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Concise Review: Multipotent Mesenchymal Stromal Cells in Blood

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ABSTRACT

Peripheral blood-derived multipotent mesenchymal stromal cells circulate in low number. They share, most although not all, of the surface markers with bone marrow-derived multipotent mesenchymal stromal cells, possess diverse and complicated gene expression characteristics, and are capable of differentiating along and even beyond mesenchymal lineages. Although their origin and physio-pathological function are still unclear, their presence in the adult peripheral blood might relate to some interesting but controversial subjects in the field of adult stem cell biology, such as systemic migration of bone marrow-derived multipotent mesenchymal stromal cells and the existence of common hematopoietic-mesenchymal precursors. In this review, current studies/knowledge about peripheral blood-derived multipotent mesenchymal stromal cells is summarized, and the above-mentioned topics are discussed. STEM CELLS *2007;25:69–77*

INTRODUCTION

Peripheral blood-derived multipotent mesenchymal stromal cells (PBMSCs) are cells isolated from adult peripheral blood (PB) by primary culture as discrete plastic-adherent colonies consisting of fibroblast-like cells, that is, colony-forming units fibroblastic (CFU-Fs), which are similar to the CFU-Fs formed in the bone marrow (BM) stromal cell culture. Bone marrow-borne CFU-Fs (BM CFU-Fs), or so-called bone marrow-derived multipotent mesenchymal stromal cells (BMMSCs) have been extensively studied in terms of their phenotype, plasticity, and transplantation since being originally identified in the guinea pig by Friedenstein et al. in 1970 [1]. The cell populations of PBMSCs were named after the BMMSCs, as many reports indicated that the two cell populations share some common characteristics. This review aims to offer a complete framework and summary of the current studies/knowledge about PBMSCs and their relationship with BMMSCs. Thus, it is helpful to give a brief summary of the current knowledge on BMMSCs before proceeding with the review of PBMSCs.

Brief Summary of Research on BMMSCs

BMMSCs reside in the nonhematopoietic components in the postnatal bone marrow and provide a suitable hematopoietic microenvironment for the hematopoietic cell population's proliferation and differentiation [2, 3]. In vitro, they are "adherent, clonogenic, nonphagocytic, and fibroblastic in habit (defined as colony-forming units-fibroblastic; CFUFs)" [4, 5]. The BM CFU-Fs are heterogeneous populations with varying colony sizes, growth rates, immunophenotypes, and differentiation abilities [5, 6]. Based on extensive studies at the level of the unfractionated cell population, immunoselected cell population, or single cell, it has been widely accepted that the BM CFU-Fs are able to differentiate under proper experimental conditions into bone, cartilage, adipocyte, fibrous tissues, and hematopoietic supporting tissues in vitro and in vivo [6–21]. Evidence also showed that BM CFU-Fs could undergo unorthodox differentiation, giving rise to cells with visceral mesoderm, neuroectoderm, and endoderm characteristics when induced [6, 22–25], could engraft in bone, muscle, brain, lung, heart, liver, gastrointestinal tract, and hematopoietic system when transplanted [20, 23, 26, 27], and could even contribute to most somatic cell types when injected into an early blastocyst [23]. Therefore, adult stem cells (generally defined as clonogenic cells capable of both self-renewal and multilineage differentiation [28, 29]) were presumed to reside in the BM CFU-Fs and thus the terms: marrow mesenchymal stem cells [30], mesenchymal stem cells [31], and multipotent adult progenitor cells [23] were proposed. However, a trend was noticed that the term mesenchymal stem cells was gradually improperly being adopted in some literature without applying the stringent criteria for stem cells. Consequently, a position statement [32] proposed to use "multipotent mesenchymal stromal cells" to designate the plastic-adherent cells isolated from bone marrow or other tissues with multipotent differentiation capacity. To date, specific markers are still lacking for isolating the BMMSC subset with predictably broad or restricted potential [5]; nevertheless, surface markers such as Strol-1+ [33], SH2+ (CD105+), CD34−, CD45, CD14−, and so on [17] were used individually or in combination as general markers to define or purify BMMSCs. In addition to the multipotent differentiation capacity, BMMSCs were also discovered to be immunologically immature. They do not appear to elicit alloreactive lymphocyte proliferative responses and may modulate immune responses [34] by suppressing the proliferation of T lymphocytes [35], thus they are able to survive in a xenoge-

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Table 1. CFE of peripheral blood-derived CFU-Fs in various species

