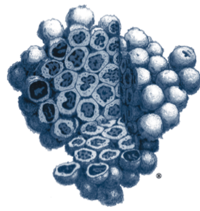
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Stefano Zangrossi, Mirko Marabese, Massimo Broggini, Rosaria Giordano, Marco D'Erasmo, Elisa Montelatici, Daniela Intini, Antonino Neri, Maurizio Pesce, Paolo Rebulla and Lorenza Lazzari

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STEM CELL GENETICS AND GENOMICS

Oct-4 Expression in Adult Human Differentiated Cells Challenges Its Role as a Pure Stem Cell Marker

STEFANO ZANGROSSI,[a] MIRKO MARABESE,[b] MASSIMO BROGGINI,[b] ROSARIA GIORDANO,[a] MARCO D'ERASMO,[a] ELISA MONTELATICI,[a] DANIELA INTINI,[c] ANTONINO NERI,[c] MAURIZIO PESCE,[d] PAOLO REBULLA,[a] LORENZA LAZZARI[a]

[a]Cell Factory, Center for Transfusion Medicine, Cellular Therapy and Cryobiology, Fondazione Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, Milan, Italy; [b]Laboratory of Molecular Pharmacology, Istituto di Ricerche Farmacologiche "Mario Negri," Milan, Italy; [c]Laboratory of Molecular Genetics and Gene Expression, Fondazione Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, Milan, Italy; [d]Laboratory of Vascular Biology and Gene Therapy, Centro Cardiologico Monzino, Istituto di Ricovero e Cura a Carattere Scientifico, Milan, Italy

Key Words. Oct-4 • Human peripheral cells • Stem cell marker

ABSTRACT

The Oct-4 transcription factor, a member of the POU family that is also known as Oct-3 and Oct3/4, is expressed in totipotent embryonic stem cells (ES) and germ cells, and it has a unique role in development and in the determination of pluripotency. ES may have their postnatal counterpart in the adult stem cells, recently described in various mammalian tissues, and Oct-4 expression in putative stem cells purified from adult tissues has been considered a real marker of stemness. In this context, normal mature adult cells would not be expected to show Oct-4 expression. On the contrary, we demonstrated, using reverse transcription-polymerase chain reaction (PCR) (total RNA, Poly A+), real-time PCR, immunoprecipitation, Western blotting, band shift, and immunofluorescence, that human peripheral blood mononuclear cells, genetically stable and mainly terminally differentiated cells with well defined functions and a limited lifespan, express Oct-4. These observations raise the question as to whether the role of Oct-4 as a marker of pluripotency should be challenged. Our findings suggest that the presence of Oct-4 is not sufficient to define a cell as pluripotent, and that additional measures should be used to avoid misleading results in the case of an embryonic-specific gene with a large number of pseudogenes that may contribute to false identification of Oct-4 in adult stem cells. These unexpected findings may provide new insights into the role of Oct-4 in fully differentiated cells. STEM CELLS *2007;25:1675–1680*

Disclosure of potential conflicts of interest is found at the end of this article.

INTRODUCTION

Since its discovery in the early nineties, it has been suggested that Oct-4 transcription factor expression is a marker of pluripotency and a master gene in the regulation of embryonic differentiation. Oct-4 (also known as Oct-3 and Oct3/4) is a member of the POU family and is expressed in the germ line and pregastrulation embryo [1, 2]. In particular, a critical level of Oct-4 expression is required for the self-renewal and totipotency maintenance of embryonic stem cells (ES) [3–5], and it has also been shown that different levels of Oct-4 expression induce divergent developmental programs, with a transient increase inducing commitment to primitive endoderm and mesoderm lineages, and repression leading to trophectoderm differentiation [6]. As gastrulation ends, Oct-4 is progressively downregulated and, 8.5 days post coitum in mouse, is only expressed in primordial germ cells. These data support a specific role of Oct-4 as a master regulator of pluripotency.

As a member of the POU transcription factor family, Oct-4 contains a bipartite DNA binding domain that consists of two subdomains connected by a flexible linker of varying length. POU transcription factors can activate the transcription of genes containing an octameric sequence (the octamer motif) within their promoter or enhancer regions. The consensus-binding motif recognized by POU factors is the sequence ATGCAAAT [3].

