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Mesenchymal stem cells can be easily isolated from bone marrow of patients with various haematological malignancies but the surface antigens expression may be changed after prolonged *ex vivo* culture

Leukemia (2005) **19,** 1505–1507. doi:10.1038/sj.leu.2403795; published online 19 May 2005

TO THE EDITOR

The stromal cells in the haematopoietic microenvironment include fibroblasts, reticular endothelial cells, adipocytes, osteogenic and chondrogenic precursors.[1] These cells provide growth factors, cell-to-cell interaction, and matrix proteins that play an important role in the regulation of haematopoiesis, and many of these cells may be derived from a common precursor – the mesenchymal stem cell (MSC).[2,3] It is therefore that the MSC population may serve as a facilitator of haematopoietic engraftment in clinical haematopoietic stem cell transplantation[4] as well as a good springboard for further studies of how stroma influences haematopoiesis.[5] It is currently recognized that the microenvironment in which a cancer cell grows comprises a critical component of the malignant disease process.[6] Elucidating the interaction between MSCs and haematological cancer cells should be an important research focus. However, the sources of MSC in most of the prior studies were normal healthy donors or patients with normal marrow. Can MSCs be isolated and *ex vivo* expanded from bone marrow (BM) of patients with various haematological malignancies? If MSCs could be isolated and *ex vivo* expanded, which passage of *ex vivo* culture is better for study? Are they all the same? To answer these basic questions, 44 BM aspirates isolated from 42 patients with various haematological malignancies at various clinical situations (at initial diagnosis, after chemotherapy, or years after successful allogeneic peripheral blood stem cell transplantation (PBSCT)) were collected for MSCs culture in this study and we

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Received 18 March 2005; accepted 4 April 2005; published online 19 May 2005

Table 1 Demographic and clinical characteristics of patients

Patients	
Total number	42
Bone marrow specimens	44
Age (years)	15–83, median 52
Sex	
Male	25
Female	17
Disease status	
AML (11)	
At diagnosis	5
After chemotherapy	1
After allogeneic transplantation	5
MM (11 patients, 13 specimens)	
At diagnosis	5
After chemotherapy	3
After thalidomide	2
After allogeneic transplantation	3
NHL with BM involvement (8)	
All at initial diagnosis	
PTCL[a]	3
DLBCL[b]	2
MALT[c]	1
MCL[d]	1
LPL[e]	1
ALL (4)	
At diagnosis	3
After allogeneic transplantation	1
CML (4)	
At diagnosis	2
After allogeneic transplantation	2
MDS (2), all at initial diagnosis	
RA[f]	1
RAEB[g]	1
CLL (1), at initial diagnosis	1
Agonogenic myeloid metaplasia, at initial diagnosis	1

[a]Peripheral T-cell lymphoma; [b]diffuse large B-cell lymphoma; [c]mucosa-associated lymphoid tissue lymphoma; [d]mantle cell lymphoma; [e]lymphoplasmacytoid lymphoma; [f]refractory anaemia; [g]refractory anaemia with excess blast.

analysed surface antigen expression, and multilineage-differentiation potentials of those MSCs successfully isolated.

Between December 2002 and July 2004, 44 BM specimens were obtained from 42 patients of haematological malignancies and the patients' characteristics were showed in Table 1. The isolation, *ex vivo* expansion of MSCs, and induction of adipogenic and osteogenic differentiation were performed using methods as described previously.[7] This study is conducted under condition approved by the Institutional Review Board of China Medical University Hospital. The cells were harvested at different passages by treatment with 0.25% trypsin-EDTA. The phenotype of MSCs was determined at passage 2 (P2) and subsequent passages by both flow cytometric analysis and immunohistochemical staining. For flow cytometric analysis, monoclonal antibodies against CD11b, CD14, CD15, CD25, CD34, CD38, CD45 (BD, San Jose, CA, USA), CD105, and CD166 (Serotec, Kidlington, Oxford, UK) were used. For immunohistochemical study, monoclonal antibodies to CD45, CD105, and CD166 (BD, San Jose, CA, USA) were used. Subsequent colour development was achieved by incubating the slides with 3,3′-diaminobenzidine tetrahydrochloride (DAB, Zymed). Positive staining was recognized under a microscope as brown colour.