Species	Reference	CFE (no. of colonies/10^6 mononuclear cells)	No. of donors with CFU-F colony forming/total no. of donors
Guinea pig	[52]	2–31	14/14
	[53]	1.1–3.9	5/5
Mouse	[57]	0.3 ± 0.2/0.2 ml of blood	Not provided
	[58]	1.1–13.6	Not provided
	[53]	0–3.8	Not provided
Rabbit	[47]	Not provided	7/11
	[53]	0–0.58	Not provided
	[54]	1.2–13	Not provided
GFP Rat	Data in our lab	0.00–0.66	13/30
Human	[60]	Not provided	11/14 (mobilized blood of breast cancer patients) 0/3 (healthy donors) 1/1 (non-breast cancer patient)
	[53]	0–0.025	2 clones in 10 (healthy donors)
	[63]	0.27 ± 0.22	Not provided
	[66]	Not provided	8/11 Samples (fibrin microbeads-based method and mobilized blood of normal donors)
	Data in our lab	0.0208–0.0286 0	5/9 (Non-union patients) 0/6 (Healthy donors)

Abbreviations: CFE, colony-forming efficiency; CFU-Fs, colony-forming units-fibroblastic; GFP, green fluorescent protein.

neic environment [20, 36]. As BMMSCs are easy to isolate and expand, they have aroused great interest as attractive candidates for cell therapy or cellular vehicles in molecular therapy to deliver genes [37–39]. The study of BMMSCs has been extended from bench to bedside with many clinical applications such as improving hematopoietic engraftment [40] and correcting genetic disorders [41].

Detection of CFU-Fs in Peripheral Blood

The detection of peripheral blood-borne CFU-Fs (PB CFU-Fs) was actually earlier than the detection of BM CFU-Fs. The observations [42, 43] made in the early 20th century on the transformation of blood leukocytes into fibroblast-like cells and further into connective tissues were probably the earliest indications for the existence of PB CFU-Fs. The existence of PB CFU-Fs with clonogenesis feature and capacity for prolonged passaging was later confirmed by many other investigators [44–48]. But it was suspected that these blood-borne fibroblast-like cells might be a result of connective tissue fragment contamination of blood when obtained by the cardiac route [49–51]. The suspicion of the contamination with endothelial or connective tissue cells during blood collection was soon eliminated by independent experiments conducted in adult male rabbits [47] and adult guinea pig [52], respectively, by comparing the number of fibroblastic colonies in the blood collected by multiple punctures versus fewer or single puncture(s). The investigators found no change in the number of the fibroblastic colonies regardless the number of heart punctures applied.

To date, PB CFU-Fs have been detected in the adult peripheral blood of a variety of mammalian species including guinea pig [43, 52, 53], rabbit [43, 45, 47, 53, 54], dog [55, 56], mouse [53, 57, 58], rat [59 and unpublished data in our laboratory], and human [46, 53, 60–66]. It was also reported that fibroblastic colonies developed in the macrophage cultures obtained from chicken cardiac blood, although the authors explained it as connective tissue fragment contamination [50], which has already been refuted [52]. The observations recorded in those old publications [42, 43] also implied that PB CFU-Fs also exist in Rhesus monkey blood; yet, no conclusive data were documented.

Colony-Forming Efficiency of PB CFU-Fs

PB CFU-Fs are extremely low in frequency. The frequency of CFU-Fs is indicated by CFE, colony-forming efficiency [1] defined as the ratio of the number of colonies to the number of cells seeded. Many investigators have calculated the CFE of CFU-Fs in primary culture. Accordingly, CFE was the ratio between the colony number and the number of seeded mononuclear cells (MNCs) in cultures. Table 1 shows clearly that the CFE of PB CFU-Fs varies widely both among and within species. Compared with BM CFU-Fs [11, 67], PB CFU-Fs have a much lower CFE. For example, human bone marrow generally yields colony numbers in the range one per 5×10^3 to 1×10^4 MNCs [33, 68–70], whereas the yield of PB CFU-Fs is usually poor, and it is quite common to fail in detecting any of them [71–73]. Moreover, maintaining PB CFU-Fs seems to be difficult also [74].

It is a putative concept that each fibroblastic colony is derived from a single cell present in the primary culture [75]. The proliferation of these freshly-isolated single cells requires growth factors produced by the accompanying hematopoietic cells in the cultures [76]. One study [77] showed that the initial proliferation of a BM CFU-F was complex, usually requiring participation of at least four growth factors, platelet-derived growth factor, basic fibroblast growth factor, transforming growth factor-β, and epidermal growth factor, and the requirement for each of the growth factors differed among species. In light of these findings, it can be conjectured that the low frequency of PB CFU-F colony may be due to insufficient or unfavorable growth factors in primary culture, as PB-derived MNCs (PBMNCs) may differ from the BM-derived MNCs (BMMNCs), or the PB CFU-Fs have different growth factor requirements from BM CFU-Fs. On the other hand, given that the CFE of CFU-Fs seemed to increase in the blood of murines with phenylhydrazine-induced hemolytic anemia [58], in the growth factor-mobilized peripheral blood of breast cancer patients [60], and in the blood of nonunion patients (unpublished data in our laboratory), the PB CFU-Fs may be low per se but may be changeable as responsive cells to systemic or local hormones, cytokines, and growth factors in certain pathological conditions. However, there was no convincing evidence for the association between PB CFU-Fs and leukapheresis technique or solid tumors as there were conflicting reports on success [60–62, 66] and failure [71–73] in detecting CFU-Fs in PB under those conditions. It was reported that the mobilized peripheral blood of healthy donors gave rise to CFU-Fs formation after consecutive CD6 depletion [61] or by using the fibrin microbead-based method [66] or contains more CFU-Fs after

