Novel fibroblast-specific marker for rapid, efficient removal of mEFs from stem cell cultures by magnetic separation

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Introduction

Mitotically inactivated primary mouse embryonic fibroblasts (mEFs) and fibroblast-derived cell lines (NIH/3T3, STO) are widely used as feeder cells, e.g., for keratinocyte cultures, and more importantly for both mouse and human pluripotent stem cells, such as embryonic stem cells (ESCs) and induced pluripotent cells (iPSCs). In coculture, mEFs support the maintenance of ESC and iPSC pluripotency by secreting extracellular matrix molecules and cytokines enabling robust expansion of ESCs and iPSCs. Conversely, mEFs can interfere with several downstream applications, such as ESC and iPSC differentiation or expression profiling.

Different methods have been advised in order to reduce the feeder cell content before such experiments. One protocol is based on the preferential adherence of mEFs to uncoated tissue culture plates (fig. 1A). Another approach consists of simply weaning ESCs/iPSCs off feeders over several passages (fig. 1B). However, these protocols are laborious, inefficient, and result in considerable loss of pluripotent ESCs. Furthermore, weaning ESCs/iPSCs off mEFs may cause mEF-adapted ESCs/iPSCs to enter a crisis characterized by a high degree of spontaneous differentiation and impaired lineage differentiation potential.
Here we show that MACS® Cell Separation constitutes a superior method to obtain pure populations of ESCs or iPSCs from mEF cocultures within less then 20 minutes (fig. 1C).