Although the identification of specific target genes has been difficult, it has been found that Oct-4 activates fibroblast growth factor-4 (FGF-4), osteopontin adhesion molecule, transcriptional coactivator Utf1, and transcription factor Rex-1. An enhancer element has been found in the 3′-UTR of the FGF-4 gene that is activated by Oct-4 in cooperation with a high mobility group box transcription factor Sox-2 [7–11]. The mechanisms involved in the Oct-4 regulation of ES have been widely investigated, but the postnatal role of Oct-4 is still not clear, although it has recently been associated with the undifferentiated pluripotent state of stem cell populations derived from various adult human tissues [12–16]. In order to clarify the role of Oct-4 in adult cells, we evaluated its expression and that of other embryonic genes in fully differentiated cells, and found that it is also expressed at the gene and protein levels in peripheral blood mononuclear cells from healthy adult donors.

Correspondence: Lorenza Lazzari, D.Sc., Cell Factory, Center for Transfusion Medicine, Cellular Therapy and Cryobiology, Fondazione Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, Via F. Sforza 35, 20122 Milano, Italy. Telephone: +39 02 5503 4053; Fax: +39 02 5503 2796; e-mail: cbbank@policlinico.mi.it Received September 28, 2006; accepted for publication March 14, 2007; first published online in STEM CELLS *EXPRESS* March 22, 2007. ©AlphaMed Press 1066-5099/2007/$30.00/0 doi: 10.1634/stemcells.2006-0611

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Materials and Methods

Cell Sources

Peripheral blood (PB) was collected from healthy donors ($n = 30$) after informed consent. Mononuclear cells (MNCs) were isolated by density gradient centrifugation (Lympholyte-H, 1.077 g/ml; Cedar Lane, Hornby, ON, Canada, http://www.cedarlanelabs.com). CD34+ ($n = 3$), CD3+, and CD14+ ($n = 3$) cells were isolated from PB MNCs using a positive selection system (Miltenyi Biotec, Bergisch Gladbach, Germany, http://www.miltenyibiotec.com), and their purity was analyzed by flow cytometry (FC500; Beckman Coulter, Fullerton, CA, http://www.beckmancoulter.com). Anti-human CD34 phycoerythrin (PE), anti-human CD3 fluorescein isothiocyanate (FITC), and anti-human CD14 PE (Becton, Dickinson and Company, Franklin Lakes, NJ, http://www.bd.com) were used. The MCF-7 human breast cancer cell line, F9 murine embryocarcinoma cell line, and human embryonic stem cell line RNA (hES-5 p33; hES-1 p33) were used as positive controls.

Reverse-Transcription Polymerase Chain Reaction

Total RNA was extracted from 1×10^8 PB MNCs and 3×10^6 PB CD3+ and PB CD14+ cells using the RNeasy Mini Kit (Qiagen, Hilden, Germany, http://www1.qiagen.com). The contaminating genomic DNA was further digested by DNase (Qiagen) according to the manufacturer's instructions. The quality, integrity, and size distribution of total RNA were evaluated by optic density (a 260/280 ratio of >1.9), denaturing agarose gel electrophoresis, and ethidium bromide staining. Poly A+ was purified from PB MNC and human ES (hES) total RNA using Dynabeads Oligo(dT)$_{25}$ (Dynal Biotech ASA, Oslo, Norway, http://www.invitrogen.com/dynal). Each reverse transcription was carried out using a High Capacity cDNA Archive kit (Applied BioSystems, Foster City, CA, http://www.appliedbiosystems.com). Reverse-transcription polymerase chain reaction (RT-PCR) was performed using GoTaq DNA Polymerase (Promega, Madison, WI, http://www.promega.com); the primers listed in Table 1 were designed to span exon junctions in order to amplify only spliced RNA. The same starting concentrations of cDNA template were used in all cases. The PCR products were separated on 1.5% agarose gel by electrophoresis. *GAPD* was used as an internal control.

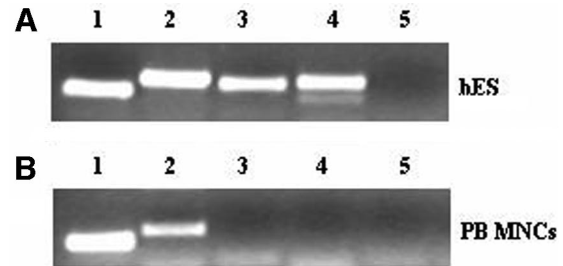

Figure 1. Qualitative reverse transcription-polymerase chain reaction for Oct-4, and its downstream genes Sox-2 and FGF-4, was performed on total RNA extracted from PB MNCs. **(A):** hES. **(B):** PB MNCs. Lane 1: GAPD. Lane 2: Oct-4. Lane 3: Sox-2. Lane 4: FGF-4. Lane 5: no template control. Abbreviations: hES, human embryonic stem cell; PB MNCs, peripheral blood mononuclear cells.

