Bone marrow mesenchymal cells: polymorphism associated with transformation of rough endoplasmic reticulum

Yong-Xin Rua,+,†, Shu-Xu Donga, Chun-Hui Xua, Shi-Xuan Zhaoa, Hua-Mei Zhanga, Hao-Yue Lianga, Min Fen†, Feng-Kui Zhang†, Ying-Dai Gaoa, Shi-Lin Qi†, Hong-Cai Shang††

State Key Laboratory of Experimental Hematology, National Clinical Research Center for Blood Diseases, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin, China; ††Key Laboratory of Chinese Internal Medicine of Ministry of Education, Beijing Dongzhimen Hospital, Beijing University of Chinese Medicine, Peking, China

Abstract
To understand the behavior and function of bone-marrow mesenchymal cells (BMMCs), we overviewed the morphological presentation of BMMCs in bone-marrow granules (b-BMMCs), isolated BMMCs (i-BMMCs), and BMMCs (c-BMMCs) cultured in semisolid methylcellulose and MEM media. All samples were derived from bone-marrow aspirates of 30 patients with hematopoietic diseases. Light microscopy exhibited b-BMMCs and i-BMMCs characterized by abundant cytoplasm and irregular shape in bone-marrow smears, as well as c-BMMCs in culture conditions. Scanning electron microscopy demonstrated cultured c-BMMCs with a sheet-like feature enveloping hematopoietic cells. Transmission electron microscopy revealed b-BMMCs constructing a honeycomb-like structure by thin bifurcate processes among hematopoietic cells. Furthermore, i-BMMCs had bifurcate parapodiums on the surface and prominent rough endoplasmic reticulum (rER) connected with the plasmalemma of the parapodiums. The detailed images suggested that rER may serve as a membrane resource for plasmalemmal expansion in BMMCs in bone marrow.

Keywords: Bone-marrow mesenchymal cell, Morphology, Plasmalemma, Rough endoplasmic reticulum, Transformation

1. INTRODUCTION
Bone-marrow mesenchymal cells (BMMCs) construct hematopoietic microenvironments to regulate hematopoietic stem/progenitor cells (HSPCs) in bone marrow. The morphologic relationship between BMMCs and HSPCs has been analyzed from the 1960s to 1990s, and the biological effects of BMMCs on HSPCs have been the object of intense study in recent decades. BMMCs participate in blood production through intimate cell-cell contacts and the release of hematopoietic factors. In a previous study, we described BMMCs characterized by fibroblastic and macrophage features constructing a hierarchical meshwork by divaricate processes in bone marrow. To understand the behavior and function of BMMCs, we examined BMMCs in bone-marrow granules and in culture by light microscopy, scanning and transmission electron microscopy (SEM and TEM). In the bone-marrow granules, which are regarded as having a good replication of the in vivo state of bone marrow, BMMCs within areas of organized bone-marrow tissue were designated as b-BMMCs, while solitary BMMCs from bone-marrow granules but not closely associated with any other cells were designated as i-BMMCs. BMMCs in culture were designated as c-BMMCs.

2. RESULTS
2.1. Morphology of b-BMMCs on light microscopy
Bone-marrow granules varied in size and contained many hematopoietic cells (erythrocytes and granulocytes) in bone-marrow smears. Most b-BMMCs were difficult to distinguish from hematopoietic cells and macrophages on account of cellular overlapping in large bone-marrow granules, but some b-BMMCs clearly exhibited a round nucleus and irregular shape with cytoplasmic processes around hematopoietic cells in small bone-marrow granules (Fig. 1A, B). Sometimes, i-BMMCs were found among hematopoietic cells in bone-marrow smears, characterized by a round nucleus, irregular processes with granular cytoplasm and vacuoles (Fig. 1C).