Feeder depletion from Pluripotent Stem Cells

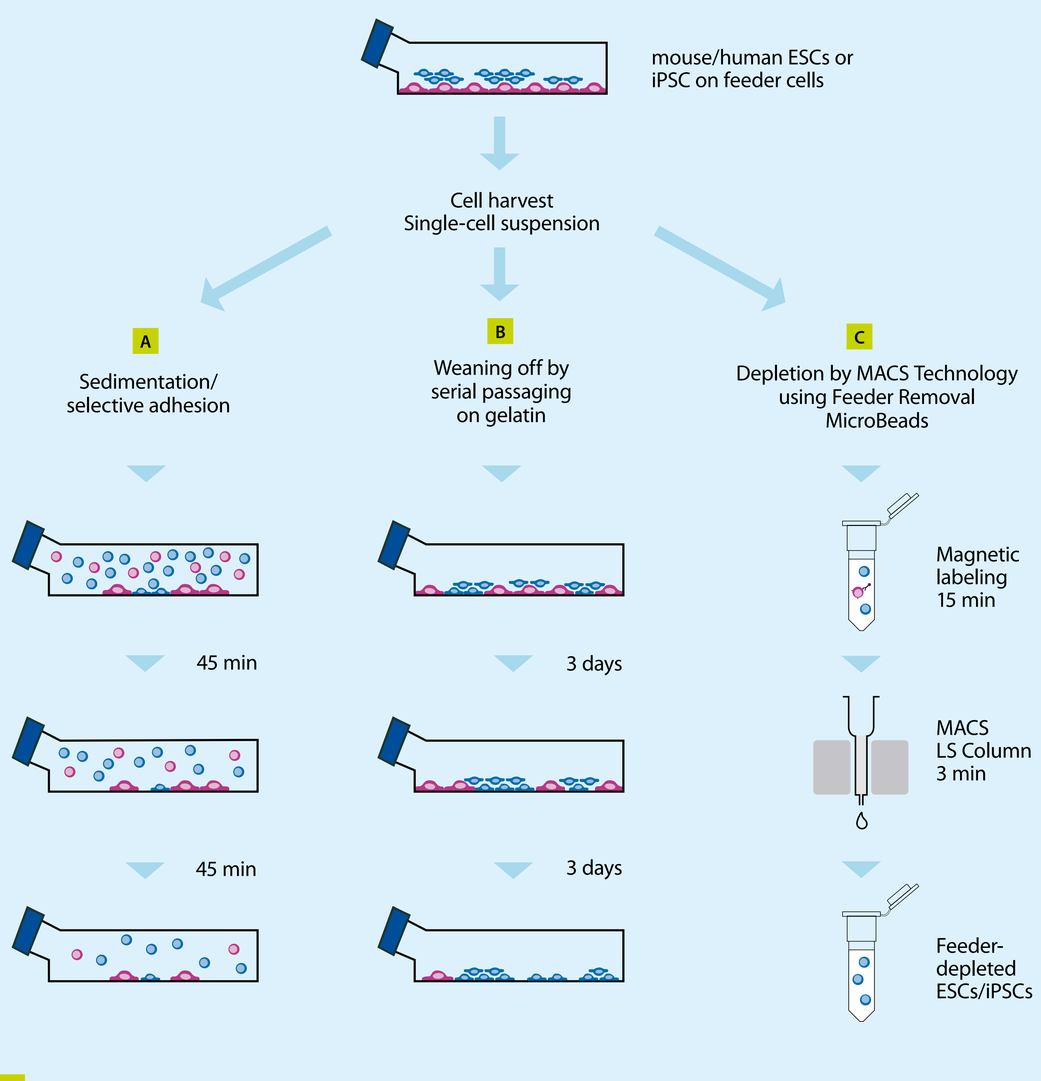

Figure 1

Results

1 Identification of a mouse fibroblast-specific surface marker

Until recently, the potential of MACS Technology for feeder depletion was hampered by the lack of a specific mouse fibroblast surface marker present on mEFs from all major mouse strains, as well as on STO and NIH/3T3 fibroblast lines.
In order to identify such a molecule, an empiric flow cytometry screening was carried out covering 100 surface molecules of known and yet unknown identity. Using mEFs from previously generated H2Kk-transgenic C57BL/6 mice, we were able to reliably discriminate mEFs from mESCs during the initial cytometry screens using anti-H2Kk antibodies. One out of 100 candidates (clone mEF-SK4, rat IgG1, κ) showed no reactivity with undifferentiated ESCs but recognized all fibroblast types as shown by flow analysis using a biotin-labeled mEF-SK4 antibody (fig. 2). Primary fibroblasts from CF-1, CF-6, DR4, C57BL/6xCD1 mouse strains (fig. 2A), as well as NIH/3T3 fibroblasts (fig. 2B) and STO fibroblasts (fig. 2C) stained positive when compared to the respective controls.
Undifferentiated mESCs (HM1, SV129, C57BL/6, J1, R1), hESCs (SA001) and hiPSCs (Cl.1 and Cl.2[1]) were negative for the identified marker (not shown).

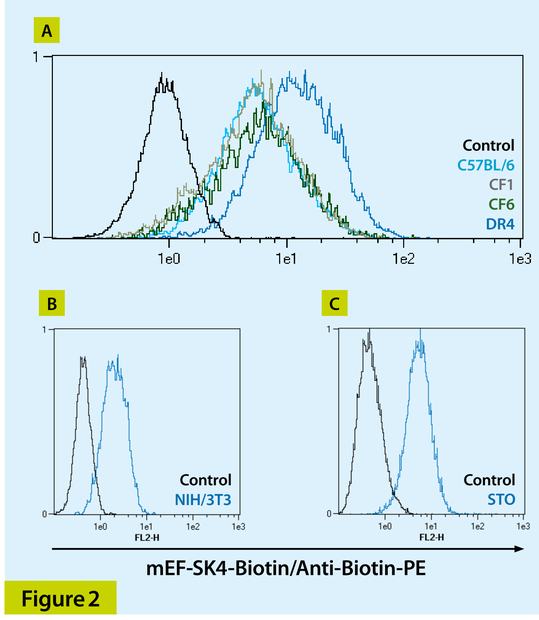

Figure 2

2 mESCs, hiPSCs, or hESCs depleted of feeder cells by MACS Technology can be differentiated into cells of the three embryonic germ layers

