

Oct-4, Rex-1, and Gata-4 expression in human MSC increase the differentiation efficiency but not hTERT expression.

Stéphane Roche, Marie-Jeanne Richard, Marie-Christine Favrot

► **To cite this version:**

Stéphane Roche, Marie-Jeanne Richard, Marie-Christine Favrot. Oct-4, Rex-1, and Gata-4 expression in human MSC increase the differentiation efficiency but not hTERT expression.. *Journal of Cellular Biochemistry*, Wiley, 2007, 101 (2), pp.271-80. 10.1002/jcb.21185 . inserm-00312819

HAL Id: inserm-00312819

<https://www.hal.inserm.fr/inserm-00312819>

Submitted on 9 Jan 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Oct-4, Rex-1, and Gata-4 Expression in Human MSC Increase the Differentiation Efficiency But Not hTERT Expression

Stéphane Roche,* Marie-Jeanne Richard, and Marie-Christine Favrot

Centre d'investigation Biologique, Centre Hospitalier Universitaire de Grenoble, Grenoble, France

Abstract Micro-environment seems to exert an important influence on human mesenchymal stem cell (MSC) differentiation and proliferative capacity in bone marrow as well as in culture *ex vivo*. Oct-4, Rex-1, and TERT genes are well-known for the maintenance of pluripotentiality differentiation and the proliferative capacity of embryonic stem cells. Some previous data report expression of these embryonic factors in selected clones from bone marrow adult stem cells. Our goal was to study expression of Oct-4, Rex-1, and TERT in primary cultured human MSC according to the serum concentration. In addition, we have studied the expression of Gata-4 since this factor plays a key role in organogenesis. We hypothesized that low serum concentration with appropriate growth factors may induce an undifferentiated status with a re-expression of embryonic factors and extend differentiation capacity. Thus, using a defined culture medium, we report on the increased expression of Oct-4, Rex-1, and Gata-4 in human MSC. We have correlated this expression to an increase in differentiation efficiency towards osteogenic and adipogenic phenotypes. Our data suggest that the culture medium used permits the emergence of adult stem cells with a high differentiation capacity and expression of embryonic factors. These cells may have important implications for cell therapy.

Key words: human mesenchymal stem cell; Oct-4; Rex-1; Gata-4; TERT; define medium; differentiation

The factors involved in the differentiation process of mesenchymal stem cells (MSC) have not been completely elucidated. Among them, the micro-environment (ME) or “niche” seems to play a critical role. The ME controls the undifferentiated status of the MSC as well as the multipotentiality in different environments including bone marrow, or the extensive *ex vivo* culture.

Various studies have identified factors normally restricted to embryonic stem cells (ESC) and which may be expressed in adult stem cells under specific conditions. Reyes et al. [2001] have isolated Multipotent Adult Progenitor Cells (MAPC) described as precursors of MSC. The MAPC express Oct-4 and Rex-1 transcrip-

tion factors and several common surface antigens with MSC. MAPC share important characteristics with embryonic stem cells, in particular large telomeres after long-term culture [Reyes et al., 2001] and probably pluripotency [Jiang et al., 2002a]. Pochampally et al. [2004] have described the selection of MSC precursors using serum deprivation. These cells express Oct-4, telomerase reverse transcriptase (TERT) and maintain large telomeres.

Oct-4 [Boiani et al., 2002] and Rex-1 [Ben-Shushan et al., 1998] are transcription factors characteristic of pluripotentiality. They have been characterized as embryonic and germinal restricted factors [Baddoo et al., 2003]. The molecular events underlying the separation of distinct cell lineages are still not well understood. However, it is known that the transcription factor Oct-4 is expressed throughout oogenesis and becomes restricted to the inner cell mass at the blastocyst stage and is later confined to developing germ cells [Scholer et al., 1990; Palmieri et al., 1994]. Niwa et al. [2000] have demonstrated a relationship between Oct-4 and Rex-1 expression. Paradoxically, an over- or under-expression of

Stéphane Roche's present address is Institut de Génétique Humaine, CNRS UPR1142 Montpellier, France

*Correspondence to: Stéphane Roche, 141, rue de la cardonille, Montpellier 34396, France.

E-mail: stephane.roche@igh.cnrs.fr

Received 8 June 2006; Accepted 22 September 2006

Oct-4 leads to a down-regulation of Rex-1 expression. These transcription factors regulate the totipotency of the ESC. Their down-regulation triggers trophoblast differentiation and their up-regulation triggers primitive endoderm and mesoderm differentiation [Niwa et al., 2000]. Among other embryonic mesendodermic transcription factors, some factors from the GATA family play a critical role in mesendoderm and endodermal differentiation. These zinc finger proteins are composed of two conserved zinc fingers and their adjacent basic domains bind to (A/T)GATA(A/G) sequences. Only six GATA factors have been isolated from vertebrates. They are subdivided in two classes, GATA 1–3 and 4–6 [Technau and Scholz, 2003]. GATA factors 4–6 are controlled by signaling pathways that act in the early specification of endoderm and mesoderm [Shoichet et al., 2000; LaVoie, 2003; Yoshida-Koide et al., 2004].

In contrast to embryonic stem cells and MSC precursors, human and murine primary MSC are negative for TERT expression [Banchi et al., 2003] and have a limited number of divisions before senescence. The unlimited propagation of ES cells is related to the expression and activity of telomerase [Kim et al., 1994]. Some studies have reported the establishment of human MSC with an infinite life span which maintain their differentiation potency and their physiological growth rate by the enforced expression of TERT [Okamoto et al., 2002; Mihara et al., 2003].

The aim of this work has been to demonstrate that the culture conditions of adult human MSC may modify the expression of embryonic genes associated with their potential for differentia-

tion. A culture medium with a low serum concentration seems to play a major role in restoring the multipotentiality of human MSC [Reyes et al., 2001; Hu et al., 2003]. Our data shows an increase in Oct-4, Rex-1, and Gata-4 by MSC and their differentiation potency.