Real-Time RT-PCR

Real-time RT-PCR on the cDNA specimens and standards was performed (in triplicate) in a 25-μl reaction mixture containing 40 ng of cDNA template, 12.5 μl of TaqMan Universal PCR Master Mix (Applied BioSystems), and the gene expression assays specified in Table 1. RNA levels were measured using an ABI PRISM 7700. The efficiency and reproducibility of the PCR were assessed using standard curves in order to measure the slope and correlation coefficient (r^2), both of which were very close to the theoretical values. The relative quantification of marker gene RNA expression was calculated using the comparative threshold cycle (Ct) method: the value of our target, normalized to an endogenous control (GAPD) and relative to a calibrator (hES), was expressed as $2^{-\Delta\Delta Ct}$ (fold difference), where ΔCt = the Ct of the target gene − the Ct of the endogenous control gene, and $\Delta\Delta Ct$ = the ΔCt of the target gene samples − the ΔCt of the target gene calibrator.

Immunoprecipitation and Western Blotting

Cell extracts obtained from the PB samples and F9 cells were prepared by lysing the cells in RIPA buffer for 30 minutes on ice in the presence of protease inhibitors. The insoluble material was pelleted at 13,000*g* for 10 minutes at 4°C, and protein concentration was determined using a Bio-Rad Assay Kit (Bio-Rad, Hercules, CA, http://www.bio-rad.com). One mg of total cell proteins were incu-

Table 1. Primer sequences used for the RT-PCR, real-time RT-PCR, and band shift assays

Gene	Primers for qualitative RT-PCR			
	Size (bp)	Primers	Primers	References
Oct-4	173	Oct-4 F Oct-4 R	cttgctgcagaagtgggtggaggaa ctgcagtgtgggtttcgggca	[21]
Oct-4	246	Oct-4 F Oct-4 R	cgtgaagctggagaaggagaagctg caagggccgcagcttacacatgttc	[22]
Oct-4	293	Oct-4 F Oct-4 R	acatgtgtaagctgcggcc gttgtgcatagtcgctgcttg	Homemade
FGF-4	275	FGF-4 F FGF-4 R	ctactgcaacgtgggcatc acatgccggggtacttgtag	Homemade
Sox-2	264	Sox-2 F Sox-2 R	accagaaaaacagcccgga tcatgagcgtcttggttttcc	Homemade
GAPD	210	GAPD F GAPD R	gcttgtcatcaatggaaatccc ttcacacccatgacgaacatg	Homemade
Assays for quantitative real-time RT-PCR TaqMan chemistry				
GAPD			Hs99999905_m1 (Applied Biosystems)	Gene Expression Assay-on-Demand
Oct-4			Hs00742896_s1 (Applied Biosystems)	Gene Expression Assay-on-Demand
Oligonucleotides used for band shift				
O-wt			cggtaatttgcatttctaa	[23]
p53			ggacatgcccgggcatgtcc	[24]

Abbreviations: bp, base pairs; F, forward; FGF, fibroblast growth factor; R, reverse; RT-PCR, reverse transcription-polymerase chain reaction.

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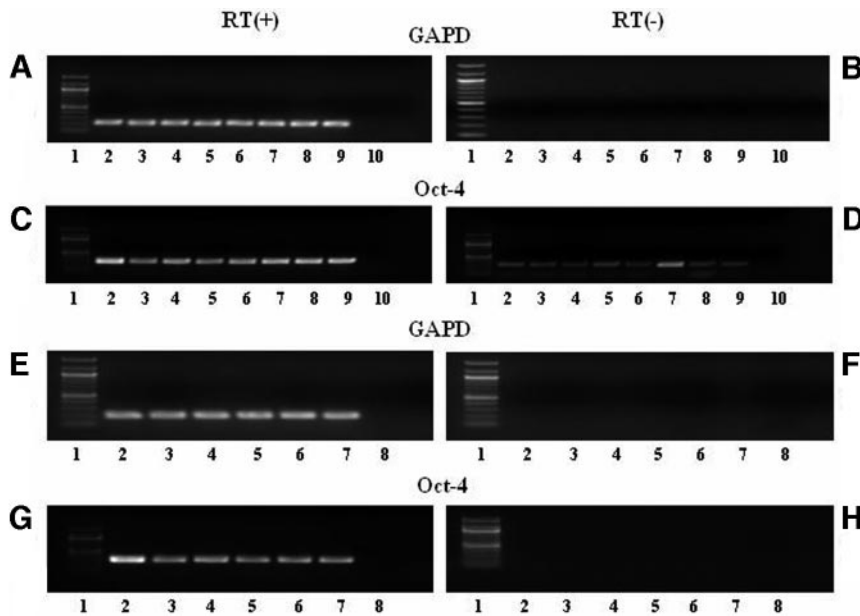