mEF-SK4 antibodies were coupled to paramagnetic particles (Feeder Removal MicroBeads) to allow for magnetic labeling and subsequent MACS Cell Separation of feeder cells from mESCs. HM1 or C57BL/6 mESCs were cultivated on H2Kk-positive mEFs. Cells were harvested using trypsin/EDTA to obtain a single-cell suspension containing approximately 10% mEFs (fig. 3.1 A). After incubation (15 min, 4 °C) with Feeder Removal MicroBeads, cells were passed over an LS Column to obtain a virtually pure mESC cell suspension (fig. 3.1 B) with less than 0,15% remaining mEFs. The purified ESCs were subsequently differentiated *in vitro*: EBs were cultivated in mass cultures in ES medium without LIF for 4 days (fig. 3.1 C). Subsequently, they were plated on gelatin-coated dishes. Endoderm and mesoderm differentiation was observed 8 days later as evidenced by FoxA2 (fig. 3.1 D) and SMA (fig. 3.1 E) expression, respectively. Using a five-step differentiation protocol[2] C57BL/6 mESCs were differentiated into neurons that expressed nestin, PSA-NCAM, beta-3-tubulin (fig. 3.1 F) as well as TH or GAD67.

Likewise, Feeder Removal MicroBeads were used for depletion of feeder cells from human ESCs and iPSCs (fig. 4). iPSC clone 2[1], grown on H2Kk-positive mEFs was enzymatically harvested to obtain a single-cell suspension containing approximately 15% mEFs (fig. 3.2 A). Depletion of mEFs by MACS Cell Separation resulted in a virtually pure population of hiPSCs containing less than 0,16% contaminating mEFs (fig. 3.2 B).
ESCs or iPSCs that were used for downstream cultivation or differentiation were incubated and separated in standard ESC/iPSC medium. Efficient generation of EBs from isolated hESCs/iPSCs was achieved by seeding cells in culture medium supplemented with 2 µM thiazovivin[3] (fig. 3.2 C). Spontaneous endoderm and mesoderm differentiation was observed 12 days after EBs had been seeded on gelatin-coated plates, as characterized by alpha-fetoprotein (fig. 3.2 D) and SMA (fig. 3.2 E) expression, respectively. Use of ITS medium resulted in spontaneous differentiation into beta-3-tubulin–expressing neuronal cells (fig. 3.2 F).