MATERIALS AND METHODS

Mesenchymal Stem Cell Isolation and Expansion

Bone marrow aspirates were collected on ACD-heparin from healthy adult volunteers after informed consent. Nucleated cells were directly plated at 50,000 cells per cm^2 on plastic culture dishes. Expansion medium consisted of α MEM basal medium (Sigma Aldrich, Saint Quentin Falavier, France) supplemented with 10% FCS (Invitrogen), 2 mM glutamine and penicillin/streptomycin (Roche Diagnostic, Meylan, France) noted α MEM medium. Two to three days later non-adherent cells were discarded and primary culture was performed for 21 days. The complete medium was changed twice a week. After reaching subconfluence, cells were lifted with 0.25% trypsin and 1 mM EDTA, suspended in fresh medium, plated at 1,000 cells/ cm^2 and incubated for 14 days at 37°C in 5% CO_2 .

Induction Phase

Two different conditions of culture were tested (see Fig. 1) for their capacity to facilitate cell differentiation. After trypsinization, cells were plated either on plastic in α MEM medium or on plastic coated with human fibronectin (Sigma Aldrich) in a modified MCDB201 medium. This modified MCDB201 medium was composed of: MCDB201/DMEM (60/40), 2% FCS, 1× ITS + 1 (insulin-transferrine

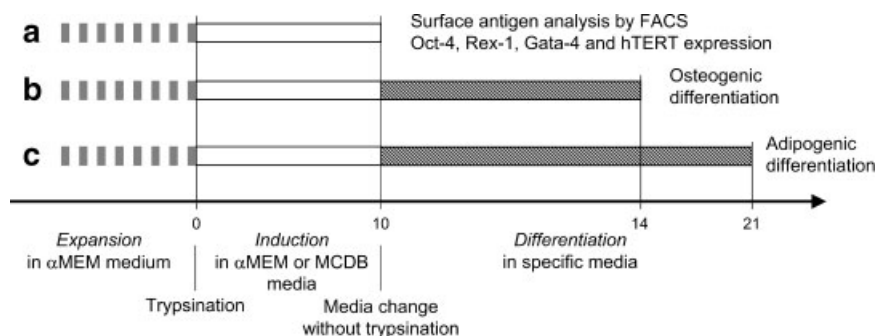


Fig. 1. Design of the study. Cells were cultured in modified MCDB201 medium or α MEM medium for 10 days. After this induction phase, RT-PCR and quantitative PCR analysis were performed (lane a) as well as differentiation to osteoblastic phenotype (lane b) and adipocyte phenotype (lane c).

Embryonic Gene Expression in Adult MSC

selenium containing linoleic acid and BSA), 10^{-9} M dexamethasone, 10 ng/ml PDGF-BB, 10 ng/ml EGF and 0.2 mM ascorbate-2-P (All from Sigma Aldrich).

Fluorescence Activated Cell Sorting (FACS) Analysis

Cells were washed once with culture medium, once with PBS, resuspended in PBS-BSA 1% and stained with coupled fluorochrome antibodies as described by the manufacturer. Analysis was performed with a FACSCalibur (Beckton Dickinson, Pont De Claix, France). CD90-PE, CD73-PE, CD45-Cy, CD34-Cy, CD44-FITC, CD49b-PE were purchased from Beckton Dickinson; CD105-PE and GlyA-FITC from Santa Cruz (Santa Cruz, CA); CD133-PE from Miltenyi Biotech (Paris, France).

RNA Extraction and RT-PCR

Total RNA was extracted from each sample with RNA plus (Qbiogen, Illkirch, France), according to the recommendations of the manufacturer. The harvested RNA was reverse transcribed using a M-MLV reverse transcriptase kit (Roche Diagnostic). The cDNA was amplified with specific primers as shown in Table I in a reaction mixture containing "FastTaq" Taq polymerase (Roche Diagnostic). PCR was performed in a DNA thermal Cycler (T3 thermocycler, Biometra, Goettingen, Germany). The amplified samples were visualized on 2% agarose gels stained with ethidium bromide and photographed under UV light using a GelDoc (BioRad, Marnes-la-Coquette, France) system.

Detection of Telomerase Components hTERT

Telomerase components hTERT were detected by quantitative, one-step RT-PCR with the LightCycler instrument (Roche Diagnostic) using the *TeloTAGGG* hTERT Quantification Kit (Roche Diagnostic) according to the manufacturer's instructions. In a separate one-step RT-PCR, mRNA encoding for porphobilinogen deaminase (PBGD) was processed for use as a housekeeping gene. The reaction product serves both as a control for RT-PCR performance and as a reference for relative quantification.

Differentiation

After cell maintenance for 10 days in α MEM or in modified MCDB201 medium, cells were cultured in specific differentiation media (Fig. 1).

To induce adipocyte differentiation, human MSC were cultured in MCDB201/DMEM (60/40) with $2 \times$ ITS + 1 (containing linoleic acid and BSA), 10% horse serum (Sigma Aldrich) 10% foetal calf serum, 10^{-7} M dexamethasone and 60 μ M indomethacine and maintained for 21 days with medium exchanges every 5 days. For oil-red staining, cells were fixed with cold methanol (-20°C) for 2 min and rinsed with propan-2-ol 50%. Cells were stained by oil-Red-O at 0.2% in propan-2-ol for 10 min. After photography, coloration was solved by propan-2-ol 100% and quantify at 500 nm [Ramirez-Zacarias et al., 1992; Sekiya et al., 2002, 2004].

