LT-HSCs is usually assessed by serial transplantation experiments in SRC assays, since the life span of mice is much shorter than that of humans.

In this study, we analyzed 74 cultured SRC clones and identified 20 clones in more than 2 recipient mice. Eleven of these 20 clones were further assessed for their self-renewal ability by secondary transplantation experiments, with 3 clones, 3-23, 3-26, and 3-36, reconstituting 2 recipients at the same time. Therefore, we can reasonably claim that ex vivo–expanded LT-HSCs were present in at least 3 of the 11 tested SRC clones. As discussed in the paper,1 we could not discount the possibility that the clones that were undetectable in the secondary recipients may correspond to lymphomyeloid short-term repopulating cells (STRC-MLs). The clinical significance of this study resides, however, in the discrepant findings between the clonal analysis and the limiting dilution analysis (LDA), which is used in most clinically approved protocols for ex vivo expansion of HSCs. Our culture conditions resulted in a 5-fold expansion of SRCs by LDA and only a 1.5-fold expansion by clonal assay. Therefore, even if SRC expansion was shown by LDA in a clinical protocol, it might not guarantee the real expansion of SRCs. Our study highlights the fact that clonal analysis is required to gain accurate results regarding expansion of HSCs.

Horn et al reported that SRCs represent STRCs but not LTRCs in nonhuman primates by retroviral gene marking.2 This represented an important trial to determine the reliability of SRCs as a model for human HSCs in preclinical studies. However, we consider their conclusion to be premature for the following 4 reasons. (1) The conclusion was derived from the analysis of only 2 clones from a baboon. This is a case report and the reproducibility is not warranted. (2) ST and LT clones are different populations, as ST clones support only early-phase hematopoiesis and LT clones support late-phase hematopoiesis after transplantation.3,4 Therefore, it is not surprising that most SRCs at 6 weeks are STRCs and thus undetected in a baboon at 6 months after transplantation. The authors should demonstrate the presence of SRC clones in the recipients of serial transplantation, but not SRCs at 6 weeks, in a baboon 6 months after transplantation. (3) Lentiviral vectors transduce LTRCs more efficiently than retroviral vectors.5 (4) The most serious flaw is that the culture condition used in the study was not demonstrated to expand SRCs. To confirm this, it is required to detect common clones in multiple recipients after aliquots of the same transduced cells were transplanted.

Nonetheless, we agree that SRC assays still provide a surrogate assay for HSCs and endorse the viewpoint that the method needs improvement. Previously, we succeeded in humanizing the hematopoietic microenvironment in nonobese diabetic (NOD)/SCID mice by transplanting human mesenchymal stem cells into bone marrow.8 We also recently demonstrated that human LT-HSC clones could produce T cells, B cells, and myeloid cells up to tertiary transplantation over 1.5 years.9 Our future aim is to obtain the “holy grail” in SRC populations through these studies.

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References


To the editor:

The stromal component of the marrow microenvironment is not derived from the malignant clone in MDS

Myelodysplastic syndromes (MDSs) represent a spectrum of disorders that are generally thought to arise from a defective hematopoietic stem cell leading to clonal, dysregulated hematopoiesis. Although it is generally agreed that the marrow microenvironment plays a role in the biology of MDSs, it is unclear whether this represents an intrinsically abnormal stromal compartment derived from the MDS clone. One report does suggest that stromal cells contain MDS-associated cytogenetic abnormalities,1 while others have contradicted these findings and suggest these cells function normally.2,3 These conflicting observations, along with the differing reports on hematopoietic stem cell plasticity, prompted us to evaluate this issue directly using long-term marrow cultures (LTCs) established from MDS patients.

We first addressed the issue of the origin of stroma in 1987 by using LTCs established with marrow aspirates from patients with functioning sex-mismatched allografts.4 By using fluorescence in situ hybridization (FISH), we were able to demonstrate that all of the stromal cells were host derived, whereas all of the cells containing donor sex chromatin were macrophages. We subsequently extended this observation to first document by nonspecific esterase (NSE) staining that up to 40% of cells in primary LTCs can be macrophages and, as expected, originate from the donor. In this case, we demonstrated that even after 27 years of 100% donor-derived hematopoiesis, the stromal cells still originated from the host.5

One could infer from these data that the stroma in patients with MDSs would not be derived from the malignant clone. To address
this issue directly, we analyzed LTCs established from 9 patients with MDSs. LTCs were cultured for 6 to 9 weeks before cells were harvested and cytocentrifuged onto glass slides. The percentage of macrophages was determined by staining with NSE. FISH was then performed on duplicate slides to determine the percentage of clonally marked cells. In every case except 1, the percent of clonally marked cells in the LTCs did not exceed that which could be accounted for by the combination of macrophages and background levels for FISH (Table 1). In case no. 7, we noted an unusual retention of myelocytes in the LTC, which probably contributed to the increased percentage of clonally marked cells noted in this patient. These data strongly suggest that stromal cells are not part of the malignant clone in MDSs and support our previous conclusion that stromal cells and hematopoietic cells are not derived from a common marrow-derived adult stem cell.

