Leukemia & Lymphoma, July 2006; 47(7): 1392 – 1393

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LETTER TO THE EDITOR

Limitations of the use of GFP transgenic mice in bone marrow transplantation studies

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(Accepted 22 November 2005)

Numerous transplantation studies used enhanced green fluorescent protein (GFP) transgenic mice (C57BL/6-Tg(ACTB-EGFP)1Osb/J, strain 003291, the Jackson Laboratory, Bar Harbor, ME, USA) for tracking the fate of exogenous murine cells [1 – 4]. In this strain, GFP cDNA is inserted under the control of a chicken β-actin promoter, a cytomegalovirus enhancer and bovine globin polyadenylation signal. GFP fluorescence is assumed to be uniformly expressed in all tissues with the exception of erythrocytes and hair [5]. Here, we demonstrate that GFP expression is variable in hematopoietic cells, maximal in primitive stem/progenitor cells and gradually lost with differentiation.

First, we collected bone marrow (BM) and peripheral blood (PB) of GFP mice. After hemolysis, the proportion of GFP^+ cells was assessed by flow cytometry in phenotypically-defined populations of primitive and differentiated cells (Table I). In the BM, GFP expression was predominant in primitive $Sca\text{-}1^+$ $ckit^+$ lin^- stem/progenitor cells and declined in $Sca\text{-}1^+$ $ckit^+$ lin^+ committed progenitors ($P = 0.014$). In the PB, the overall GFP expression was lower than in the BM. When present, GFP fluorescence was of similar intensity in all cell lineages examined contrarily to what has been reported in other strains of GFP transgenic mice in which T cells have a 2-fold higher GFP expression than B cells (strain 004353, Jackson Laboratory).

We then evaluated the presence of functional clonogenic GFP^+ progenitors. BM cells were seeded in a standard semi-solid medium (M3434, Stem Cell Technologies, Vancouver, Canada). Among 238 ± 15 hematopoietic colonies (10^4 bone marrow cells seeded, $n = 4$), only 81 ± 5 (34% of total colonies) were GFP^+ when examined in fluorescence microscopy.

The lower proportion of GFP^+ cells in mature cell fractions could result from an increased apoptosis rate of GFP^+ primitive stem/progenitors and a subsequent enrichment of differentiated cells generated from GFP^- stem/progenitor cells. Alternatively, GFP^+ stem/progenitors could lose GFP expression along with multiplication and differentiation. To assess the apoptotic rate, $sca\text{-}1^+$ $ckit^+$ lin^- cells isolated from GFP and wild-type mice were subjected to annexin V/propidium iodide FACS analysis. GFP^+ sca^+ $ckit^+$ lin^- cells did not present a significant higher apoptotic rate ($2.2 \pm 1.1\%$) than wild-type $sca\text{-}1^+$ $ckit^+$ lin^- cells ($1.6 \pm 0.69\%$, $n = 3$). To test the second hypothesis, we performed in vitro cell tracking experiments of GFP^+ $sca\text{-}1^+$ $ckit^+$ lin^- cells. Cells were labeled with PKH26 (Sigma, St-Louis, MO, USA) and seeded in suspension culture with cytokines mSCF, mIL3, mIL6 and mG-CSF (Peprotech, Rocky Hill, NJ, USA) for 3 days, after which retention of PKH26 staining and GFP expression were analyzed by FACS (Figure 1). After culture, GFP expression was present in a minor population (6.9%), while the majority of cells were GFP negative after the execution of a variable number of cell divisions. Interestingly, GFP fluorescence intensity was either present or totally absent, suggesting a complete suppression of transgene expression in most dividing cells rather than a gradual increase in GFP degradation.

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ISSN 1042-8194 print/ISSN 1029-2403 online © 2006 Informa UK Ltd.
DOI: 10.1080/10428190500513512

Table I. Distribution of GFP expression in hematopoietic cells.