Figure 2. **(A–H):** Qualitative reverse transcription-polymerase chain reaction (PCR) for Oct-4 was performed on total RNA extracted from peripheral blood mononuclear cells (PB MNCs) and reverse transcribed by standard methods with or without addition of reverse transcriptase **(C, D)**. PCR amplification of total RNA yielded products contributed by both genomic DNA contamination and mRNA transcripts encoding Oct-4 **(D)**. **(A):** GAPD RT(+). **(B):** GAPD RT(−). **(C):** Oct-4 RT(+). **(D):** Oct-4 RT(−). Lane 1: marker. Lane 2: human embryonic stem cell (hES). Lanes 3–9: PB MNCs. Lane 10: no template control (NT). The poly A+ fraction of RNA was enriched from PB MNCs and reverse transcribed by standard methods with or without addition of reverse transcriptase **(G, H)**. PCR amplification of the poly A+ mRNA fraction confirmed that human embryonic stem cells and PB MNCs express Oct-4 mRNA **(G)**. **(E):** GAPD RT(+). **(F):** GAPD RT(−). **(G):** Oct-4 RT(+). **(H):** Oct-4 RT(−). Lane 1: marker. Lane 2: hES. Lanes 3–7: PB MNCs. Lane 8: NT. Abbreviation: RT, reverse transcriptase.

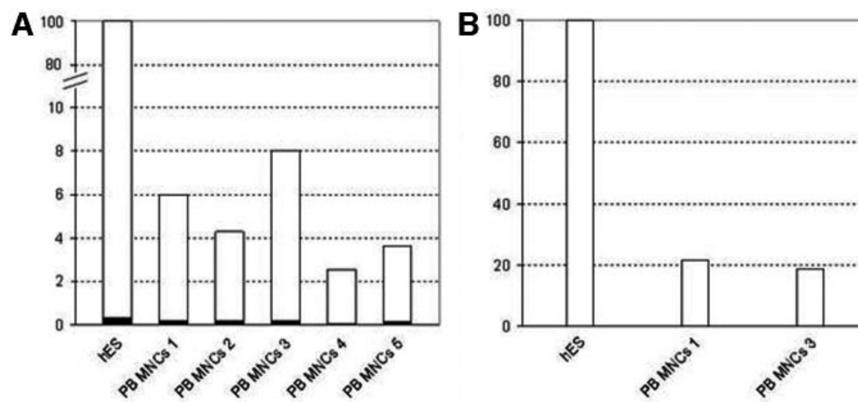

Figure 3. **(A, B):** Quantitative real-time polymerase chain reaction for Oct-4 was performed with the TaqMan assay on reverse transcriptase (RT)(+) and RT(−) reverse transcribed products from both the total **(A)** and poly A+ **(B)** RNA templates. The $2^{-\Delta\Delta Ct}$ (fold difference) was calculated using the equation indicated in Materials and Methods. **(A):** Fold difference in percentage Oct-4 expression in RT(+) (white bars) and RT(−) (black bars) total RNA samples. **(B):** Fold difference in percentage Oct-4 expression in poly A+ RNA samples. Abbreviations: hES, human embryonic stem cell; PB MNCs, peripheral blood mononuclear cells.

bated overnight with 2 μg of goat anti-Oct-4 antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA, http://www.scbt.com) and for 2 hours with A/G agarose beads (Santa Cruz Biotechnology). The antibody neutralization was performed trough specific blocking peptide (Santa Cruz Biotechnology) following the manufacturer's instructions. For blocking, the antibody was combined 1:5 with the blocking peptide before the immunoprecipitation. The lysates with bound proteins were washed five times with RIPA buffer. The bound complexes were released by heating at 95°C for 5 minutes, resolved on 12% sodium dodecyl sulfate-polyacrylamide gels, and analyzed by Western blotting using rabbit polyclonal anti-Oct-4 antibody (Chemicon, Temecula, CA, http://www.chemicon.com).

