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Hematopoietic progenitors express embryonic stem cell and germ layer genes

Expression par des progéniteurs hématopoïétiques de gènes de cellules souches embryonnaires et de gènes de différenciation des feuillets embryonnaires

Bernard Pessac [a,*], Mehdi Ait Bara [b], David Ford [c,d], Gopi K. Patibandla [c], David Trisler [c,d]

[a] *CNRS UMR 8118, université Paris Descartes, 45, rue des Saints-Pères, 75270 Paris cedex 06, France*
[b] *Centre de recherche des Cordeliers, UMRS 872, 15, rue de l'École de médecine, 75006 Paris, France*
[c] *Department of Neurology, University of Maryland School of Medicine, Baltimore, MD 21201, USA*
[d] *Multiple Sclerosis Center of Excellence, East VAMHCS, Baltimore, MD 21201, USA*

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ABSTRACT

Cell therapy for tissue regeneration requires cells with high self-renewal potential and with the capacity to differentiate into multiple differentiated cell lineages, like embryonic stem cells (ESCs) and adult somatic cells induced to pluripotency (iPSCs) by genetic manipulation. Here we report that normal adult mammalian bone marrow contains cells, with the cell surface antigen CD34, that naturally express genes characteristic of ESCs and required to generate iPSCs. In addition, these CD34+ cells spontaneously express, without genetic manipulation, genes characteristic of the three embryonic germ layers: ectoderm, mesoderm and endoderm. In addition to the neural lineage genes we previously reported in these CD34+ cells, we found that they express genes of the mesodermal cardiac muscle lineage and of the endodermal pancreatic lineage as well as intestinal lineage genes. Thus, these normal cells in the adult spontaneously exhibit characteristics of embryonic-like stem cells.
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Mots clés :
Cellule souche CD34+
Cellule pluripotente
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Cellules pancréatiques endodermiques

RÉSUMÉ

La régénération tissulaire par thérapie cellulaire nécessite des cellules avec un potentiel élevé d'auto-renouvellement et une capacité de différenciation en de multiples lignages de cellules différenciées, comparable à celle des cellules souches embryonnaires (CSE) et des cellules somatiques induites à la pluripotence (CSIP) par manipulation génétique. Nous rapportons ici que la moelle osseuse de mammifères adultes contient des cellules, avec l'antigène de surface CD34, qui expriment naturellement, sans induction, des gènes caractéristiques des CSE et nécessaires à la génération des CSIP. De plus, ces cellules CD34+ expriment spontanément, sans manipulation génétique, des gènes caratéristiques des trois couches embryonnaires : ectoderme, mésoderme et endoderme. En plus des gènes du lignage neural dont nous avons précédemment rapporté l'expression, nous avons observé que ces cellules CD34+ expriment des gènes du lignage mésodermique caractéristiques du myocarde ainsi que des gènes du lignage endodermique propres au développement du pancréas endocrine et au tube digestif Ainsi ces cellules CD34+ présentes normalement

* Corresponding author.
E-mail address: bernard.pessac@wanadoo.fr (B. Pessac).

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chez l'adulte expriment spontanément des caractéristiques comparables à celles des CSE et des CSIP.

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1. Introduction

Pluripotency of stem cells is important for cell replacement therapy. Embryonic stem cells (ESCs) exhibit pluripotency [1,2], but because of practical issues, they are problematic for human cell replacement therapy. This led laboratories to induce pluripotency in normal adult somatic cells such as skin fibroblasts (iPSCs) [3,4]. However, the induction of pluripotency may evoke unknown genetic changes in the cells and cause tumorigenicity [4]. Therefore, we investigated whether there are natural adult stem cells that express ESC genes and share pluripotency with ESCs. Indeed, we find that a subpopulation of stem cells from adult bone marrow naturally expresses an array of ESC genes including *Oct-4*, *Rex-1*, *Sox-2*, *Klf-4* and *LIN-28*–genes that have been used to induce pluripotent stem cells from adult fibroblasts.

