Brief Report

Mesenchymal Stem Cells and Endothelial Cells: A Common Ancestor?

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Abstract

Bone marrow mesenchymal stem cells (BM-MSCs) are commonly known as nonhematopoietic-nonendothelial cells based on *in vitro* expressed markers and properties. Despite the massive research on *ex vivo* expanded MSCs, their *in vivo* identity remains elusive. In this study, we report the existence of large multinuclear CD31 positive cells in the beginning of human BM-MSCs culture. Interestingly, the adjacent multinuclear cells occasionally formed tube-like structures. The large multinuclear cells then gave rise to mononuclear cells that fulfilled the criteria for BM-MSCs and were negative for CD31 but positive for other endothelial markers, CD54, CD106, and CD144. These observations, although primitive, imply that MSC ancestors may directly participate in the formation of new vessels. Further studies on BM-MSCs in the first few days of culture are definitely required to investigate the exact role of these cells in the vascular system.

Keywords: Angiogenesis, bone marrow, mesenchymal stem cells, multinucleation,

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Introduction

Mesenchymal stem cells (MSCs) were first isolated from bone marrow (BM) in 1970 by Friedenstein *et al.* as stromal non-hematopoietic non-endothelial cells.[1] Since then, these cells could be isolated from different sources and many animal studies and clinical trials have been conducted to assess their regenerative potential.[2] In spite of this huge effort, our understanding of the *in vivo* identity of these cells is yet primitive.[3] MSCs are commonly cultured for at least 2–3 weeks before they can be used for characterization experiments or assessment of regenerative potential. Therefore, identification of these cells is solely based on *in vitro* parameters[4] and little is known about their *in vivo* properties. It has been shown that many properties of MSCs, including their surface markers, differentiation potential, and genome content, can change dramatically during *ex vivo* expansion.[5] Therefore, they can be best investigated in the first few days of culture, when they are more similar to their *in vivo* counterparts. We have previously shown that mouse and rabbit MSCs are derived from large multinuclear cells (LMCs) that are enriched in cell aggregates in BM.[5–7] Here, we report our observation of LMCs in the first few days of human BM culture which give rise to mononuclear MSCs. We also present initial evidences that they may also participate in the formation of new vessels.

Materials and Methods

The study was approved by our institute's ethics committee and informed consent was obtained before the procedure. BM aspirates were harvested under local anesthesia from iliac crest of healthy male and female donors (15–55 years old) participating in a clinical trial on autologous transplantation of MSCs for orthopedic disorders. Heparinized human BM samples were diluted 1:1 (v/v) with CliniMACS PBS–EDTA buffer (Miltenyi Biotec, Bergisch Gladbach, Germany) and carefully laid over Ficoll (Innotrain, Kronberg, Germany) at a ratio of 10:3 (v/v) in 15-mL conical tubes. After centrifugation (20 min, 600 g), the upper fraction containing mononuclear cells (MNCs) was collected and washed with PBS. After isolation, the MNCs were suspended in Dulbecco's Modified Eagle's Medium (DMEM; PAA Laboratories, Pasching, Austria) containing 10% autologous serum and cultured at 37°C and 5% CO2. Flowcytometry, immunocytochemistry (ICC), karyotype analysis and differentiation assays were performed as described previously.[5–7] Data are presented as mean ± standard deviation.

Results

Five days after culture of human BM-MNCs, the flask was washed and fresh medium added. Interestingly, numerous multinuclear round cells were observed, some of which were very large and had tens of prominent nuclei. In some cases, the nuclei were concentrated in one region of the cells. Interestingly, occasionally two adjacent LMCs were connected by tube-like structures made of nuclei protrusion (Figure 1). As this process was reminiscent of vessel formation, we decided to assess the expression of CD31 endothelial marker. The cells were moved to 4-well plates and ICC was performed on days 6, 15 and 25 after isolation. CD31 was strongly expressed by LMCs on day 6. On days 15 and 25, LMCs gave rise to mononuclear cells with MSC morphology and gradually became negative for this marker (Figure 2). The mononuclear cells derived from LMCs fulfilled the criteria for MSCs as they were positive for CD73, CD90 and CD105 and negative for CD31, CD34 and CD45 markers on day 30. Furthermore, they had the potency to differentiate into adipose