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Table 2. Histochemical characteristics of peripheral blood-derived colony-forming units-fibroblastic

Marker	Human	Mouse	Rabbit	Guinea pig	Dog	Rat
Hydroxyproline	–	–	Pos ([47])	–	–	–
Alkaline phosphatase	Neg ([53, 60])	Pos ([58])	Neg ([53])	Neg ([53])	–	–
Acid phosphatase	Pos ([53, 60])	lPos ([58])	Pos ([53])	Pos ([53])	–	–
α-Naphthyl-acetate esterase	Pos ([53])	–	Neg ([53])	–	–	–
α-Naphthyl-butyrate esterase	Pos ([53])	–	–	–	–	–
PAS	Pos ([60])	–	–	–	–	–
Sudan Black	Pos ([60])	–	–	–	–	–

— indicates not tested.
Abbreviations: lPos, low expression, limited expression, dull expression, or indicates −/+ staining in literature; Neg, negative expression by ICC and/or flow cytometry; PAS, periodic acid Schiff; Pos, positive expression by histochemical staining or by ICC and/or flow cytometry.

CD133 selection [62], suggesting that immunoselection may enrich PB CFU-Fs.

Histochemical Characteristics and Immunophenotype of PB CFU-Fs

PB CFU-Fs shared many phenotypic characteristics with BM CFU-Fs and showed an immunophenotypic profile that was similar overall between species (Tables 2 and 3). Human PB CFU-Fs synthesized a series of collagens and other extracellular matrix molecules [53, 60]. They lacked or possessed low levels of the hematopoietic progenitor marker CD34 and lacked the macrophage marker CD14 and the leukocyte common antigen CD45 [53, 60–63]. It was also shown that human PB CFU-Fs had low-level CD117 (c-kit) [61] and did not express human leukocyte antigen (HLA)-DR [61, 62], VIII-factor-associated antigen [53], CD31 [63], and neurofilament [53], but exhibited a range of mesenchymal lineage phenotypes [53, 60–63]. Human PB CFU-Fs expressed CD106 (vascular cell adhesion molecule-1) and intercellular adhesion molecule-1 [53, 60], which are adhesive molecules "used by stromal cells to interact with marrow hematopoietic progenitors" [78, 79]. However, the human PB CFU-Fs were negative for Stro-1 [53], which is a human marrow stromal marker widely used for purifying the BMMSCs [33, 80]. Human PB CFU-Fs did not express Muc-18(53) as BM CFU-Fs did [81]. And there were also inconsistent findings for endoglin (CD105) expression [53, 60, 62, 63]. More interestingly, PB CFU-Fs could be enriched in CD133+ cell populations [62], which has been used as a strategy to isolate cells with hematopoietic, endothelial potential, or hemangioblasts [82].

Gene Expression of PB CFU-Fs

A gene expression survey [61, 83] carried out by using reverse transcriptase polymerase chain reaction (RT-PCR) method on the human CD34−/lowCD105+-adherent cell line V54/2 and murine CD34− adherent cell line RM26 cloned from PBMNCs showed some interesting results at the transcription level. It has been found that the human CD34−/lowCD105+ cell population transcribed the following factors: Myb, Tie-1, and vascular endothelial growth factor (VEGF). Its subpopulation, a minority Rh123lowCD34+ fraction transcribed significantly higher levels of GATA-1, GATA-2, GATA-3, GATA-6, ζ-globin, and PECAM1 compared with the majority Rh123highCD34− fraction. Moreover, the purified Rh123highCD34− cells could give rise to the small population of Rh123lowCD34+ cells during the culture [61]. The murine CD34− adherent cell line RM26 cloned from PBMNCs expressed mesodermal markers: HPRT, BMP RIA, BMP-4, Smad1; erythroid/myeloid genes: Nglobin, Oglobin, Lysozyme, Epo R; transcription factors: Scl, GATA-2, GATA-3, GATA-5, GATA-6, EKLF, c-myb, LMO2, Ets1, Ets2, Fli1, Elf-1, Tel; endothelial markers: VEGF-R, CD31, Tie-2, thrombomodulin, vascular endothelial-cadherin, von Willebrand factor, VEGF; and HSC markers: CD34 and c-kit [83]. The GATA transcription factor family either regulate proliferation and differentiation of multiple hematopoietic cell types or activate cardiac muscle structural genes. The *Scl* gene contributes to the development of hematopoietic lineages by encoding a basic helix-loop-helix transcription factor [84]. The expression of both mesodermal markers and erythroid/myeloid transcription factors in the murine CD34− adherent cell line, along with the conversion from Rh123highCD34− cells to Rh123lowCD34+ within the human CD34−/lowCD105+ adherent cell line may imply an even more primitive cell population. This was supported by the detection of Octomer-binding transcription factor four (Oct4) expression in the CFU-Fs from the mobilized human PB-derived CD133-positive cell fraction [62]. Oct4 is an important binding transcription factor present in pluripotent embryonic stem cells [85]. It was reported that Oct4 was detected at a lower level in multipotent adult progenitor cells (MAPCs), a subpopulation of BM CFU-Fs [86]. The numerous and complicated phenotypic and gene expression characteristics make the PB CFU-Fs more elusive. According to such a bewildering phenotypic and gene expression profile, we naturally wonder: do they have a broad spectrum of plasticity?