Of the 44 collected specimens, MSCs were successfully isolated and *ex vivo* expanded from 41. In all, 10 of the 11 specimens obtained from recipients of high-dose chemotherapy and allogeneic PBSCT were also successful. Primary culture failure was noted in three patients and they are patients of AML 2 years after allogeneic PBSCT, agnogenic myeloid metaplasia, and diffuse large B-cell lymphoma with BM involvement at initial diagnosis, respectively. A morphologically homogenous population of fibroblast-like cells was detected in primary and subsequent passages of *ex vivo* cultures. Induction of adipogenic and osteogenic differentiation was performed on P2 or P3 MSCs in 21 specimens (six AML, six MM, three NHL with bone marrow involvement, two ALL, two CML, one MDS and one CLL, and five of these were recipients of allogeneic PBSCT) and was successful. Cytogenetic study by using standard G-banding was performed on P2 MSCs from four sex-mismatched allogeneic PBSCT recipients and showed that the MSCs were completely recipient-derived.

Flow cytometric analysis of the 41 successfully expanded MSCs at P2 cells showed that 32 of them were $CD11b^-$, $CD14^-$, $CD15^-$, $CD25^-$, $CD34^-$, $CD38^-$, $CD45^-$, $CD105^+$, $CD166^+$. The other nine specimens had atypical surface antigens