To induce osteoblast differentiation, human MSC were cultured in DMEM low glucose (1 g/L) with 10% FCS, 10 mM β -glycerophosphate,

TABLE I. Sequence of Primer, Size of Product, Number of PCR Cycle and Annealing Temperature

Gene	Primers	PCR cycle	Annealing temperature ($^{\circ}\text{C}$)
RPL27	5' GAACATTGATGATGGCACCTC 3' GGGGATATCCACAGAGTACC	26	55
CD90	5' TCGCTCTCCTGCTAACAGTCTTG 3' GCCCTCACACTTGACCAAGTTTG	30	58
CD73	5' TCGGCTCTTACCAAGGTTTCAG 3' CCCACAACATTCATCACCAACAGG	30	58
CD105	5' CATGTGGCATCCTTCGTGG 3' AACTTGTACCCCTGTCTCTG	30	58
Oct-4	5' CGACCATCTGCCGCTTTGAG 3' CCCCCTGTCCCCATTCCTA	36	58
Rex-1	5' GCGTACGCAAATFAAAGTCCAGA 3' CAGCATCCTAAACAGCTCGCAGAAT	36	58
Gata-4	5' GATGCCTTTACACGCTGATG 3' TGGGTTAAGTGCCCTGTAG	34	60

Oct-4 and Rex-1 primers were defined by Henderson et al. [2002].

10^{-7} M dexamethasone, and 0.2 mM ascorbic acid with medium changes twice a week. After 14 days, human MSC were fixed with methanol. Mineralization was assayed using 2% alizarine red for 2 min. Alkaline phosphatase activity was detected by 5-bromo-4-chloro-3-indoly phosphate and nitroblue tetrazolium for 5–10 min and then washed with 100 mM Tris HCl pH 9.5 100 mM NaCl and 10 mM $MgCl_2$ buffer [Stanford et al., 1995; Sakaguchi et al., 2004; Abdallah et al., 2006].

RESULTS

We first checked that human Mesenchymal Stem Cells (human MSC), selected and expanded in α MEM medium, were not contaminated with hematopoietic cells. Indeed, CD45 positive cells represent $5\% \pm$ in the primo culture, $1\% \pm$ of cells above second sub-culture and were not detectable after this sub-culture. Human MSC cultured in α MEM medium did not express other hematopoietic markers such as CD34 and CD133 and expressed CD90 (Thy-1), CD73, CD105 (endoglin), CD44 and CD49b

(Fig. 2a) in agreement with the expression pattern of surface antigens previously described for human MSC. The proportion of positive cells for these markers was $97.6 \pm 2.3\%$ CD90, $97.7 \pm 2.2\%$, CD73 and $97.5 \pm 1.9\%$ CD105 (Fig. 2a). This expression was maintained during the expansion phase in each sub-culture and we checked that cells have osteogenic, adipogenic, and chondrogenic differentiation capability (data not shown). The surface antigens on the human MSC expanded 10 days in modified MCDB201 medium were analyzed by flow cytometry and compared with cells expanded in α MEM. No differences were observed in the level of CD90, CD73, and CD105 antigens (Fig. 2b). CD49b expression was not modified by culture on plastic coated with 10 μ g/ml human fibronectin (Fig. 3a). A previous study observed a modification of CD44 antigen expression in relation to the percentage of FCS in culture [Lodie et al., 2002]. To test whether our culture conditions could interfere with CD44 expression, we performed an additional experiment using modified MCDB201 medium supplemented or not with 2 or 10% FCS