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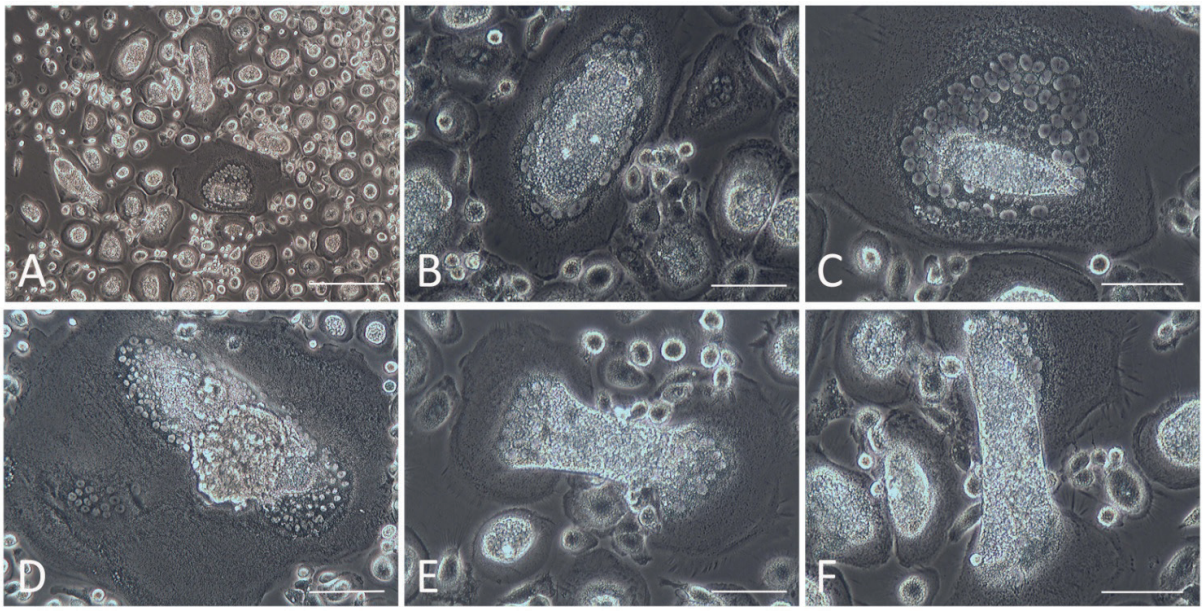

Figure 1. Multinuclear cells are visible in the beginning of the culture and make vessel-like structures. After five days of BM-MNCs culture, medium was exchanged and non-adherent cells were removed. The remaining cells were mostly round with different sizes **(A)**. However, some cells were large and multinuclear **(B-D)**. Occasionally two adjacent multinuclear cells were connected by tube-like structures made of their nuclei **(E, F)**. Scale bars: **A**: 200 µm; **B-F**: 100 µm.

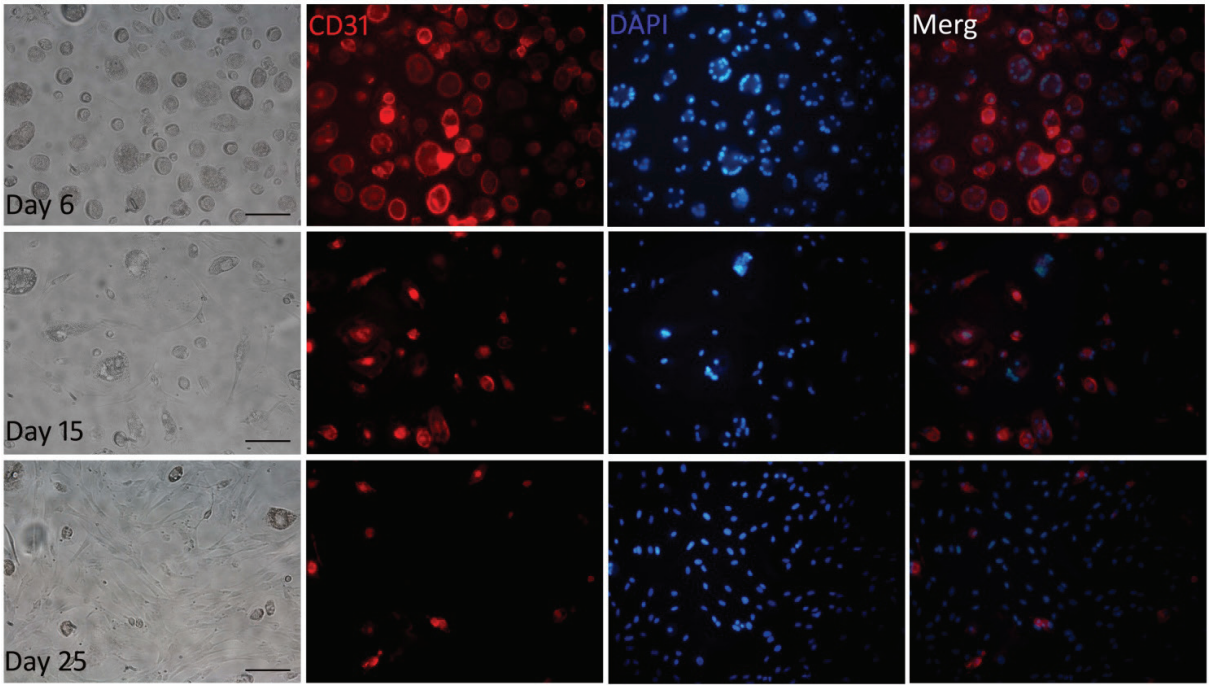

Figure 2. CD31-positive large multinuclear cells turn into mononuclear CD31-negative cells. ICC with PE-conjugated anti-human CD31 antibody showed that BM-derived multinuclear cells, observed in day 6 after isolation, were positive for this endothelial marker. The derivative mononuclear cells gradually lost this marker from day 15 to 25. Nuclei are stained with DAPI. Scale bars: 100 µm

and osteogenic lineages. In addition, karyotype analysis showed no chromosomal aberrations (Figure 3).

