Multipotent mesenchymal stromal cells from amniotic fluid: solid perspectives for clinical application

Nadia Sessarego,[1] Alessia Parodi,[1,2] Marina Podestà,[1] Federica Benvenuto,[3,4] Massimo Mogni,[5] Valentina Raviolo,[1] Mario Lituania,[5] Annalisa Kunkl,[6] Guido Ferlazzo,[7] Franca Dagna Bricarelli,[5] Antonio Uccelli,[3,4] and Francesco Frassoni[1]

[1]Centro Cellule Staminali e Terapia Cellulare, Ospedale San Martino, Genova; [2]Laboratorio di Biologia Cardiaca e Vascolare, Clinica delle Malattie Cardiovascolari, DIMI, Università di Genova; [3]Unità di Neuroimmunologia, Dipartimento di Neuroscienze Oftalmologia e Genetica and [4]Centro di Eccellenza per la Ricerca Biomedica, Università di Genova; [5]Dipartimento di Scienze Genetiche, Perinatali e Ginecologiche, Ospedale Galliera, Genova; [6]Divisione di Anatomia Patologica, Ospedale San Martino and [7]Dipartimento di Patologia Umana, Università di Messina, Italy

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Flow cytometry

Direct staining was performed by incubating MSC with fluorochrome-labeled antibodies for 30 min at 4°C. Indirect staining was performed by incubating a first step antibody for 30 min at 4°C; cells were then washed in phasphate-buffered saline (PBS) 0.01M and incubated with FITC- goat anti mouse Ig. Stained cells were washed and fixed with 1% paraformaldeyde solution in PBS, pH 7.2.

The size of the MSC was also evaluated by flow cytometry using a Size Calibration Kit (Molecular Probes). A linear regression plot of forward side scatter geometric mean per bead against assigned size was generated and used to extrapolate mesenchymal cell size.

To follow the AF–MSC in vitro growth rate according to different plating cell-densities (400 cells/cm^2 and 4000 cells/cm^2), we used the intracellular fluorescent label CFDA-SE, as previously described 27.

All flow cytometric analyses were performed using a FacsCalibur flow cytometer (BD Biosciences, Milan Italy); 5000 events were acquired and data were analyzed using CellQuest or Mod Fit software (BD Bioscences).

Karyotype analysis

The MSC culture was set up at 25% confluence in a SlideFlask (NUNC). The cells were then incubated in Ham's F10 (Celbio) with colcemid (0.1 mg/mL, Sigma) for 3 h. The medium was removed and hypotonic solution (0.075 M kcl; 0.017 M Na-citrate) added. Cells were fixed with ethanol: methanol: acetic acid (1:2:1, v/v/v) and dried at 25°C and 45% humidity. Metaphase spreads were analyzed after staining with quinacrine (Sigma) for karyotyping. At least 20 cells from each sample were examined.

Inhibition of T-cell proliferation

PBMC from healthy donors were stimulated by antiCD3 (R&D Systems, Milan, Italy) and antiCD28 (BD Biosciences), both used at the concentration of 2 μg/mL. Phytohemagglutinin was also used as control (1μg/mL). Cells were cultured alone or in the presence of irradiated (at 20Gy, 2000 rad) MSC at three different concentrations (MSC: T-cell ratios of 1:1, 1:4, and 1:10) for 4 days. The cells were then pulsed with 0.5 μCi of 3H thymidine for 8 hours (5 Ci/mmole specific activity, GE Healthcare Europe GmbH, Milan, Italy) and harvested on Multiscreen Harvest plates (Millipore, Billerica, MA, USA). Results are given as the mean value of triplicate wells and expressed as Kcpm (cpm x 1000).

References

27. Urbani S, Caporale R, Lombardini L, Bosi A, Saccardi R. Use of CFDA-SE for evaluating the in vitro proliferation pattern of human mesenchymal stem cells. Cytotherapy 2006;8:243-53.

Supplementary Figure 1

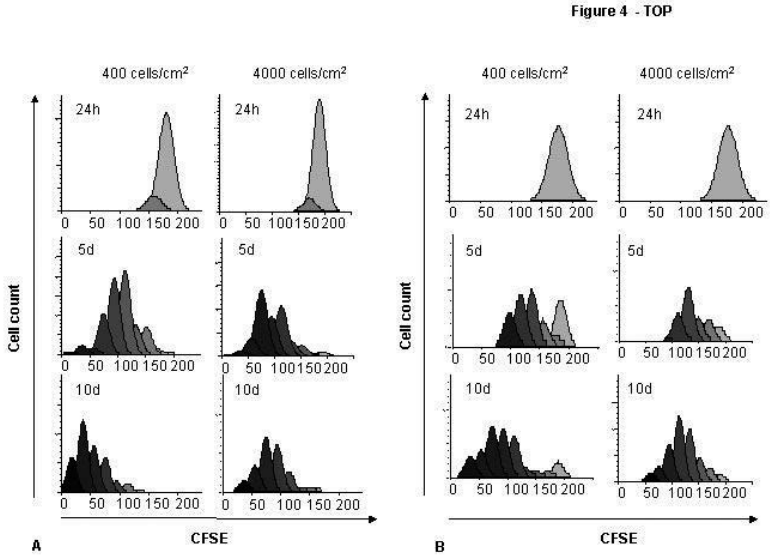

Figure 4. Proliferation pattern of fetal and adult MSCs. (A): proliferation profile of AF-MSCs grown at 400/cm² and 4000/cm² was evaluated at 24 hours, 5 days and 10 days. (B): proliferation profile of BM-MSCs at 400/cm² and 4000/cm² was analyzed at 24 hours, 5 days and 10 days. Abbreviations: d, days