In semithin sections of Epon 812 blocks, b-BMMCs showed a main body with a nucleus and scanty cytoplasm, and long thin processes among hematopoietic cells. Proximate processes were usually thick and divaricated into thinner ones between hematopoietic cells. Additionally, there was a circular crevice between hematopoietic cells and processes. The circular crevices and processes constructed a single compartment for each hematopoietic cell (Fig. 2).
2.2. Morphology of c-BMMCs on light microscopy

Clusters of c-BMMCs were scattered over the bottoms of dishes in H4434 methylcellulose semisolid medium. Most were spindle shaped, while some, adherent to hematopoietic cells, were flat and showed AKP activity on inverted microscopy (Fig. 3A, B). On the other hand, c-BMMCs in MEM media were flat and polygon-like, and positive for CD44 and α-SMA (Fig. 3C, D). By SEM, these c-BMMCs looked like a huge thin blanket, enveloping and supporting blood cells (Fig. 4).

2.3. Ultrastructure of b-BMMCs on TEM

A few b-BMMCs shared undeveloped features with a prominent nucleolus and fewer processes around the main cell bodies (Fig. 5A), but most b-BMMCs were fully developed, characterized by a smaller main body with an ovoid nucleus and more bifurcated processes among hematopoietic cells. They included plentiful rER, lysosomes and phagosomes in the peripheral cytoplasm and proximate processes (Fig. 5B). Sometimes, a few b-BMMCs connected together and enclosed hematopoietic cells by processes (Fig. 5C, D). The processes usually divaricated hierarchically and became thinner and thinner between hematopoietic cells. Distal processes were from 60 to 80 nm thick and contained few cellular organelles, which constructed a honeycomb-like structure for hematopoietic cell in bone-marrow granules (Fig. 6).

2.4. Ultrastructure of i-BMMCs on TEM

i-BMMCs were about 60 μm in diameter, including a round nucleus, plentiful lysosomes, rER, and many bifurcate parapodiums on the cell surface (Fig. 7A, C and Fig. 8A). Most rER cisternae were expanded and divaricated, demarcating cytoplasm into segments like those in megakaryocytes.13,14 The segments of cytoplasm were of the same size as parapodiums on the cell surface. In higher-power views, rER often appeared to open on to the cell surface and connect with the plasmalemma of i-BMMCs (Fig. 8B–D).

3. DISCUSSION

Bone-marrow stromal cells (BMSCs) are a heterogeneous group of cells with distinctive morphological features that construct hematopoietic microenvironments by varied physical structures and releasing different hematopoietic factors in bone marrow. Each BMSC is located in a specific region, for example, osteoblasts, osteoclasts and chondrocytes line the endosteal surface, whereas adventitial cells and pericytes are adjacent to the outer wall of sinusoids and perivascular regions.15–17 As such, they compose dynamic hematopoietic microenvironments of HSPCs in bone marrow. BMMCs are pleomorphic depending not only on its inherent entity but also external factors.

In the present study, b-BMMCs showed irregular processes with blurred boundaries among hematopoietic cells; however, i-BMMCs exhibited a round nucleus and coarse processes with granular cytoplasm in bone-marrow smears. The above appearances were seldom described because of the difficulty in identifying them in obscure images in bone-marrow smears by light microscopy. In semithin sections of Epon 812 blocks, b-BMMCs exhibited the original morphology of those in vivo, characterized by a main body with an oval nucleus and many bifurcate processes projecting out between hematopoietic cells. In
culture conditions, most c-BMMCs were spindle shaped, but some of them were flat. The flat BMMCs often adhered to hematopoietic cells and showed AKP activity in H4434 methylcellulose semisolid medium. All c-BMMCs in MEM media were flat and polygonal, and positive for CD44 and α-SMA. The results were consistent with other observations.\textsuperscript{5,18} The above appearances suggested that the morphology of BMMCs was affected predominantly by local microenvironments and culture conditions.