Immunofluorescence

MCF-7 cell cultures and cytospin preparations of PB MNCs, PB CD3+ and PB CD14+ cells, and PB CD34+ cells and their negative fraction were fixed with methanol at −20°C for 5 minutes. After washing with phosphate buffered-saline (PBS; Gibco, Grand Island, NY, http://www.invitrogen.com), the cells were permeabilized with 0.1% Triton X-100, blocked for 30 minutes with PBS containing 2% bovine serum albumin (Sigma-Aldrich, St. Louis, http://www.sigmaaldrich.com), and then incubated with primary mouse monoclonal anti-human Oct-4 antibody (1:500; Chemicon) for 1 hour at room temperature. Negative control was performed with FITC-conjugated goat anti-mouse IgG secondary antibodies (1:1,000 dilution; Chemicon) and PE-conjugated goat anti-rabbit IgG secondary antibodies (1:50 dilution; Vector Laboratories, Burlingame, CA, http://www.vectorlabs.com). In order to validate our results, the same experiment was performed using a primary mouse monoclonal anti-human Oct-4 antibody (1:100) kindly provided by Hans Scholer. The primary antibody was removed, and the slides were washed with PBS and incubated for 1 hour at room temperature with FITC-conjugated goat anti-mouse IgG secondary antibody (1:1,000 dilution; Chemicon), after which the cells were counterstained with 4′,6′-diamidino-2-phenylindole dihydrochloride (Roche Diagnostics, Basel, Switzerland, http://www.roche-applied-science.com) for 15 minutes at room temperature. A polyclonal rabbit anti-human CD3 antibody (1:70; DakoCytomation, Glostrup, Denmark, http://www.dakocytomation.com), PE-conjugated goat anti-rabbit IgG secondary antibody (1:50), and a monoclonal anti-human CD14-PE antibody (1:10; Miltenyi Biotec) were used after Oct-4 staining on cytospin preparations of CD3+ and CD14+ cells. A monoclonal anti-human CD34-PE antibody (1:50; Becton, Dickinson and Company) was used after Oct-4 staining on cytospin preparations of CD34+ cells and their negative fraction. After incubation, the coverslipped slides were examined using a fluorescence microscope (Eclipse 80I; Nikon, Tokyo, http://www.nikon-

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instruments.jp/eng) equipped with a Plan Fluor (Nikon) 100×/1.30 oil numeric aperture objective, and photographs were collected with a Digital Sight camera control (DS-5M; Nikon). The images were acquired using a Nikon Digital Slide DS-L1 and merged and analyzed using Adobe Photoshop 5.5 software (Adobe, San Jose, http://www.adobe.com).

Band Shift Assays

Nuclear Extracts. Ten million PB MNCs or F9 mouse cells were collected, washed in ice-cold PBS, and resuspended in 400 μl of 10 mM HEPES pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, and 1 mM dithiothreitol (DTT) ice-cold buffer containing a protease inhibitor cocktail (Sigma-Aldrich). After 15 minutes on ice, 25 μl of a 10% solution of Nonidet NP40 were added, and the tube was vigorously vortexed for 10 seconds. The homogenate was centrifuged for 30 seconds at 13,000 rpm. The nuclear pellet was resuspended in 50 ml of 20 mM HEPES, pH 7.9, 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, and 1 mM DTT ice-cold buffer containing a protease inhibitor cocktail and vigorously rocked at 4°C for 15 minutes on a shaking platform. The nuclear homogenate was centrifuged for 5 minutes at 13,000 rpm at 4°C, and the supernatant containing the nuclear proteins was frozen at −80°C.

Electrophoretic Mobility Shift Assay. The binding conditions for the electrophoretic mobility shift assay were 10 mM Tris-HCl, pH 7.4, 50 mM NaCl, 1 mM EDTA, 5% glycerol, 1 mM DTT, 3 μg poly d(I-C), and 10,000 cpm of the O-wt labeled oligonucleotide. A p53 oligonucleotide was used as a nonspecific competitor (Table 1). The final reaction volume was 15 μl. After incubation for 30 minutes at room temperature, the binding reactions were loaded for gel electrophoresis. For the supershift, 1 μg of mouse monoclonal antibody Oct-4 (Santa Cruz Biotechnology) was used.