To further assess the endothelial markers, three human BM aspirate were cultured in standard culture conditions and the expression of CD31, CD54, CD106, and CD144 was measured by flowcytometry on days 10, 15, 30, and 60 after isolation. In agreement with ICC data, the cells were only slightly positive for CD31 on day 10 and were negative on the next days. In contrast, the emerged MSCs were positive for CD54, CD106, and CD144 endothelial markers in the examined time points, though with variable expression rates (Table 1).

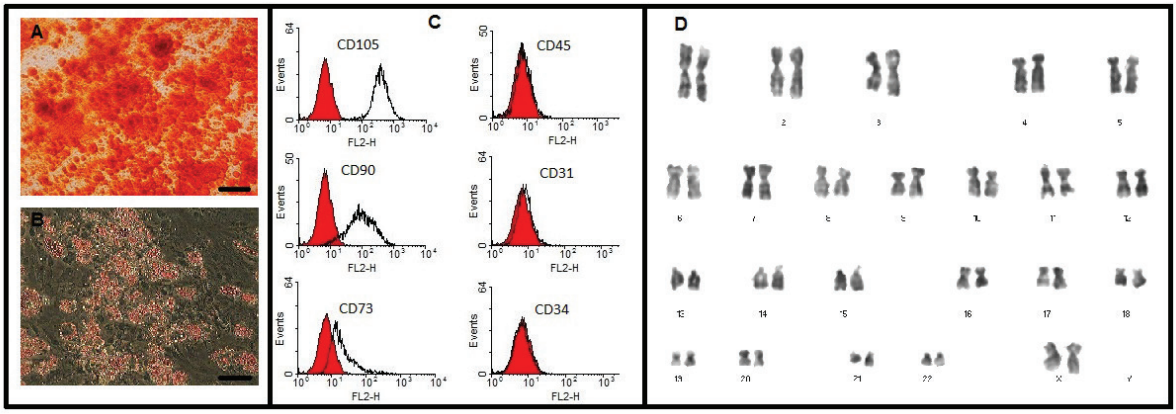

Figure 3. The mononuclear cells derived from LMCs fulfill the criteria of MSCs. The derivative cells had the potential of differentiation into osteocyte and adipocyte lineages as revealed by Alizarin red S and Oil red O staining (**A** and **B**, respectively). Scale bars: 100 µm. Surface markers assessed by flowcytometry were compatible with MSC marker panel (**C**). In addition, the cells did not demonstrate any chromosomal aberrations (**D**).

Table 1. Cytofuorometric assessment of surface markers on MSCs in Days 10–60 after isolation from BM.

Marker	Expression of Surface Markers (%)			
	Day 10	Day 15	Day30	Day 60
CD31	2.7 ± 1.5	0 ± 0	0.3 ± 0.5	0 ± 0
CD54	60.4 ± 32.5	50.4 ± 7.2	24 ± 16.3	12 ± 6.5
CD106	82 ± 19.6	18.4 ± 6.5	4.4 ± 1.5	18.4 ± 6.5
CD144	6 ± 5.2	66.7 ± 14.5	82.7 ± 18.6	39 ± 29.8

Discussion

In the current study, we observed the formation of LMCs at the beginning of human BM-MSC culture. The special arrangement of nuclei of multinuclear cells resulted in the formation of tube-like structures. The multinuclear cells were CD31-positive but their derivative mononuclear cells were CD31-negative and fulfilled the criteria for MSC. However, the emerged MSCs were positive for other endothelial markers, CD54, CD106, and CD144.

We have previously noticed the appearance of multinuclear cells in the culture of mouse and rabbit BM-derived cells and shown that they are the origin of MSCs.[5–7] It seems that a special form of internal nuclear duplication is responsible for the formation of these cells. Although several years ago, it has been suggested that this type of division named *neosis* is responsible for the resistance of malignant cells to chemotherapy,[8] the molecular basis and biological significance of this phenomenon are yet unexplained. The observation of LMCs in the first few days of BM culture in this study suggests that they reside in native BM. Further studies are definitely required to elucidate the *in vivo* significance of these cells.

An interesting observation in this study was the special arrangement of nuclei of neighbor LMCs that formed tube-like structures. This phenomenon in association with the expression of endothelial markers provides a clue that LMC formation may be essential for angiogenesis. In agreement with this hypothesis, it has been shown that the paracrine effects of perivascular multinuclear cells are crucial for angiogenesis.[9–11] Also, in line with our observations, it has been previously shown that MSCs and endothelial cells originate from a common precursor, named mesenchymoangioblast, during embryonic development.[12–13]

In conclusion, shortage of knowledge on *in vivo* counterparts of MSCs and classification of these cells as non-hematopoietic non-endothelial cells, based on *in vitro* expressed markers, has raised ambiguity about the entity, location and natural physiological function of these cells. The current study provides primitive evidence that MSCs are derivatives of multinuclear cells and are parts of the vascular system.

Conflict-of-interest disclosure

The authors declare no competing financial interests.

Acknowledgments

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