On TEM, b-BMMCs stretched out many bifurcate processes among hematopoietic cells, most of them about 60 nm thick, more than 3 times that of the plasmalemma, and constructed a honeycomb-like meshwork for hematopoietic cells in bone-marrow spaces. The question arises: how do b-BMMCs obtain

\textbf{Figure 3.} Characteristics of c-BMMCs in culture. (A) Wright staining shows a cluster of c-BMMCs attached to hematopoietic cells on the dish bottoms in H4434 methylcellulose semisolid medium; (B) some flat c-BMMCs show AKP activity in H4434 methylcellulose semisolid medium; (C) and (D) c-BMMCs in MEM media are flat and polygonal, and positive for CD44 and α-SMA respectively.

\textbf{Figure 4.} Scanning electron micrographs of c-BMMCs co-cultured with hematopoietic cells. (A) c-BMMCs (arrows) show a sheet-like feature enveloping 3 hematopoietic cells, ×2.5K; (B) sheet-like process (arrow) supports a hematopoietic cell, ×3K; (C) a hematopoietic cell is isolated by c-BMMCs (arrow), ×3K.
the large amount of membrane to supplement the plasmalemma on these processes in bone marrow?

In attempting to answer this question, it should be noted that i-BMMCs had many parapodiums on the surface and plentiful rER in the cytoplasm compared with the processes and cytoplasm of b-BMMCs. The morphologic differences might be associated with external factors: parapodiums on i-BMMCs resulted from the release of hematopoietic cells and process retraction of b-BMMCs during bone-marrow aspiration. In contrast, processes of b-BMMCs resulted from the squeezing and pressing together of hematopoietic cells in bone marrow. Furthermore, detailed TEM images demonstrated that rER were bifurcated in the peripheral cytoplasm and connected with plasmalemma of i-BMMCs. It suggested that the huge plasmalemma of b-BMMCs may be derived from cytoplasmic rER. In other words, rER may contribute the membrane component and, in effect, be the origin of the processes of BMMCs in bone marrow.

Theoretically, plasmalemma is supplemented by the enveloping membrane of recycling phagosomes and vesicles from Golgi apparatus; membrane of the Golgi apparatus was mainly derived from rER during protein transport in well-developed cells. In fact, the conventional view has been that vesicles and phagosomes act predominantly as transporters or carriers of proteins rather than being a membrane resource for the plasmalemma.

Cell morphology and structure was not only determined by cytoskeleton components of actin, tubulin, myosin, dynamin, actinin and supervillin filaments, but also was influenced by other cells in multicellular tissues. In particular, the morphology of b-BMMCs was influenced by environmental factors, that is, bifurcate processes were associated with a pressing together of hematopoietic cells. However, this specific network demands a large amount of membrane to supplement the plasmalemma of b-BMMCs in bone marrow. In this study, evidence has been provided for the phenomenon of prominent rER connecting with plasmalemma, which suggested that plasmalemmal expansion was closely related to transformation of rER in the cytoplasm of b-BMMCs in the same way as occurs in yeast cells.

As a multifaceted organelle, rER is transported between the Golgi apparatus and nuclear envelope through a stripping of membrane-bound ribosomes in mammalian cells. Some...
Figure 6. Bifurcate processes of b-BMMCs. (A) Bifurcated processes about 60 nm thick (arrows) isolate erythroblasts in various developmental stages (E), ×4000. (B) Thick proximate processes (PP) of a b-BMMC (MC) contain more lysosomes in the cytoplasm, but thinner processes (arrowheads) are bifurcated at a locus (stars) among granulocytes (G) and erythrocytes (E), ×4000.