Results

Qualitative RT-PCR for Oct-4, and its downstream genes Sox-2 and FGF-4, was performed on total RNA extracted from PB MNCs ($n = 15$), and hES lines were used as positive controls. Surprisingly, all of the PB MNC samples were Oct-4 positive using homemade and previously published primers (Table 1), whereas no Sox-2 or FGF-4 expression was found (Fig. 1). In order to confirm this unexpected Oct-4 positivity, we evaluated mRNA and protein expression by qualitative and quantitative RT-PCR, immunoprecipitation/Western blot analysis, and immunocytochemistry of PB MNCs ($n = 5$). In the first RT-PCR assays, several steps were employed to eliminate genomic DNA contamination, including designing the primers to span exon junctions, treating the samples with DNase, and assigning a minimum ratio of 1.9 to assess RNA quality. Alternatively, the poly A+ fraction of RNA was enriched from cells and reverse transcribed by standard methods with or without addition of reverse transcriptase. This strategy was used to unequivocally determine if Oct-4 mRNA or contaminating Oct-4 genomic DNA-encoding sequences were amplified by PCR. Our results demonstrate that PCR amplification of total RNA yielded products contributed by both genomic DNA contamination and mRNA transcripts encoding Oct-4 (Fig. 2D). PCR amplification of the poly A+ mRNA fraction confirmed that human ES and PB MNCs express Oct-4 mRNA (Fig. 2G). This approach provided a more reliable semiquantitative evaluation of Oct-4 due to the lack of products generated by contaminating genomic DNA sequences (Fig. 2H). These data were confirmed by sequencing the poly A+ PCR products. The sequencing results gave a 100% match with the Oct-4 gene. When blasted against the different pseudogenes reported to be potentially transcribed, we found that amplification products contained several mismatches, indicating that, in these cells, the PCR products correspond to Oct-4, and that we did not pick up transcribed pseudogenes.

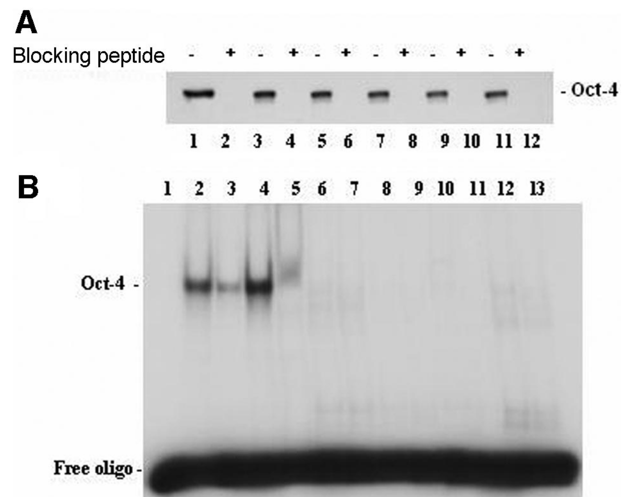

Figure 4. (A, B): Immunoprecipitation/Western blot and band shift assays. **(A):** Lanes 1–2: F9 murine embryocarcinoma cell line (used as a positive control). Lanes 3–12: peripheral blood mononuclear cells (PB MNCs). Specificity of antibodies was provided by using specific blocking peptide for each sample (lanes 2, 4, 6, 8, 10, and 12). **(B):** A gel shift assay was carried out by matching the highly specific oligo(O-wt) with nuclear extracts from F9 cells and PB MNCs. The radiolabeled oligonucleotide O-wt was incubated in the absence (lane 1) or presence of F9 nuclear extract (ne) (lane 2), ne and O-wt cold competitor oligo (lane 3), ne and p53 cold competitor oligo (lane 4), and ne and Oct-4 antibody (lane 5). PB MNC nuclear extracts from four different donors were incubated with O-wt radiolabeled oligo in the absence (lanes 6, 8, 10, 12) or presence of Oct-4 antibody (lanes 7, 9, 11, 13). For each lane, 3 μg of extract were used.

In order to quantify the genomic contribution of Oct-4 expression in adult differentiated cells, we measured the level of Oct-4 by quantitative real-time PCR with the TaqMan assay on reverse transcriptase (RT)(+) and RT(−) reverse transcribed products from both the total and poly A+ RNA templates. The total RNA results showed that the amount of Oct-4 in the PB MNCs was 2%–8% of that observed in the hES (Fig. 3A, white bars), with the genomic component quantitatively negligible (Fig. 3A, black bars). When poly A+ RNA was used to quantify Oct-4 expression, the PB MNCs contained approximately 20% of that present in hES (Fig. 3B).

Oct-4 protein expression in PB MNCs was evaluated by immunoprecipitation/Western blot analysis and immunocytochemistry on cytospin preparations. As shown in Figures 4A and 6A, all of the samples were positive. In order to verify whether Oct-4 could bind its consensus sequence, we carried out a gel shift assay by matching the highly specific oligo(O-wt) with nuclear extracts from F9 cells and PB MNCs ($n = 4$). A clear, Oct-4-specific retarded band was obtained when F9 cell nuclear extracts were used, whereas no specific retarded band was detected in the nuclear extracts of PB MNCs isolated from different donors (Fig. 4B).