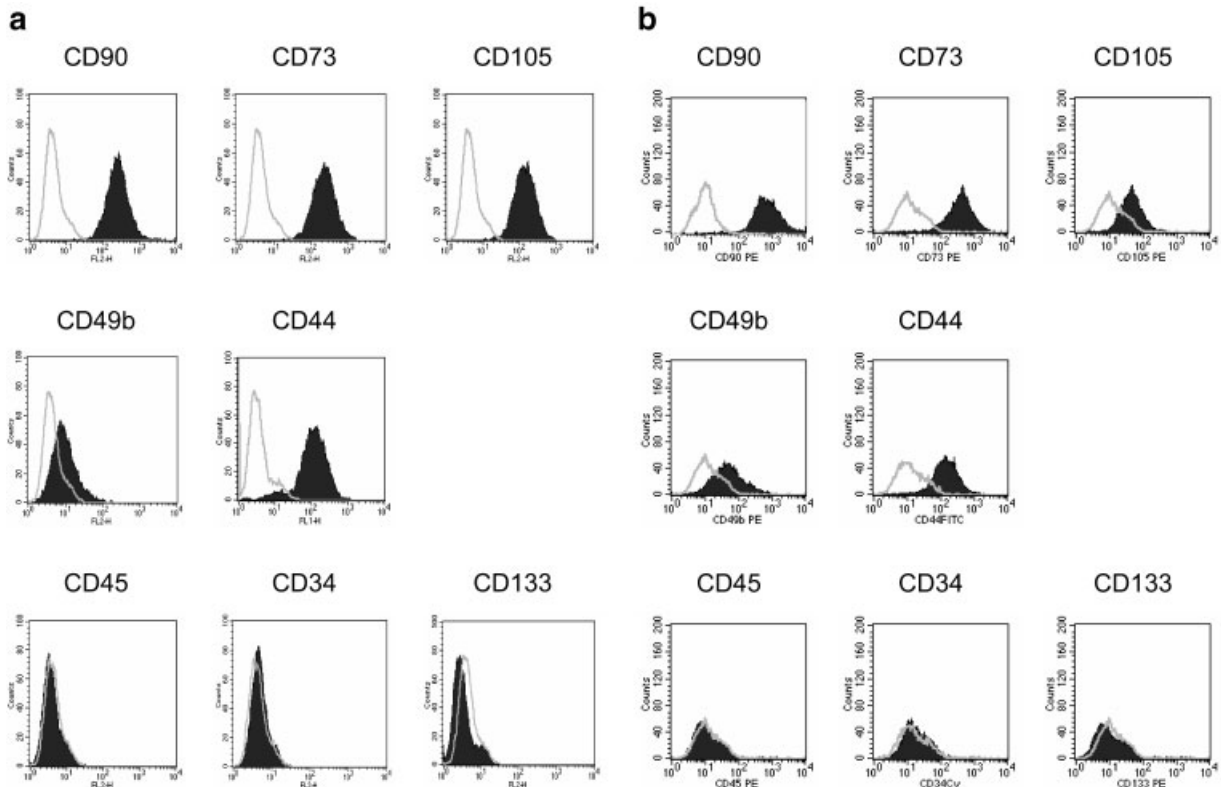


Fig. 2. Phenotype of human mesenchymal stem cell (human MSC) in culture in (a) α MEM medium and (b) modified MCDB201. The black shaded curve represents antigen specific recognition and the unshaded curve the negative isotopic control. These blots are representative of three different experiments.

Embryonic Gene Expression in Adult MSC

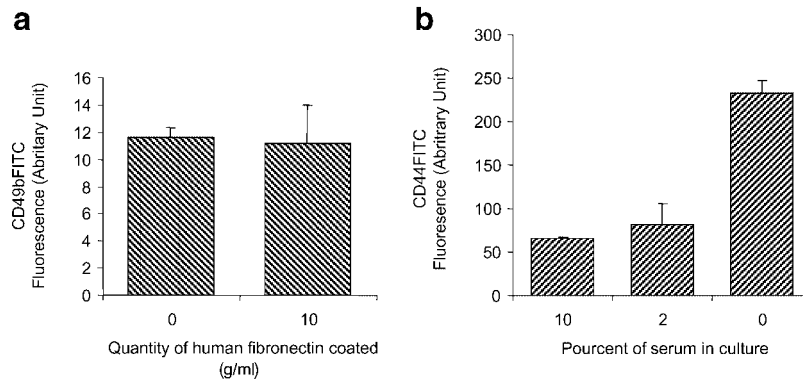


Fig. 3. Quantification of matrix receptor CD49b (panel a) and CD44 (panel b) level of expression by flow cytometry. Plots show isotype control versus specific antibody staining profile.

(Fig. 3b). Cells cultured in serum free medium (0% FCS) for 10 days expressed a high level of CD44 (level 233 ± 13 in arbitrary unit of fluorescence). During culture with 2 or 10% of FCS, CD44 expression diminished to 65 ± 1 arbitrary unit (Fig. 3b).

Cells cultured in modified MCDB medium show a more rapid growth than cells cultured in α MEM medium until the 6th passage analyzed (Fig. 4). To further determine the extent of differentiation of cells cultured for 10 days in modified MCDB201 or in α MEM medium, the

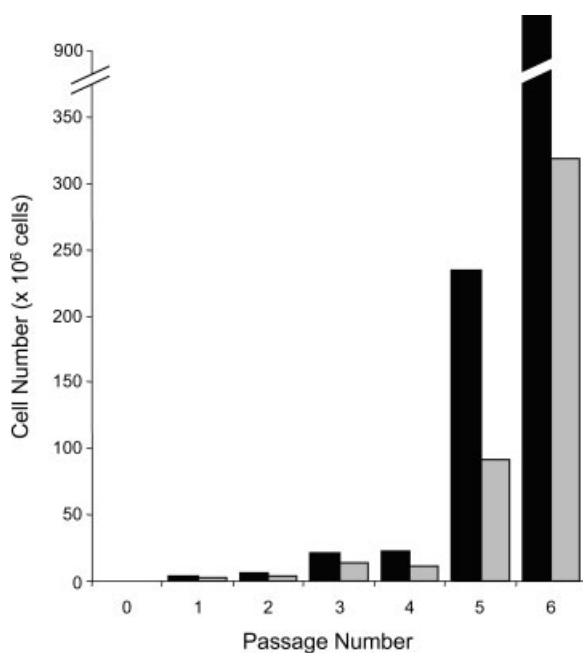


Fig. 4. Cell growth capability of human mesenchymal stem cells in α MEM medium (gray bar) and modified MCDB201 (black bar). After each passage, cells were plated at 1,000 cells per cm^2 and the culture medium changed every 3 days. This experiment is representative of three independent cultures.

expression of Oct-4 and Rex-1 was studied. In MSC cultured in α MEM medium, no expression of Rex-1 and very low levels of Oct-4 were detected (Fig. 5 column a) whereas Rex-1 and Oct-4 were expressed in human MSC after the induction phase obtained by expansion in modified MCDB201 medium (Fig. 5 column b).

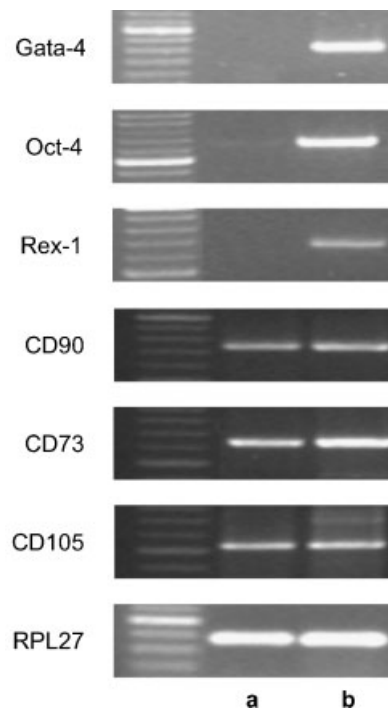


Fig. 5. Expression of transcription factors by RT-PCR. Reverse transcription was performed using 0.5 μg of total RNA and oligo dT nucleotides. Primers and PCR conditions are described in Table I. RPL27 is a ubiquitous protein; CD90, CD73, and CD105 genes were positive controls of MSC. We carried out a 10-day culture in α MEM (column a) or in modified MCDB201 (column b) media before RNA extraction. This figure is representative of three independent experiments.