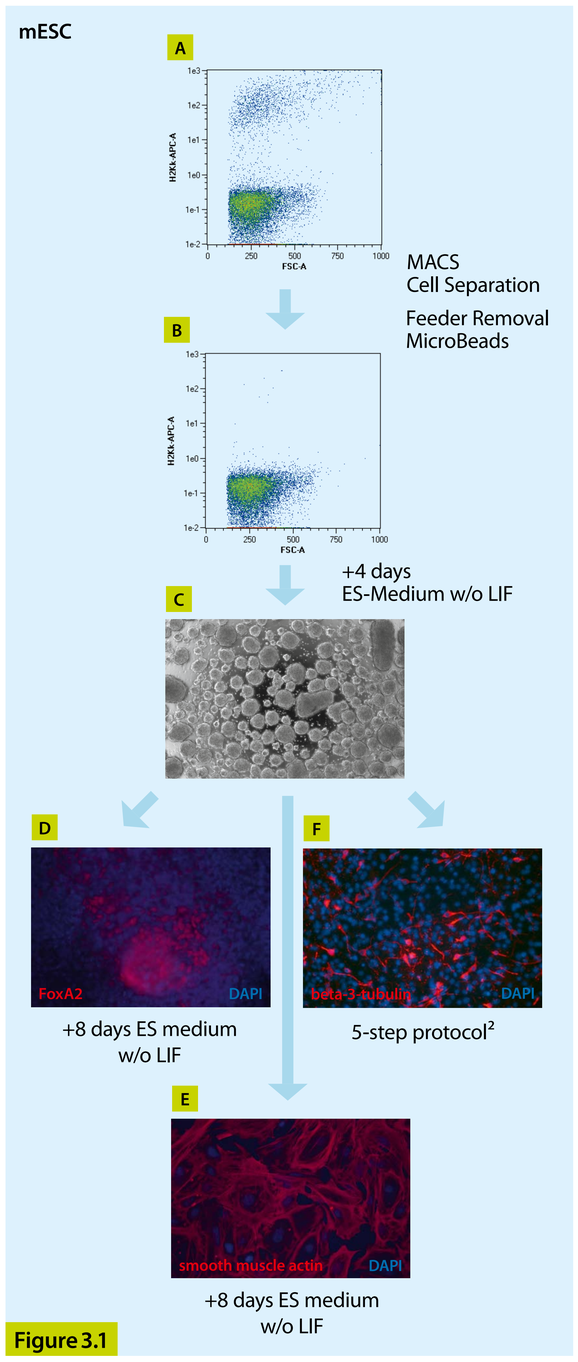

Figure 3.1

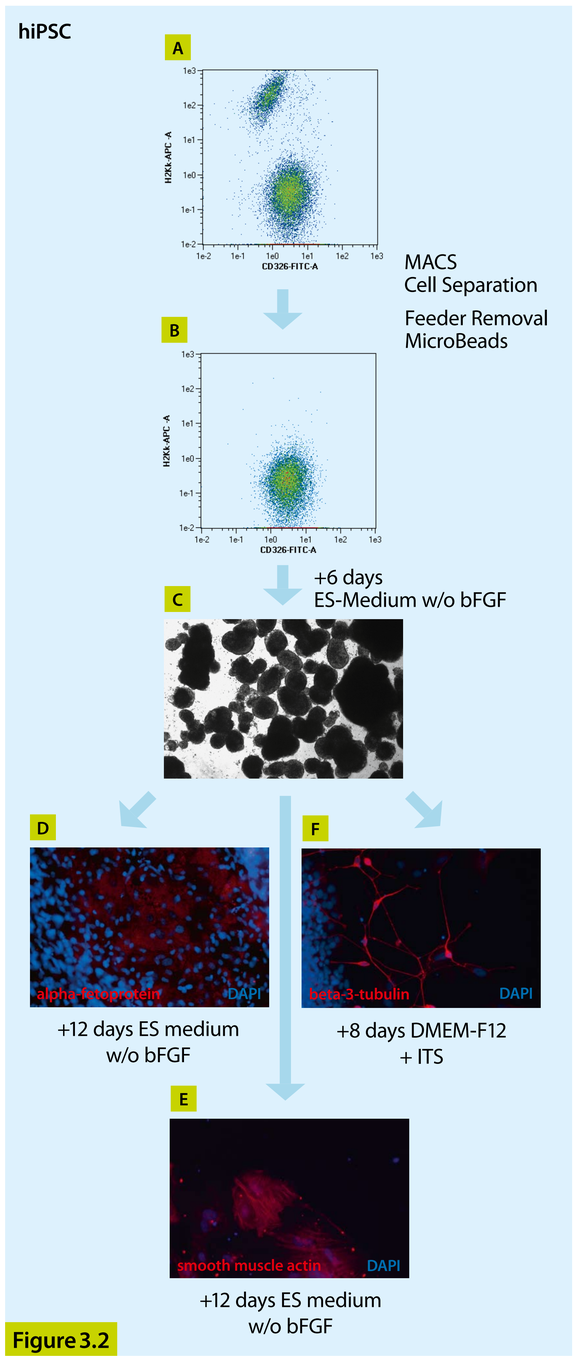

Figure 3.2

Conclusion

- An emipirical flow cytometry screening led to the identification of a monoclonal antibody recognizing:
  - primary mouse embryonic fibroblasts,
  - NIH/3T3 fibroblasts,
  - STO fibroblasts.
- Depletion of feeder cells by MACS Technology constitutes a rapid, efficient, and reliable method to obtain pure ESCs/iPSCs from feeder cocultures.
- Mouse and human ESCs/iPSCs purified by MACS Technology, without being biased by contaminating feeders, can be:
  - differentiated into cells representing all three embryonic germ layers,
  - subjected to molecular analyses.

References

1. Haase, A. *et al.* (2009) Cell Stem Cell 5: 434–441.
2. Lee, SH *et al.* (2000) Nat. Biotechnol. 18: 675–9.
3. Xu, Y. *et al.* (2010) Proc. Natl. Acad. Sci. USA 107: 8129–8134.