Figure 7. Ultrastructure of i-BMMCs. (A) An i-BMMC about 50 μm in diameter has numerous bifurcate parapodia on its cell surface (arrows), ×2.5K. (B) an i-BMMC shows a meshwork feature resulting from rER expansion (pairs of arrows) in the peripheral cytoplasm, ×10K. (C) Parapodiums connect with peripheral cytoplasm of an i-BMMC, ×3K. (D) a high magnification of (C), rER (arrows) demarcate cytoplasm into segments as large as parapodiums on the surface, ×30K.
experiments have recently revealed that rER contacted the plasmalemma and participated in macrophage phagocytosis. Gagnon and colleagues showed that phagosome membranes were derived from rER in addition to plasmalemma during macrophage phagocytosis. The relationship between the plasmalemma and endoplasmic reticulum has also been investigated and identified in various cells in recent decades. These studies reinforce the presumption that rER of BMMCs may be transformed into plasmalemma in human bone marrow (Fig. 9).

4. CONCLUSIONS

BMMCs show a degree of polymorphism dependent on their different environments. Their extensive slender processes appear to have a role in the development of adjacent hematopoietic cells, and the rER of BMMCs may in turn have a role as a membrane resource for the plasmalemma of these processes. Confirmation of the direct interaction between rER and plasmalemma awaits further high-resolution ultrastructural studies in the future.

5. MATERIAL AND METHODS

5.1. Light microscopy

All bone-marrow aspirates were from of 30 patients with hematocytopenia presenting at our hospital. Bone-marrow smears were prepared and surveyed according to our routine diagnostic schedule, but bone-marrow granules were prospectively collected from bone-marrow aspirates and processed for TEM. Semithin sections of bone-marrow granules in Epon 812 blocks were stained by the Wright-Giemsa method and observed by light microscopy.

5.2. BMMCs culture and cytochemical staining

Mononuclear cells were isolated from bone-marrow aspirates by lymphoprep following gradient centrifugation. Part of them was cultured in H4434 methylcellulose semisolid medium, and c-BMMCs were demonstrated on the bottom surface of dishes by Wright staining and the activity of alkaline phosphatase (AKP) was detected by cytochemistry after 14 days. Other mononuclear cells were incubated in MEM media with 10% fetal bovine serum
for a week, and c-BMMCs on the bottom surfaces of dishes were also identified by monoclonal antibodies for CD44 and α-smooth muscle actin (α-SMA) immunocytochemically.

5.3. Electron microscopy

The c-BMMCs, adhering with hematopoietic cells on the bottom surfaces of dishes with MEM media, were processed for SEM. Mononuclear cells and bone-marrow granules isolated from bone-marrow aspirates were processed according to TEM procedures. Briefly, the samples were fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, washed in phosphate-buffered saline, dehydrated in graded alcohols and embedded in Epon 812. Ultrathin sections at 60nm were stained with uranyl acetate and lead citrate. The b-BMMCs and i-BMMCs were observed by TEM.

ACKNOWLEDGMENTS

We would like to thank Dr Brian Eyden (Christie NHS Foundation Trust, UK) for English language assistance.

