Tech-Trends

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siRNA transfection of human mesenchymal progenitor cells by electroporation

Activin A expression regulates multipotency of mesenchymal progenitor cells

Background:
Bone marrow (BM) stroma currently represents the most common and investigated source of mesenchymal progenitor cells (MPCs); however, comparable adult progenitor cells or stem cells have also been isolated from a wide variety of tissues. This study aims to assess the functional similarities of MPCs from different tissues and to identify factor(s) related to their multipotency.

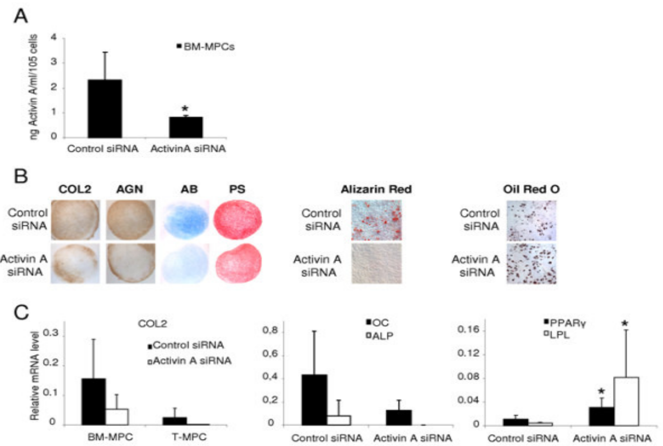
Methods:
For this purpose, we directly compared MPCs isolated from different adult tissues, including bone marrow, tonsil, muscle, and dental pulp. We first examined and compared proliferation rates, immunomodulatory properties, and multidifferentiation potential of these MPCs *in vitro*. Next, we specifically evaluated activin A expression profile and activin A:follistatin ratio in MPCs from four sources.

Results:
The multidifferentiation potential of the MPCs is correlated with activin A level and/or the activin A:follistatin ratio. Interestingly, by siRNA-mediated activin A knockdown, activin A was shown to be required for the chondrogenic and osteogenic differentiation of MPCs. These findings strongly suggest that activin A has a pivotal differentiation-related role in the early stages of chondrogenesis and osteogenesis while inhibiting adipogenesis of MPCs.

Conclusions:
This comparative analysis of MPCs from different tissue sources also identifies bone marrow-derived MPCs as the most potent MPCs in terms of multilineage differentiation and immunosuppression, two key requirements in cell-based regenerative medicine. In addition, this study implicates the significance of activin A as a functional marker of MPC identity.

ECM 830 Square Wave Electroporation System includes ECM 830 Generator, 630B Safety Stand, Cuvettes 1 mm, 2 mm and 4 mm pkg. of 30 and Cuvette Rack 660

Cat. 450002



Role of activin A in MPC multipotency. BM-MPCs transiently transfected either with control siRNA or with activin A siRNA were analyzed. **(a)** Activin A secretion level in the supernatant of transfected BM-MPCs quantified by ELISA 3 days (D3) after transfection showed the efficiency of knockdown by using activin A siRNA. **(b, c)** Effect of activin A knockdown on BM-MPC and T-MPC chondrogenic potential. Chondrogenic differentiation was evaluated after 21 days in micropellet culture. **(b)** Immunohistochemical analysis of cartilage markers (COL2 and AGN) in pellet cultures of BM-MPCs and T-MPCs as well as alcian blue (AB) and picrosirius red (PS) staining revealed that, compared with the untransfected and Control siRNA, MPCs transfected with activin A siRNA exhibited less chondrogenic potential. **(c)** Quantitative RT-PCR also showed decreased expression of COL2 in MPCs transfected with activin A siRNA, compared with the cells transfected with control siRNA (c). **(b, c)** Effect of activin A knockdown on BM-MPC osteogenic potential. Both the expression levels of the osteogenic markers (OC and ALP), determined by quantitative RT-PCR, and alizarin red staining, revealed a decrease in the osteogenic activity of BM-MPCs with activin A knockdown. **(b, c)** Effect of activin A knockdown on BM-MPC adipogenic potential. Both expression levels of the adipogenic markers (PPAR-γ and LPL), determined by quantitative RT-PCR and oil red O staining, revealed an increase in the adipogenic potential of BM-MPCs with activin A knockdown, compared with BM-MPCs transfected with the irrelevant Control siRNA. RT-PCR data represent the mean ± SD of three independent experiments. $^{*}P \leq 0.05$, versus Control siRNA.

ECM 830 ELECTROPORATION PROTOCOL

Cell Preparation:

For adipogenic differentiation, cells were seeded at a density of 20,000 cells/cm^2 and treated for 3 weeks with adipogenic medium consisting of DMEM with 10% FBS, and supplemented with 0.5 mmol/L 3-isobutyl-1-methyl-xanthine (IBMX), 1 µg/ml insulin, and 1 µmol/L dexamethasone. For osteogenic differentiation, cells were seeded into 6-well plates at a density of 20,000 cells/cm^2, and treated for 3 weeks with osteogenic medium, consisting of DMEM with 10% FBS, and supplemented with 10 mmol/L β-glycerol-phosphate, 10 nmol/L dexamethasone, 50 µg/ml ascorbic acid-2-phosphate, and 10 nmol/L 1,25 di-hydroxyvitamin D_3. To induce chondrogenic differentiation, 96-microwell polypropylene plates were seeded with a density of 300,000 cells per well, and cell pellets formed by centrifugation at 1100 rpm for 6 minutes. The pellet cultures were treated for 3 weeks with chondrogenic medium, consisting of high-glucose DMEM supplemented with 100 nmol/L dexamethasone, 50 µg/ml ascorbic acid-2-phosphate, 100 µg/ml sodium pyruvate, 40 µg/ml L-proline, 10 ng/ml recombinant human transforming growth factor-β3, and 50 mg/ml ITS-premix stock.

Electroporation settings:

Voltage:	1075 V
Pulse Length:	105 µs
Pulse Interval:	5 s
Pulse Number:	2
Electrode Gap:	2 mm
Pulse:	Press Start to Activate automatic charge and pulse
Post treatment:	Incubate for 15 min at 37ºC in a 5% CO2/95% air atmosphere.

Electroporation procedure:

Total volume: 300 ul
Cell density: 750,000
Transfectant: 45 ul of 22.5 ug siRNA

References:
Farida Djouad*[1,2], Wesley M Jackson1, Brent Bobick[1,3], Sasa Janjanin[1,4], Yingjie Song1, George TJ Huang[1,5] and Rocky S Tuan[1,6]
[1]Cartilage Biology and Orthopaedics Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Department of Health and Human Services, 9000 Rockville Pike, Bethesda, Maryland 208992, USA. [2]Current address: Inserm U 844, Hopital Saint-Eloi, Batiment INM, 34295 Montpellier, France. [3]Current address: Department of Biological Sciences, 2500 University Drive NW, Calvary, Alberta, T2N 1N4, Canada. [4]Current address: Department of Otorhinolaryngology, Head & Neck Surgery, Zagreb Clinical Hospital Center, Zagreb University School of Medicine, Salata 4, 10000 Zagreb, Croatia. [5]Current address: Department of Molecular and Cell Biology, Boston University Henry M. Goldman School of Dental Medicine, 72 East Concord Street, Boston, MA 02118, USA. 6Current address: Department of Orthopaedic Surgery, Center for Cellular and Molecular Engineering, University of Pittsburgh School of Medicine, 450 Technology Drive, Room 221, Pittsburgh, PA 15219, USA

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