Pluripotency means that the stem cells have the potential to differentiate into a variety of different lineages. We examined, in this subset of bone marrow stem cells, the expression of lineage genes for each of the three embryonic germ layers. We previously reported that these bone marrow stem cells express an array of ectodermal neural genes of both neural progenitors and differentiated neurons and oligodendrocytes [5]. Here, we find that the same subset of adult stem cells expresses

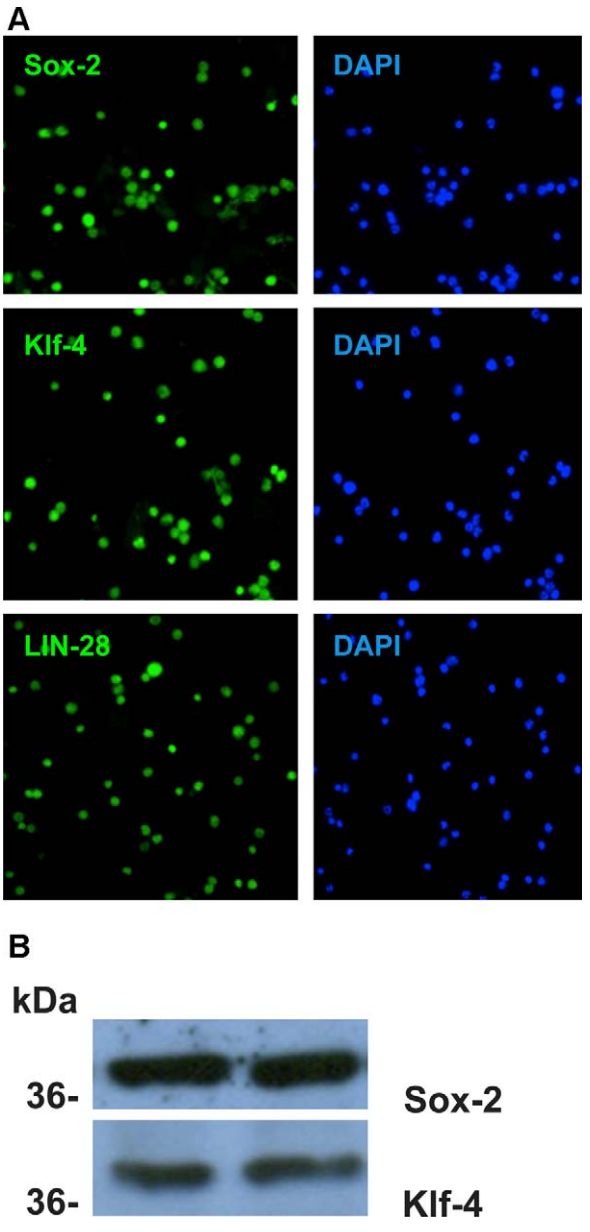

Fig. 1. Embryonic stem cell genes Sox-2, Klf-4 and LIN-28 are expressed in all non-adherent CD34+ cells cultured from adult bone marrow as reported in M&M.

A. Immunocytochemistry. Cells were suspended in 4% paraformaldehyde fixative for 15 min. at 4°C, washed 3 times in PBS and then deposited on microscope slides by cytospin and blocked with PBS containing 0.025% Tween-20 for 30 min. at RT. Cells were incubated for from 2 hours to overnight in primary antibody in Block buffer at RT, then washed with PBS 3 times quickly and three 5 min washes. The primary antibodies used were: anti-Sox-2 (Santa Cruz sc-20088), anti-Klf-4 (R&D Systems AF3158) and anti-LIN-28 (Santa Cruz sc-67266). The secondary antibodies FITC-anti-rabbit IgG (KPL202-1516) or FITC-anti-goat IgG (KPL 02-13-06) were added in Block buffer for 2 hrs. at RT and washed as above. The cells were mounted in Vectashield containing the nuclear dye DAPI, the coverslip sealed and cells were examined by flurescence microscopy. All cells with DAPI+ nuclei were labeled by each antibody.

B. Western blot analysis of the non-adherent CD34+ cells in culture with antibodies Sox-2 and Klf-4. Protein expression was determined on 4-12% polyacrylamide gradient gells (Invitrogen) by standard methodology (5,20). Duplicate lanes are shown for each protein. The MW standard positions (kDa) are shown on the left. The gels were run in triplicate. Both antibodies were strongly reactive.

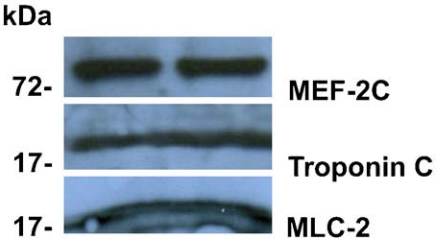

Fig. 2. Mouse hematopoietic progenitors express mesodermal lineage cardiac genes. The non-adherent CD34+ cells in culture were analyzed by Western blot with antibodies to the cardiac lineage transcription factor MEF-2 C (Santa Cruz sc-13266) and the differentiated cardiac genes troponin C (Santa Cruz sc-7672) and myosin light chain MLC-2 (Santa Cruz sc-48414). The procedure used was identical to that used in Fig. 1. The MW standard positions (kDa) are shown on the left. The three proteins are expressed at a high level.

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