Cell fraction	% GFP^+ cells
Bone marrow	
Sca-1^+ ckit$^+$ lin$^-$	84.6 ± 3.6
Sca-1^+ ckit$^+$ lin$^+$	64.9 ± 9.1
Peripheral blood	
CD5 (Ly-1)$^+$	46.1 ± 9.9
CD45R/B220$^+$	32.7 ± 4.6
Gr (Ly-6G)$^+$	36.2 ± 2.2
CD11b/Mac-1^+	36.0 ± 1.7
Total	37.8 ± 2.9

GFP expression was measured in combination with indicated phenotypic markers ($n = 5$).

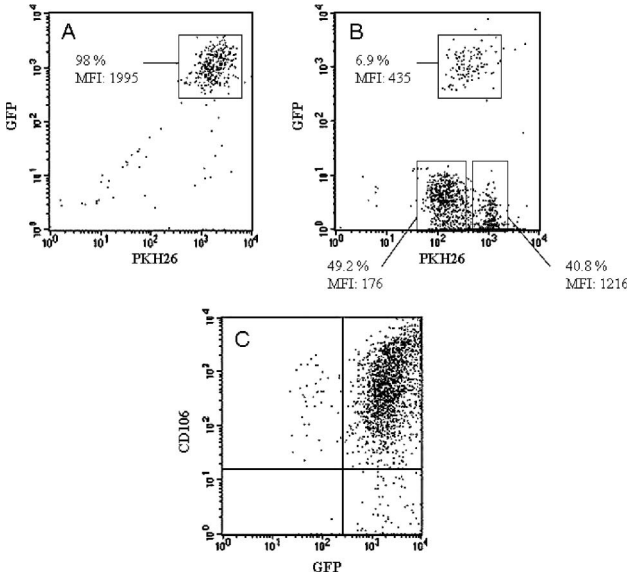

Figure 1. Stability of GFP expression in hematopoietic progenitors and MSC cultured ex vivo. (A) GFP^+ sca-1^+ ckit$^+$ lin$^-$ cells were sorted and labeled with PKH26. Uncultured stained cells were saved as control of baseline fluorescence. (B) GFP expression and PKH26 staining after 3 days in culture. Medium consisted of α-MEM, 10% FBS, 10 ng/ml each of IL-3, IL-6, SCF, and G-CSF. Mean PKH26 fluorescence intensity (MFI) and percentage of distinct cell clusters are indicated. (C) GFP expression by $CD106^+$ MSC after seven passages.

In contrast, GFP expression was conserved in ex vivo-amplified BM mesenchymal stem cells (MSC). Indeed, a progressive increase in the proportion of GFP-expressing cells was observed during culture of BM mononuclear cells in DMEM plus 10% fetal bovine serum: % GFP^+ cells was 33.4 ± 4.0 cells at culture initiation to reach >95% after 7 passages. This was correlated with the emergence of a phenotypically pure population of $CD106^+$ Sca-1^+ $CD45^-$ $CD11b^-$ MSCs, retaining osteocytic, adipocytic and chondrocytic differentiation capacity in adequate induction mediums [6].

In conclusion, we show that in this transgenic GFP-expressing mouse strain, transgene expression is stable in MSCs but not in hematopoietic cells. Indeed, while present in the large majority of primitive stem/progenitor cells, GFP is absent in a sizeable fraction of BM committed progenitors and PB differentiated leucocytes. Our data support the hypothesis that GFP expression is lost upon cell division and differentiation. If used as reporter gene in transplantation experiments of BM cells, measure of GFP expression in recipients could vastly underestimate the cell output of hematopoietic origin while the generation of MSC-derived cells would be correctly determined. The elucidation of molecular mechanisms underlying the observed differences in transgene expression between these two cell lineages requires further investigation.

Acknowledgements

This work was supported by grants from the Fonds National de la Recherche Scientifique (F.N.R.S., Brussels, Belgium), the University of Liège Center against Cancer and the Belgian Federation against Cancer (a non-profit organization). N.V.O. was supported by a Télévie fellowship. M.D. is a fellow of the Fonds pour la Formation à la Recherche dans l'Industrie et l'Agriculture (F.R.I.A.). Y.B. and A.G. are Research Director and Research Associate, respectively, of the F.N.R.S.

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