In addition, we observed an increase in Gata-4 gene expression during the same induction phase. The phenotype of cells was confirmed by measuring expression of CD90, CD73, and CD105 mRNA in the same experiments. According to the FACS analysis, no difference was observed between expression of RPL27 and CD90, CD73 and CD105 in the two media.

Using a real time PCR detection kit, we compared the expression of the hTERT gene in cells maintained in α MEM versus cells induced by culture in the modified MCDB201 medium. Human MSC maintained in α MEM medium, do not express the hTERT gene (Fig. 6). Similarly, no hTERT mRNA was observed in cells cultured in modified MCDB201 medium.

As shown in Figure 7, we observed a greater deposition of mineral in cells pre-treated in modified MCDB201 medium (panel B) than cells maintained in α MEM medium (panel A). The same observation can be made concerning the activity of alkaline phosphatase. These levels are higher in modified MCDB201 medium pre-treated cells (panel D) compared with α MEM medium (panel C).

Adipogenesis as shown in Figure 7 panel E and F is significantly higher in modified MCDB201 medium pre-treated cells. These observations were confirmed by measuring fat

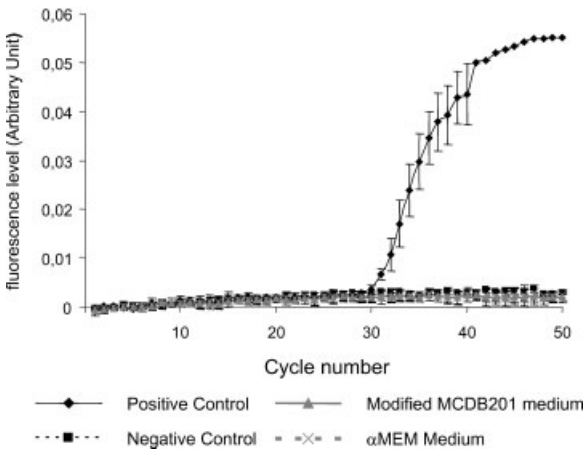


Fig. 6. Detection of hTERT mRNA by realtime PCR. A one-step detection kit and a LightCycler system were used in this study. The plain black line illustrates the positive control provided in the kit, the dotted black line the negative control, dotted gray line shows α MEM medium and the plain gray line shows the modified MCDB201 medium. As supplied by the manufacturer, we used the PBGD housekeeping gene as a control of RT-PCR reaction. This graph is representative of three independent experiments.

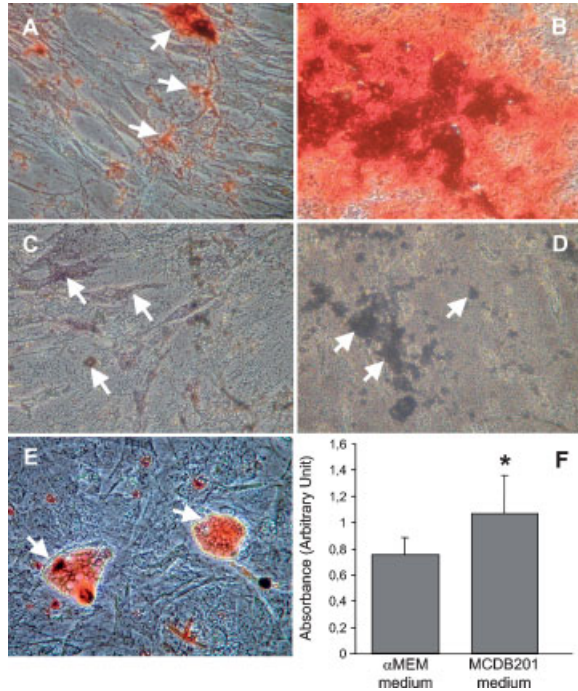


Fig. 7. Differentiation into osteoblastic and adipogenic phenotype: influence of culture medium. (1) Mineral deposition (panels A and B) and phosphatase alkaline (PA) activity (panels C and D) were observed either in modified MCDB201 (panels B and D) or α MEM (panels A and C). The mineral deposition is observed with red alizarin coloration phospho-calcic crystal. The PA activity is measured using BCIP/NBT substrate transformation. This is representative of three independent experiments. (2) Adipocytic vesicles are colored by Oil Red O (white arrow in panel E). A quantification by solubilization of coloration in 2 ml propan-2-ol and measurement of absorbance at 500 nm demonstrated a significant increase of differentiation in modified MCDB201 medium (*t*-test, $P < 0.0025$, represented by an asterisk in panel F). These results are a mean of three representative independent experiments.

droplets stained with fresh Oil Red-O (*t*-test, $P < 0.0025$).

DISCUSSION

In this study, using adult MSC, we demonstrate that their culture in modified MCDB lead to (1) the transcription of embryonic factors involved in totipotency (e.g., Oct-4 and Rex-1) and organogenesis (e.g., Gata-4) but not TERT; and (2) a facilitation of the osteogenic and adipogenic differentiation potential. These physiologic modifications were not correlated with modification of classical membrane antigens expressed by MSC in culture.