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Table 1. Histochemical analysis (NSE) and FISH of LTCs from 9 patients with MDS

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>MDS marker chromosome</th>
<th>% FISH false positive, Mean ± 3 SD</th>
<th>Long-term marrow cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>% NSE* cells</td>
</tr>
<tr>
<td>1</td>
<td>3q-</td>
<td>3.8</td>
<td>0.9</td>
</tr>
<tr>
<td>2</td>
<td>5q-</td>
<td>3.8</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>5q-</td>
<td>3.8</td>
<td>0.5</td>
</tr>
<tr>
<td>4</td>
<td>-7</td>
<td>3.5</td>
<td>5.4</td>
</tr>
<tr>
<td>5</td>
<td>7q-</td>
<td>5.7</td>
<td>7.8</td>
</tr>
<tr>
<td>6</td>
<td>7q-</td>
<td>5.7</td>
<td>4.4</td>
</tr>
<tr>
<td>7</td>
<td>-8</td>
<td>2.1</td>
<td>0.4</td>
</tr>
<tr>
<td>8</td>
<td>-8</td>
<td>2.1</td>
<td>0.8</td>
</tr>
<tr>
<td>9</td>
<td>5q-</td>
<td>3.8</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>-7</td>
<td>3.5</td>
<td>1.4</td>
</tr>
</tbody>
</table>

The cytogenetic marker specific for the MDS clone from each of 9 patients is listed together with the false-positive rate for the probe used to label that chromosome marker. Each of these background levels was determined with cells from 6 healthy individuals. The macrophage component of the LTC was estimated by the percentage of NSE-positive cells. A previous study showed that NSE is comparable to labeling with CD14 or CD45 for detecting the macrophage component of LTC.5 The data indicate that, with the exception of patient no. 7, the percentage of LTC cells derived from the MDS clone can be accounted for by the combination of macrophages and background levels of the FISH probe. In patient no. 7, we noted an unusual retention of myelocytes in the LTC, which explains the higher percentage of clonally marked cells.

References


To the editor:

Steady remission of scleromyxedema 3 years after autologous stem cell transplantation: an in vivo and in vitro study

We successfully treated a 66-year-old patient with life-threatening scleromyxedema and an immunoglobulin G (IgG) lambda monoclonal spike with the BEAM regimen (BCNU, etoposide, cytarabine [Ara-C], and melphalan) and autologous stem cell transplantation (ASCT). Six months later, he had fully recovered, and at 3 years he remains asymptomatic without any other treatment, while the IgG lambda monoclonal spike is still detectable.

ASCT following treatment with high-dose melphalan alone appeared effective in 3 previous reports,1-3 although in several cases, skin lesions relapsed and had to be retreated with melphalan.

To understand the mechanisms involved in the impressive steady remission of our patient, we performed an in vitro study using the patient’s fibroblasts before and after treatment. The assays demonstrated abnormal proliferation of fibroblasts before ASCT (8879 ± 398 [SD] counts per minute [cpm]) independently of soluble factors or immunoglobulins. Importantly, proliferation normalized to rates similar to that of controls (2853 ± 398 cpm) in the fibroblasts from a skin biopsy after ASCT (856 ± 90 cpm). We also incubated pre-ASCT fibroblasts with the different conditioning drugs. The proliferation reversed, after a 4-week washout, from 18 844 cpm in the untreated cultures to 1118 cpm in cultures treated with BCNU, 584.8 cpm in cultures treated with Ara-C, and 1059 cpm in cultures treated with melphalan. Previous studies using fibroblast primary cultures suggested that a serum factor stimulates fibroblasts proliferation.4 It has been reported that elimination of the monoclonal band was the cause of the improvement,5 although the isolated IgG fraction showed no stimulatory activity in a different study.5 Our patient recovered completely, although the gammopathy remains present.

We performed a 2-dimensional (2D) analysis (BioRad, Palo Alto, CA) using fibroblast extracts from all of the previously described conditions. The Ludesi-2D Interpreter software (Ludesi, Lund, Sweden) was used for image analysis, and selected spots were analyzed using a

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