REFERENCES

[1] McCuskey RS, McClugage SG Jr, Younker WJ. Microscopy of living bone marrow in situ. Blood 1971;38(1):87–95. doi: 10.1182/blood-1971-08-731927.
[2] Umezawa A, Maruyama T, Segawa K, Shadduck RK, Waheed A, Hata J. Multipotent marrow stromal cell line is able to induce hematopoiesis in vivo. J Cell Physiol 1992;151(1):197–205. doi: 10.1002/jpc.1041510125.
[3] Lemischka IR. Microenvironmental regulation of hematopoietic stem cells. Cell 1994;77(7):891–894. doi: 10.1016/0092-8674(94)90115-5.
[4] Dexter TM, Sponsor E. Growth and differentiation in the hematopoietic system. Annu Rev Cell Biol 1987;3:432–441. doi: 10.1146/annurev.cb.03.110187.002331.
[5] Majumdar MK, Thede MA, Haynesworth SE, Bruder SP, Gerson SL. Human marrow-derived mesenchymal stem cells (MSCs) express hematopoietic cytokines and support long-term hematopoiesis when differentiated toward stromal and osteogenic lineages. J Hematother Stem Cell Res 2000;9(6):841–848. doi: 10.1089/152581600750062264.
[6] Chitteti BR, Cheng YH, Potetz B, et al. Impact of interactions of cellular components of the bone marrow microenvironment on hematopoietic stem and progenitor cell function. Blood 2010;115(16):3239–3248. doi: 10.1182/blood-2009-09-246173.
[7] Naji A, Etoku M, Favier R, Deschaseaux F, Rouas-Freiss N, Suganuma N. Biological functions of mesenchymal stem cells and clinical implications. Cell Mol Life Sci 2019;76(17):3323–3348. doi: 10.1007/s00018-019-03125-1.
[8] Taichman RS. Blood and bone: two tissues whose fates are intertwined to create the hematopoietic stem-cell niche. Blood 2005;105(7):2631–2639. doi: 10.1182/blood-2004-06-2480.
[9] Domon T, Yamazaki Y, Fukui A, et al. Ultrastructural study of cell-cell interaction between osteoclasts and osteoblast/stroma cells in vitro. Ann Anat 2002;184(3):221–227. doi: 10.1016/S0940-9602(02)80107-8.
[10] Ru YX, Dong SX, Zhao SX, et al. One cell one niche: hematopoietic microenvironments constructed by bone marrow stromal cells with fibroblastic and histiocytic features. Ultrastruct Pathol 2019;43(2–3):117–125. doi: 10.1080/01913123.2019.1620394.
[11] Jiang HY, Shen M, Feng DY. An improved deresining method for semithin sections embedded epoxy resin. Human Yi Ke Da Xue Xue Bao 2000;25(1):85–86.
[12] Feng M, Xia Y, Cui W, Lin Y, Yang Q, Ru Y. Proliferation and differentiation characteristics of bone marrow mesenchymal stem cells in patients with hematological diseases. Tianjin Medical Journal (in Chinese) 2009;37(3):167–169.
[13] Ru YX, Zhao SX, Dong SX, Yang YQ, Eyden B. On the maturation of megakaryocytes: a review with original observations on human in vivo megakaryocyte morphometry and ultrastructure. Ultrastruct Pathol 2015;39(2):79–87. doi: 10.3109/01913123.2014.980482.
[14] Ru YX, Dong SX, Liang HY, Zhao SX. Platelet production of megakaryocyte: a review with original observations on human in vivo cells and bone marrow. Ultrastruct Pathol 2016;40(4):163–170. doi: 10.3109/01913123.2016.1170744.
[15] Kiel MJ, Yilmaz OH, Iwashita T, Yilmaz OH, Terhorst C, Morrison SJ. SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. Cell 2005;121:1109–1121. doi: 10.1016/j.cell.2005.05.026.
[16] Sugiyama T, Kohara H, Noda M, Nagasewa T. Maintenance of the hematopoietic stem cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches. Immunity 2006;25(6):977–988. doi: 10.1016/j.immuni.2006.10.016.
[17] Anthony BA, Link DC. Regulation of hematopoietic stem cells by bone marrow stromal cells. Cell Mol Life Sci 2014;71(3):32–37. doi: 10.1007/s00018-013-0748-4.
[18] Castro-Malaspina H, Gay RE, Resnick G, et al. Characterization of human bone marrow fibroblast colony-forming cells (CFU-F) and their progeny. Blood 1980;56(2):289–301.

Figure 9. Diagrammatic representation of BMMC transformation associated with rER. (1) Isolated BMMCs without processes contain prominent rER, Golgi apparatus and mitochondria. (2) BMMCs send out short processes owing to the pressing together of undeveloped hematopoietic cells. (3) With hematopoietic cells maturation and proliferation, BMMCs extrude more bifurcate processes around varied hematopoietic cells in different developmental stages. (4) Model of rER transformation into plasmalemma on bifurcate processes of BMMCs.
[19] Tareste D, Shen J, Melia TJ, Rothman JE. SNAREpin/Munc18 promotes adhesion and fusion of large vesicles to giant membranes. *Proc Natl Acad Sci U S A* 2008;105(7):2380–2385. doi: 10.1073/pnas.0712125105.