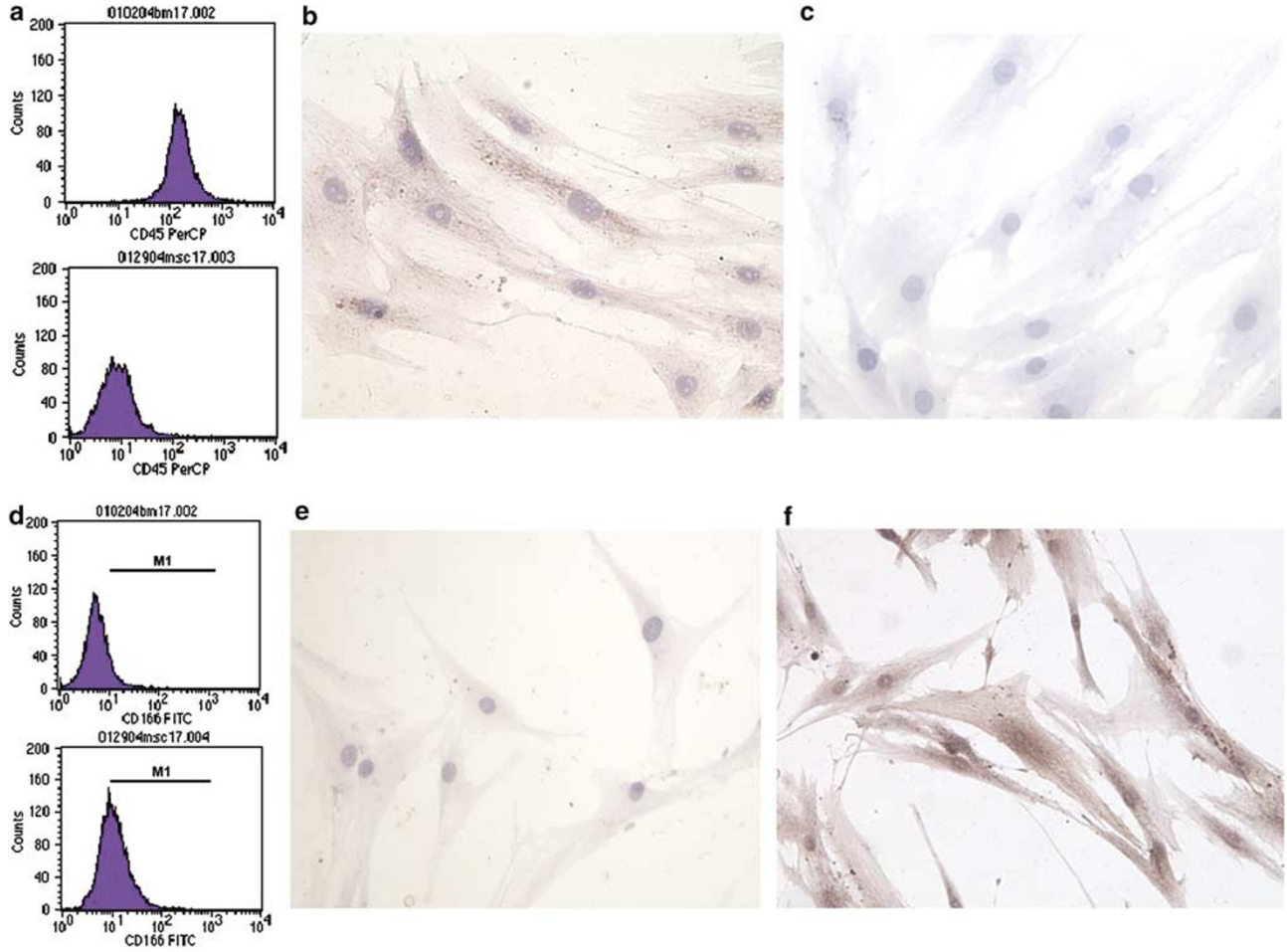

Figure 1 MSCs isolated from a patient of MM had atypical phenotype ($CD45^+$, $CD166^-$) on P2 and typical phenotype ($CD45^-$, $CD166^+$) on P3. (a) Flow cytometric analysis. CD45 was expressed on P2 cells (upper) but lost expression when the cells were successively cultured to passage 3 (lower). (b/c) Direct demonstration of CD45 expression on the 'fibroblast-like' cells on P2 cells (b; ×200) but not P3 cells (c; ×200) by using immunohistochemical staining. (d) Flow cytometric analysis. CD166 was not expressed on P2 cells (upper; M1: 8%) but was expressed when the cells were successively cultured to passage 3 (lower; M1: 47%). (e/f) Direct demonstration of CD166 expression on the 'fibroblast-like' cells on P3 cells (f; ×200) but not P2 cells (e; ×200) by using immunohistochemical staining.

expression such as $CD45^+$, $CD105^-$, and/or $CD166^-$. The 'atypical' phenotypes determined by flow cytometry were further confirmed by immunohistochemical staining. These nine specimens were isolated from BM of five patients of MM, two patients of ALL, and one patient of CLL and AML. The morphology and capacity of multidifferentiation of these atypical cells were, however, similar to those with 'typical' phenotype. Besides, with successive culture to the 3rd to 5th passages, the phenotypes changed to the 'typical' phenotype ($CD45^-$, $CD105^+$, $CD166^+$). Figure 1a–c demonstrates the atypical phenotype ($CD45^+$, $CD166^-$) of P2 MSCs isolated from BM of a patient with MM. Figure 1d–f demonstrates the phenotype changing to $CD45^-$ $CD166^+$ after successive culture to the P3 from the same marrow specimen.

It is currently not known why some of the MSCs isolated from patients with haematological malignancies have atypical phenotype ($CD105^-$, $CD166^-$, and/or $CD45^+$). The expression of CD45 on MSCs is especially of great interest because it has never been reported in the literature. Five of the 41 MSCs analysed in this study had expression of CD45 at variable degree. The direct demonstration of CD45 on 'fibroblast-like' cells by immunohistochemical study (Figure 1b) indicates that the expression of CD45 on these MSCs was 'true' positive and was not due to contamination of haematopoietic cells. Since all the MSCs with atypical phenotype at the 2nd passage of cell culture turned to 'typical' phenotype ($CD105^+$, $CD166^+$, and $CD45^-$) after successive culture to the 3rd to 5th passage, we hypothesize that the abnormal marrow microenvironment of patients with haematological malignancies may have some influence on the surface antigens expression of MSCs and this effect may persists during early *ex vivo* culture. With prolonged *ex vivo* culture in a 'standard' condition, this influence decreased and the phenotype turned to typical pattern. An example was the study conducted by Mahmoud *et al*,[8] who showed CD45 expression could be induced in U-266 myeloma cell line by adding IL-6 to the cell culture and withdrawal of IL-6 led to reduction of CD45 expression. Since the cytogenetics study showed that BM-derived MSCs of patients who had received allogeneic PBSCT were complete recipient origin, further study comparing MSCs isolated at diagnosis of leukaemia and after successful allogeneic transplant could probably know what is the impact of cancer cells on MSCs.

In summary, MSCs can be easily isolated and *ex vivo* expanded successfully from BM of patients with various haematological malignancies, including patients having received high-dose chemotherapy and allogeneic PBSCT, and they can therefore be good candidates to investigate the interaction between malignant cells and stroma. However, the phenotype, and probably other characteristics, of MSCs may be changed after prolonged *ex vivo* culture. This novel finding is quite important in designing the study involving MSCs and shorter duration of *ex vivo* culture may be better for examining the role of MSCs in the process of malignant haematopoiesis.

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