The MSC phenotype needs to be clearly defined and checked to avoid any contamination

Embryonic Gene Expression in Adult MSC

by precursor cells from other sources. Previous studies have defined the phenotype of MSC as negative for CD45 antigen and progenitor specific markers such as CD133 and CD34, and positive for CD44, CD73, CD90, CD105, and low for CD49b [Reyes et al., 2001; Lodie et al., 2002; Jiang et al., 2002b; Baddoo et al., 2003]. MSC used for this study express CD44, CD73, CD90, CD105, and were low for CD49b and negative for CD45, CD34, and CD133 as expected. Various precursor cells within bone marrow, negative for CD45, may also be found as endothelial precursor cells (EPC). EPC are CD45⁻ CD133⁺ and become CD133⁻ CD34⁺ during differentiation to mature endothelial cells [Quirici et al., 2001; Salven et al., 2003]. In bone marrow, these CD133⁺ cells are the common precursors to endothelial and hematopoietic lineages [Quirici et al., 2001]. Conversely, MSC are negative for CD133 [Vogel et al., 2003; Oswald et al., 2004]. This MSC phenotype is stable in the two types of medium used in this study. Reyes et al. [2001] observed with MAPC that the increase of FCS concentration induces an increase in CD44 and a decrease in CD49b expression in relation with a loss of differentiation potentiality and of proliferation potential beyond 30 cell doublings [Jiang et al., 2002a,b]. Thus, we analyzed the expression level of these two antigens under our culture conditions. In contrast with MAPC, we demonstrate that the level of CD49b expression was not modified by culture of MSC on fibronectin. In human MSC, CD49b associates non-covalently with CD29 [Baddoo et al., 2003; Meirelles Lda and Nardi, 2003; Zimmermann et al., 2003] and form the integrin $\alpha 2\beta 1$, a receptor for collagen and laminin [Miyake et al., 1994] and not fibronectin. This could explain the absence of the influence of fibronectin on CD49b expression. The second matrix receptor analyzed was CD44, a member of the hyaluronan-binding proteins, with structural similarities to selectins. Differences may be related to the concentration of FCS [Lodie et al., 2002]. However, between 10 and 2% (inverse!) of FCS, no significant modification was showed but we could demonstrate an important increase in the absence of FCS.

Our work demonstrating the expression of Oct-4, Rex-1, and Gata-4 is likely to be due to the culture conditions used. The expression of these embryonic factors has rarely been reported in adult cells [Moriscot et al., 2005]. Data using

embryonic stem cells in murine models demonstrated the importance of Oct-4 expression for generating the three primordial tissues (endoderm, ectoderm, and mesoderm) [Niwa et al., 2000; Niwa, 2001]. This transcription factor was expressed only in cells which can differentiate in these three embryonic layers or in an adult organism but are restricted to the germinal cell line. Regulation of this factor seems to be critical because its up or down-regulation reduces the totipotency of embryonic cells [Niwa et al., 2000; Niwa, 2001], represses target genes such as Rex-1 and induces differentiation [Niwa et al., 2000]. In our study, we both induced expression of Oct-4 and Rex-1 by culturing human MSCs with low serum concentration in a modified MCDB201 medium.

The presence of TERT expression in adult stem cells is subject to debate. The expression of TERT in cells is associated with immortal cells [Kim et al., 1994]. Several studies have demonstrated that MSC do not express TERT catalytic subunits. However, one study has demonstrated TERT expression during a limited time but failed to identify the activity of this enzyme [Bianchi et al., 2003]. Although regarded as tissue-specific stem cells, human MSCs have a relative low proliferative ability with a limited life span. The average number of population doublings is reported to be approximately 38 at which time cells finally cease to divide, being broad and flattened [Bruder et al., 1997]. Verfaillie's group has demonstrated that MAPC expressed Oct4 and have extended growth capacity due to presence of large telomeres without any evidence of hTERT expression [Reyes et al., 2001; Jiang et al., 2002a]. Prockop's group has identified in the primary cultures of hMSCs a subpopulation (named RS) which has a greater potential of proliferation and differentiation [Pochampally et al., 2004]. This population can be isolated by serum deprivation. They have demonstrated that their RS cells express Oct-4 and have an extended growth capacity due to higher TERT expression and larger telomeres than hMSCs. In our culture conditions, with PDGF and EGF growth factors, we have demonstrated that the total population of hMSCs can express embryonic transcription factors such as Oct-4 and Rex-1 and display a greater growth potential but not express hTERT. In addition, we have previously demonstrated that MSCs showed large telomeres [Moriscot et al., 2005].

We did not observe Gata-4 expression in MSC cultured in α MEM medium as previously described [Lodie et al., 2002] but we were able to demonstrate an increase in the expression of Gata-4 transcription factor in a modified MCDB201 medium. This factor plays an important role in the mechanism of differentiation into meso- and endodermal tissues [Shoichet et al., 2000; Yoshida-Koide et al., 2004] in organogenesis of most vertebrates. The expression of this transcription factor into MSC was observed after treatment by the 5-azacytidine drug [Hakuno et al., 2002; Xu et al., 2004] and after hepatocyte growth factor treatment of adult stem cells [Schwartz et al., 2002; Forte et al., 2006]. Gata-4 expression may play a key role in the differentiation of these stem cells due to its role in the development of various foetal [Arceci et al., 1993; Technau and Scholz, 2003] and adult tissues [Gillio-Meina et al., 2003] such as heart, intestinal epithelium, primitive endoderm, and gonads.

The presence of Gata-4 in conjunction with the expression of Oct-4 and Rex-1 may be related to the pluripotentiality of these cells and their capacity to differentiate to primitive endoderm and mesoderm. We analyzed the differentiation potential of osteoblastic and adipocytic phenotypes. Although induction was observed in the two differentiation pathways in most MSCs maintained in α MEM containing 10% FCS, we never observed differentiation in 100% of cells. When cells were cultured in modified MCDB201 medium, their capacities of differentiation were increased both for osteoblastogenesis and adipogenesis. We hypothesized that culture in a modified MCDB201 medium, with specific growth factors such as PDFG BB or EGF helps to maintain cells in an undifferentiated state as demonstrated by the re-expression of embryonic markers. Reyes et al. [2001] suggest that FCS reduce MAPC differentiation capacity. Similarly, in our work, we demonstrate a more efficient and complete differentiation with low FCS concentration. On the other hand, it was suggested that Oct-4 could be a co-activator of an osteoblastic gene like osteopontin (OPN) in embryonic stem cells from a potential binding site within the cis-regulatory sequences (named Palindromic Oct Regulatory Element or PORE) [Botquin et al., 1998; Pesce and Scholer, 2001]. We may hypothesize that Oct-4 is a co-activator of some genes involved in the expression of differentia-

tion programs which may increase the outcome of differentiated phenotypes.