[20] Emr S, Glick BS, Linstedt AD, et al. Journeys through the Golgi—taking stock in a new era. *J Cell Biol* 2009;187(4):449–453. doi: 10.1083/jcb.200909011.

[21] Cancino J, Capalbo A, Di Campli A, et al. Control systems of membrane transport at the interface between the endoplasmic reticulum and the Golgi. *Dev Cell* 2014;30(3):280–294. doi: 10.1016/j.devcel.2014.06.018.

[22] Brandizzi F, Barlowe C. Organization of the ER-Golgi interface for membrane traffic control. *Nat Rev Mol Cell Biol* 2013;14(6):382–392. doi: 10.1038/nrm3588.

[23] Vieira OV, Botelho RJ, Rameh L, et al. Distinct roles of class I and class III phosphoinositidase 3-kinases in phagosome formation and maturation. *J Cell Biol* 2001;155(1):19–25. doi: 10.1083/jcb.200107069.

[24] Hirschberg K, Miller CM, Ellenberg J, et al. Kinetic analysis of secretory protein traffic and characterization of Golgi to plasma membrane transport intermediates in living cells. *J Cell Biol* 1998;143(6):1485–1503. doi: 10.1083/jcb.143.6.1485.

[25] Szymanski WG, Zauber H, Erban A, Gorka M, Wu XN, Schulze WX. Cytoskeletal components define protein location to membrane microdomains. *Mol Cell Proteomics* 2015;14(9):2493–2509. doi: 10.1074/mcp.M114.046904.

[26] Head BP, Patel HH, Roth DM, et al. Microtubules and actin microfilaments regulate lipid rafts/caveolae localization of adenylylic cyclase signaling components. *J Biol Chem* 2006;281(36):26391–26399. doi: 10.1074/jbc.M602577200.

[27] West M, Zarek N, Hoenger A, Voeltz GK. A 3D analysis of yeast ER structure reveals how ER domains are organized by membrane curvature. *J Cell Biol* 2011;193(2):333–346. doi: 10.1083/jcb.201011039.

[28] Friedman JR, Voeltz GK. The ER in 3D: a multifunctional dynamic membrane network. *Trends Cell Biol* 2011;21(12):709–717. doi: 10.1016/j.tcb.2011.07.004.

[29] English AR, Voeltz GK. Endoplasmic reticulum structure and interconnections with other organelles. *Cold Spring Harb Perspect Biol* 2013;5(4):a013227. doi: 10.1101/cshperspect.a013227.

[30] Voeltz GK, Rolls MM, Rapoport TA. Structural organization of the endoplasmic reticulum. *EMBO Rep* 2002;3(10):944–950. doi: 10.1093/embo-reports/kvi202.

[31] Desjardins M. ER-mediated phagocytosis: a new membrane for new functions. *Cell* 2002;110(1):119–131. doi: 10.1016/s0092-8674(02)00797-3.

[32] Wu H, Carvalho P, Voeltz GK. Here, there, and everywhere: the importance of ER membrane contact sites. *Science* 2018;361(6401): eaan5835. doi: 10.1126/science.aan5835.

[33] Guido D, Demaurex N, Nunes P. Junction boosts phagocytosis by recruiting endoplasmic reticulum Ca2+ stores near phagosomes. *J Cell Sci* 2013;126(22):4074–4082. doi: 10.1242/jcs.172310.

[34] Nunes-Hasler P, Demaurex N. The ER phagosome connection in the era of membrane contact sites. *Biochim Biophys Acta Mol Cell Res* 2017;1864(9):1513–1524. doi: 10.1016/j.bbamacr.2017.04.007.