Dev 2001;122:713–734.

9 Zvaifler NJ, Marinova-Mutafchieva L, Adams G et al. Mesenchymal precursor cells in the blood of normal individuals. Arthritis Res 2000;2:477–488.

10 Erices A, Conget P, Minguell JJ. Mesenchymal progenitor cells in human umbilical cord blood. Br J Haematol 2000;109:235–242.

11 Romanov YA, Svintsitskaya VA, Smirnov VN. Searching for alternative sources of postnatal human mesenchymal stem cells: candidate MSC-like cells from umbilical cord. Stem Cells 2003;21:105–110.

12 Campagnoli C, Roberts IAG, Kumar S et al. Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. Blood 2001;98:2396–2402.

13 Almeida-Porada G, Shabrawy D, Porada C et al. Differentiative potential of human metanephric mesenchymal cells. Exp Hematol 2002;30:1454–1462.

14 Kitamura T, Tange T, Terasawa T et al. Establishment and characterization of a unique human cell line that proliferates dependently on GM-CSF, IL-3, or erythropoietin. J Cell Physiol 1989;140:323–334.

15 Hosoda M, Makino S, Kawabe T et al. Differential regulation of the low affinity Fc receptor for IgE (Fc epsilon R2/CD23) and the IL-2 receptor (Tac/p55) on eosinophilic leukemia cell line (EoL-1 and EoL-3). J Immunol 1989; 143:147–152.

16 Levenberg S, Golub JS, Amit M et al. Endothelial cells derived from human embryonic stem cells. Proc Natl Acad Sci U S A 2002;99:4391–4396.

17 Kaufman DS, Hanson ET, Lewis RL et al. Hematopoietic colony-forming cells derived from human embryonic stem cells. Proc Natl Acad Sci U S A 2001;98:10716–10721.

18 Shamblott MJ, Axelman J, Littlefield JW et al. Human embryonic germ cell derivatives express a broad range of developmentally distinct markers and proliferate extensively in vitro. Proc Natl Acad Sci U S A 2001;98:113–118.

19 Bellamy WT, Richter L, Frutiger Y et al. Expression of vascular endothelial growth factor and its receptors in hematopoietic malignancies. Cancer Res 1999;59:728–733.

20 Kehat I, Kenyagin-Karsenti D, Snir M et al. Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. J Clin Invest 2001;108:407–414.

21 Schuldiner M, Yanuka O, Itskovitz-Eldor J et al. Effects of eight growth factors on the differentiation of cells derived from human embryonic stem cells. Proc Natl Acad Sci U S A 2000;97:11307–11312.

22 Vescovi AL, Parati EA, Gritti A et al. Isolation and cloning of multipotential stem cells from the embryonic human CNS and establishment of transplantable human neural stem cell lines by epigenetic stimulation. Exp Neurol 1999;156:71–83.

23 Zulewski H, Abraham EJ, Gerlach MJ et al. Multipotential nestin-positive stem cells isolated from adult pancreatic islets differentiate ex vivo into pancreatic endocrine, exocrine, and hepatic phenotypes. Diabetes 2001;50:521–533.

24 Reyes M, Dudek A, Jahagirdar B et al. Origin of endothelial progenitors in human postnatal bone marrow. J Clin Invest 2002;109:337–346.

25 Deschaseaux F, Gindraux F, Saadi R et al. Direct selection of human bone marrow mesenchymal stem cells using an anti-CD49a antibody reveals their $CD45^{med.low}$ phenotype. Br J Haematol 2003;122:506–517.

26 Ben-Shushan E, Thompson JR, Gudas LJ et al. Rex-1, a gene encoding a transcription factor expressed in the early embryo, is regulated via Oct-3/4 and Oct-6 binding to an octamer site and a novel protein, Rox-1, binding to an adjacent site. Mol Cell Biol 1998;18:1866–1878.

27 Niwa H, Miyazaki J, Smith AG. Quantitative expression of Oct-3/4 defines differentiation or self-renewal of ES cells. Nat Genet 2000;24:372–376.

28 Clark E, Wognum AW, Marciniak R et al. Mesenchymal cell precursors from human bone marrow have a phenotype that is direct from cultured mesenchymal cells and are exclusively present in a small subset of $CD45^{lo}$ $SH2^{+}$ cells. Blood 2001;98:85a.