CONCLUSION

Our data suggest that modification of culture conditions (serum concentration) as well as cell density may contribute to the emergence of stem cells with a greater potentiality. These cells are characterized by the expression of Oct-4 and Rex-1 totipotency factors and by the expression of Gata-4 organogenesis transcription factor. This may have implications for reconstructive surgery using adult stem cells.

REFERENCES

- Abdallah BM, Haack-Sorensen M, Fink T, Kassem M. 2006. Inhibition of osteoblast differentiation but not adipocyte differentiation of mesenchymal stem cells by sera obtained from aged females. *Bone* 39:181–188.
- Arceci RJ, King AA, Simon MC, Orkin SH, Wilson DB. 1993. Mouse GATA-4: A retinoic acid-inducible GATA-binding transcription factor expressed in endodermally derived tissues and heart. *Mol Cell Biol* 13:2235–2246.
- Baddoo M, Hill K, Wilkinson R, Gaupp D, Hughes C, Kopen GC, Phinney DG. 2003. Characterization of mesenchymal stem cells isolated from murine bone marrow by negative selection. *J Cell Biochem* 89:1235–1249.
- Ben-Shushan E, Thompson JR, Gudas LJ, Bergman Y. 1998. 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* 18:1866–1878.
- Bianchi G, Banfi A, Mastrogiacomo M, Notaro R, Luzzatto L, Cancedda R, Quarto R. 2003. Ex vivo enrichment of mesenchymal cell progenitors by fibroblast growth factor 2. *Exp Cell Res* 287:98–105.
- Boiani M, Eckardt S, Scholer HR, McLaughlin KJ. 2002. Oct4 distribution and level in mouse clones: Consequences for pluripotency. *Genes Dev* 16:1209–1219.
- Botquin V, Hess H, Fuhrmann G, Anastasiadis C, Gross MK, Vriend G, Scholer HR. 1998. New POU dimer configuration mediates antagonistic control of an osteopontin preimplantation enhancer by Oct-4 and Sox-2. *Genes Dev* 12:2073–2090.
- Bruder SP, Jaiswal N, Haynesworth SE. 1997. Growth kinetics, self-renewal, and the osteogenic potential of purified human mesenchymal stem cells during extensive subcultivation and following cryopreservation. *J Cell Biochem* 64:278–294.
- Forte G, Minieri M, Cossa P, Antenucci D, Sala M, Gnocchi V, Fiaccavento R, Carotenuto F, De Vito P, Baldini PM, Prat M, Di Nardo P. 2006. Hepatocyte growth factor effects on mesenchymal stem cells: Proliferation, migration and differentiation. *Stem Cells* 24:23–33.
- Gillio-Meina C, Hui YY, LaVoie HA. 2003. GATA-4 and GATA-6 transcription factors: Expression, immunohistochemical localization, and possible function in the porcine ovary. *Biol Reprod* 68:412–422.