In order to clarify whether Oct-4 could be mainly attributed to a specific cell lineage, immunocytochemistry and real-time PCR for Oct-4 were performed on highly purified CD3+ and CD14+ cells. The percentages of CD3+ and CD14+ cells after selection were, respectively, 97.6% ± 0.4% and 95.3% ± 3.4% (Fig. 5A). Real-time PCR showed that Oct-4 expression was higher in the CD3+ than in the CD14+ cells (Fig. 5B), and the immunocytochemistry experiments also showed that Oct-4 was mainly expressed in CD3+ cells (Fig. 6B). Moreover, the immunocytochemistry showed the Oct-4 expression on both the

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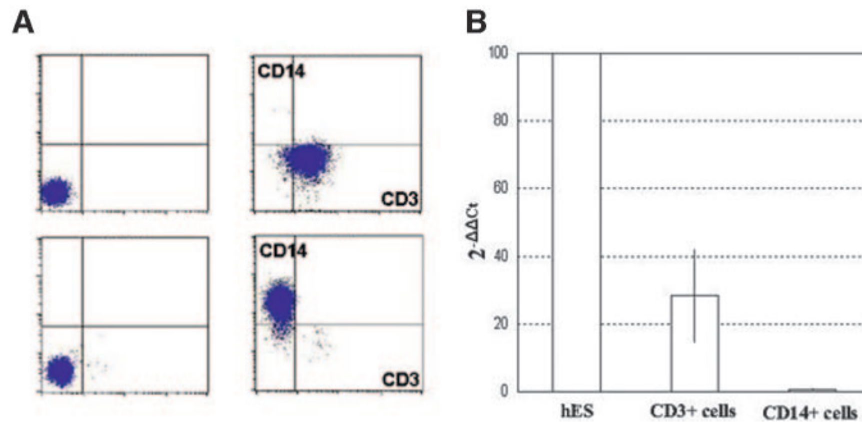

Figure 5. **(A, B):** Fluorescence-activated cell sorter (FACS) analysis and real-time reverse transcription-polymerase chain reaction (PCR) on human peripheral blood (PB) CD3+ and PB CD14+ cells. A representative FACS analysis of negative controls (**[A]**, up and low left), of isolated CD3+ cells costained with CD14 (**[A]**, up right), and of isolated CD14+ cells costained with CD3 (**[A]**, low right). The percentages of CD3+ and CD14+ cells after selection were, respectively, 97.6% ± 0.4% and 95.3% ± 3.4%. Quantitative real-time PCR for Oct-4 was performed with the TaqMan assay on human PB CD3+ and PB CD14+ cells. The $2^{-\Delta\Delta Ct}$ (fold difference) was calculated using the equation indicated in Materials and Methods **(B)**. Abbreviation: hES, human embryonic stem cell.