Embryonic Gene Expression in Adult MSC

- Hakuno D, Fukuda K, Makino S, Konishi F, Tomita Y, Manabe T, Suzuki Y, Umezawa A, Ogawa S. 2002. Bone marrow-derived regenerated cardiomyocytes (CMG Cells) express functional adrenergic and muscarinic receptors. *Circulation* 105:380–386.
- Henderson JK, Draper JS, Baillie HS, Fishel S, Thomson JA, Moore H, Andrews PW. 2002. Preimplantation human embryos and embryonic stem cells show comparable expression of stage-specific embryonic antigens. *Stem Cells* 20:329–337.
- Hu Y, Liao L, Wang Q, Ma L, Ma G, Jiang X, Zhao RC. 2003. Isolation and identification of mesenchymal stem cells from human fetal pancreas. *J Lab Clin Med* 141:342–349.
- Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M, Du J, Aldrich S, Lisberg A, Low WC, Largaespada DA, Verfaillie CM. 2002a. Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* 418:41–49.
- Jiang Y, Vaessen B, Lenvik T, Blackstad M, Reyes M, Verfaillie CM. 2002b. Multipotent progenitor cells can be isolated from postnatal murine bone marrow, muscle, and brain. *Exp Hematol* 30:896–904.
- Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, Coviello GM, Wright WE, Weinrich SL, Shay JW. 1994. Specific association of human telomerase activity with immortal cells and cancer. *Science* 266:2011–2015.
- LaVoie HA. 2003. The role of GATA in mammalian reproduction. *Exp Biol Med* (Maywood) 228:1282–1290.
- Lodie TA, Blickarz CE, Devarakonda TJ, He C, Dash AB, Clarke J, Gleneck K, Shihabuddin L, Tubo R. 2002. Systematic analysis of reportedly distinct populations of multipotent bone marrow-derived stem cells reveals a lack of distinction. *Tissue Eng* 8:739–751.
- Meirelles Lda S, Nardi NB. 2003. Murine marrow-derived mesenchymal stem cell: Isolation, in vitro expansion, and characterization. *Br J Haematol* 123:702–711.
- Mihara K, Imai C, Coustan-Smith E, Dome JS, Dominici M, Vanin E, Campana D. 2003. Development and functional characterization of human bone marrow mesenchymal cells immortalized by enforced expression of telomerase. *Br J Haematol* 120:846–849.
- Miyake S, Sakurai T, Okumura K, Yagita H. 1994. Identification of collagen and laminin receptor integrins on murine T lymphocytes. *Eur J Immunol* 24:2000–2005.
- Moriscot C, de Fraipont F, Richard MJ, Marchand M, Savatier P, Bosco D, Favrot M, Benhamou PY. 2005. Human bone marrow mesenchymal stem cells can express insulin and key transcription factors of the endocrine pancreas developmental pathway upon genetic and/or microenvironmental manipulation in vitro. *Stem Cells* 23:594–603.
- Niwa H. 2001. Molecular mechanism to maintain stem cell renewal of ES cells. *Cell Struct Funct* 26:137–148.
- Niwa H, Miyazaki J, Smith AG. 2000. Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. *Nat Genet* 24:372–376.
- Okamoto T, Aoyama T, Nakayama T, Nakamata T, Hosaka T, Nishijo K, Nakamura T, Kiyono T, Toguchida J. 2002. Clonal heterogeneity in differentiation potential of immortalized human mesenchymal stem cells. *Biochem Biophys Res Commun* 295:354–361.
- Oswald J, Boxberger S, Jorgensen B, Feldmann S, Ehninger G, Bornhauser M, Werner C. 2004. Mesenchymal stem cells can be differentiated into endothelial cells in vitro. *Stem Cells* 22:377–384.
- Palmieri SL, Peter W, Hess H, Scholer HR. 1994. Oct-4 transcription factor is differentially expressed in the mouse embryo during establishment of the first two extraembryonic cell lineages involved in implantation. *Dev Biol* 166:259–267.
- Pesce M, Scholer HR. 2001. Oct-4: Gatekeeper in the beginnings of mammalian development. *Stem Cells* 19:271–278.
- Pochampally RR, Smith JR, Ylostalo J, Prockop DJ. 2004. Serum deprivation of human marrow stromal cells (hMSCs) selects for a sub-population of early progenitor cells with enhanced expression of Oct-4 and other embryonic genes. *Blood* 103:1647–1652.
- Quirici N, Soligo D, Caneva L, Servida F, Bossolasco P, Deliliers GL. 2001. Differentiation and expansion of endothelial cells from human bone marrow CD133(+) cells. *Br J Haematol* 115:186–194.
- Ramirez-Zacarias JL, Castro-Munozledo F, Kuri-Harcuch W. 1992. Quantitation of adipose conversion and triglycerides by staining intracytoplasmic lipids with Oil red O. *Histochemistry* 97:493–497.
- Reyes M, Lund T, Lenvik T, Aguiar D, Koodie L, Verfaillie CM. 2001. Purification and ex vivo expansion of postnatal human marrow mesodermal progenitor cells. *Blood* 98:2615–2625.
- Sakaguchi Y, Sekiya I, Yagishita K, Ichinose S, Shinomiya K, Muneta T. 2004. Suspended cells from trabecular bone by collagenase digestion become virtually identical to mesenchymal stem cells obtained from marrow aspirates. *Blood* 104:2728–2735.
- Salven P, Mustjoki S, Alitalo R, Alitalo K, Rafi S. 2003. VEGFR-3 and CD133 identify a population of CD34+ lymphatic/vascular endothelial precursor cells. *Blood* 101:168–172.
- Scholer HR, Dressler GR, Balling R, Rohdewohld H, Gruss P. 1990. Oct-4: A germline-specific transcription factor mapping to the mouse t-complex. *EMBO J* 9:2185–2195.
- Schwartz RE, Reyes M, Koodie L, Jiang Y, Blackstad M, Lund T, Lenvik T, Johnson S, Hu WS, Verfaillie CM. 2002. Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells. *J Clin Invest* 109:1291–1302.
- Sekiya I, Larson BL, Smith JR, Pochampally R, Cui JG, Prockop DJ. 2002. Expansion of human adult stem cells from bone marrow stroma: Conditions that maximize the yields of early progenitors and evaluate their quality. *Stem Cells* 20:530–541.
- Sekiya I, Larson BL, Vuoristo JT, Cui JG, Prockop DJ. 2004. Adipogenic differentiation of human adult stem cells from bone marrow stroma (MSCs). *J Bone Miner Res* 19:256–264.
- Shoichet SA, Malik TH, Rothman JH, Shivdasani RA. 2000. Action of the *Caenorhabditis elegans* GATA factor END-1 in *Xenopus* suggests that similar mechanisms initiate endoderm development in ecdysozoa and vertebrates. *Proc Natl Acad Sci USA* 97:4076–4081.
- Stanford CM, Jacobson PA, Eanes ED, Lembke LA, Midura RJ. 1995. Rapidly forming apatitic mineral in an

Roche et al.

- osteoblastic cell line (UMR 106-01 BSP). *J Biol Chem* 270:9420–9428.
- Technau U, Scholz CB. 2003. Origin and evolution of endoderm and mesoderm. *Int J Dev Biol* 47:531–539.
- Vogel W, Grunebach F, Messam CA, Kanz L, Brugger W, Buhring HJ. 2003. Heterogeneity among human bone marrow-derived mesenchymal stem cells and neural progenitor cells. *Haematologica* 88:126–133.
- Xu W, Zhang X, Qian H, Zhu W, Sun X, Hu J, Zhou H, Chen Y. 2004. Mesenchymal stem cells from adult human bone marrow differentiate into a cardiomyocyte phenotype in vitro. *Exp Biol Med (Maywood)* 229:623–631.
- Yoshida-Koide U, Matsuda T, Saikawa K, Nakanuma Y, Yokota T, Asashima M, Koide H. 2004. Involvement of Ras in extraembryonic endoderm differentiation of embryonic stem cells. *Biochem Biophys Res Commun* 313:475–481.
- Zimmermann S, Voss M, Kaiser S, Kapp U, Waller CF, Martens UM. 2003. Lack of telomerase activity in human mesenchymal stem cells. *Leukemia* 17:1146–1149.