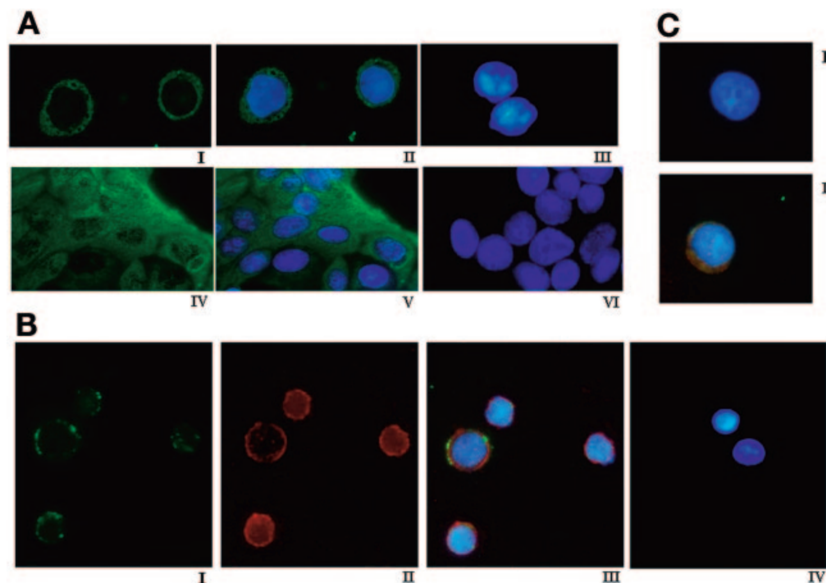

Figure 6. **(A–C):** Immunofluorescence. **(A):** Oct-4 protein expression in adult human peripheral blood (PB) mononuclear cells was observed in the cytoplasm (I and II) by fluorescence microscopy using immunocytochemistry as described in Materials and Methods. MCF-7 breast carcinoma cell line was used as a positive control (IV and V). Negative control was performed with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG secondary antibodies and phycoerythrin (PE)-conjugated goat anti-rabbit IgG secondary antibodies (III and VI). The nuclei were counterstained with 4′,6′-diamidino-2-phenylindole dihydrochloride (DAPI) (blue) (magnification: ×100). **(B):** Coexpression of Oct-4 and CD3 in adult human PB CD3+ purified cells as shown by fluorescence microscopy (I: Oct-4 FITC; II: CD3 PE; III: merge; IV: negative control). The nuclei were counterstained with DAPI (blue) (magnification: ×100). **(C):** Coexpression of Oct-4 and CD34 in adult human PB CD34+ purified cells as shown by fluorescence microscopy (I: negative control; II: merge, Oct-4 FITC, and CD34 PE). The nuclei were counterstained with DAPI (blue) (magnification: ×100).

CD34+ cell preparations (Fig. 6C) and their negative fraction (data not shown).

Discussion

Oct-4 expression, together with its cDNA sequence and gene organization, was originally detected in adult tissues by Takeda et al. using RT-PCR in 1992 [17] and, more recently, its expression has been considered a marker of pluripotency in various adult tissues. In this regard, Oct-4 expression has been found in stem cells from different adult human tissues [18], in bone marrow (BM)-derived multipotent adult progenitor cells [12], in a subpopulation of serum-deprived BM-derived mesenchymal stem cells [13], and in human marrow-isolated adult multilineage inducible cells [15], thus supporting their in vitro and in vivo multilineage differentiation potential. In addition, CD133+ pluripotent progenitors isolated from cord blood (CB) have also been found to be Oct-4 positive, with a significant reduction in expression following endothelial cell differentiation in vitro [14]. Even more recently, CB-derived embryonic-like stem cells, a supposedly rare multipotent cell population still present after embryonic and fetal development, have been found to be positive for primitive human embryonic stem cell markers, including Oct-4 [16].

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Furthermore, like other embryonic genes, Oct-4 is expressed in a number of tumor tissues and cell lines, a finding that has been correlated with the reactivation of embryonic molecular pathways in carcinogenesis and the definition of cancer as a stem cell disease [18]. In this context, normal mature adult cells would not be expected to show Oct-4 expression.

Our observations raise the question as to whether the role of Oct-4 as a marker of pluripotency should be challenged. The consideration that PB MNCs include genetically stable and mainly terminally differentiated cells with well defined functions and a limited lifespan led us to question the real meaning of Oct-4, at least in this cell type. Our finding that Oct-4 in PB MNCs does not bind to the O-wt sequence in the nucleus and that FGF-4 and Sox-2 are not expressed might support the hypothesis that Oct-4 function is different in embryonic and adult cells. Alternatively, the lack of Oct-4 nuclear binding capacity might be due to post-transcriptional mechanisms such as the activation of specific phosphorylation pathways [19].

The characteristics and even the existence of pluripotent stem cells in adult differentiated tissues are still a matter of debate, and our findings suggest that the presence of Oct-4 is not sufficient to define a pluripotent cell, and that additional measures should be used to avoid misleading results in the case of a gene with a large number of pseudogenes. In this regard, it is well known that pluripotent cell-specific genes, such as Nanog, Stella, GDF3, and Oct-4 itself, show the presence of retropseudogenes, which could explain the high probability of genomic contamination in gene expression assays. Their presence has been correlated with the high transcription levels of these genes, which lead to frequent retrotransposition events in pluripotent and germ-line cells. In particular, six pseudogenes for Oct-4 have already been identified in different chromosomal locations and, although their role is still unknown, it has been hypothesized that they may be involved in regulating cell type-specific expression, probably by epigenetic mechanisms [20]. The high degree of homology between the coding sequence of Oct-4 and its pseudogenes should be taken into account, especially when a nonquantitative assay is used and low gene expression is detected. In this regard, the reports demonstrating the expression of Oct-4 in novel putative adult stem cells [12–15, 18] should be confirmed and validated by quantitative assay avoiding the contribution of genomic DNA by at least using poly A+ transcripts and sequencing the PCR products to be sure not to pick up transcribed pseudogenes. In conclusion, thirteen years after the first report by Takeda et al. [17], our results challenge the value of Oct-4 as a pluripotent cell marker gene in adult cells and open up new perspectives concerning its function and regulation under normal and pathological conditions.

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Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

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Stefano Zangrossi, Mirko Marabese, Massimo Broggini, Rosaria Giordano, Marco D'Erasmo, Elisa Montelatici, Daniela Intini, Antonino Neri, Maurizio Pesce, Paolo Rebulla and Lorenza Lazzari

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