Multidifferentiation Potential of PB CFU-Fs

There is accumulating evidence from both in vivo and in vitro experiments suggesting that the PB CFU-Fs possess mesenchymal lineage differentiation capability. A single clone strain cloned from canine species indicated that immortalized PB CFU-F cells gave rise to "bone-lining cells" expressing osteocalcin after autologous i.v. transplantation [56]. PB CFU-Fs from human, guinea pig, mouse, rabbit, and rat proved to be able to develop into osteoblasts, reticular cells, lipocytes, chondrocytes, myotubes, and fibroblasts [53, 59, 62]. There are also other studies showed that the PB CFU-Fs may contain other precursor cells or more immature cells capable of turning into neuronal/glial cells [61, 62], generating hematopoietic progenies in lethally irradiated animals [87], forming extensive networks in vitro when growing on BD Matrigel (BD Biosciences, San Diego, http://www.bdbiosciences.com) [83], significantly improving the collateral blood flow (arteriogenesis) and neoangiogenesis formation in a murine hind limb ischemia transplant model after i.v. infusion and integrating into the skeletal muscle in the affected limb [83]. A recent study indicated that the differentiation direction of both PB CFU-Fs and BM CFU-Fs to osteogenesis, angiogenesis, and neovessel formation was milieu-dependent and could be adjusted by modification of favorable conditions [64]. In the studies on BM CFU-Fs, it has been revealed that only a small proportion of single colonies could form bone marrow organs when grafted heterotopically [2]. Analogously, current data of PB CFU-Fs suggest a heterogeneous cell population probably composed of different types of progenitor/precursor cells or cells at different differentiation stages. It is unknown for how many passages the multidifferentiation ability of these cells could be preserved. Although it is

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Table 3. Immunophenotype of peripheral blood-derived colony-forming units-fibroblastic

Marker type	Marker	Human	Mouse	Rabbit	Guinea pig	Dog	Rat
Hematopoietic progenitors	CD34	Neg ([53, 60, 62, 63, 66])/ lPos ([61])	Neg ([87])/ lPos ([83])[a]	–	–	lPos	–
	HLA-DR	Neg ([61, 62])	–	–	–	–	–
	CD117 (c-Kit)	lPos ([61])	Pos ([87])	–	–	–	–
	Sca-1	–	Pos ([87])	–	–	–	–
	CD133	Pos ([62])	–	–	–	–	–
Monocyte/macrophage	CD14	Neg ([53, 60–63])	–	–	–	–	Neg ([53])
	Mac	–	Neg ([58])	–	Neg	–	–
	RAM11	–	–	Neg ([53])	–	–	–
	MR-1	–	–	–	Neg ([53])	–	–
Leukocyte	CD45	Neg ([53, 60–63, 66])	–	Neg ([53])	Neg ([53])	–	Neg[b]
	CD6	Neg ([61])	–	–	–	–	–
	CD10	Pos ([61])	–	–	–	–	–
Endothelial	Flt-1(VEGF-R1)	–	lPos ([83])[a]	–	–	–	lPos[b]
	Flk-1(VEGF-R2)	–	–	–	–	–	Neg[b]
	CD31(PECAM)	Neg ([63])	lPos ([83])[a]	–	–	–	Neg[b]
	Muc-18	Neg ([53])	–	–	–	–	–
	VIII-Factor-associated antigen	Neg ([53])	Neg ([58])	–	Neg	–	–
	PAL-E	–	–	Neg ([53])	–	–	–
	EN4	–	–	–	Neg ([53])	–	–
Mesenchymal stromal	Strol-1	Neg ([53])	–	–	–	–	–
	Endoglin(CD105)SH2	Neg ([53])/Pos ([60–63, 66])	–	–	–	–	–
	SH3	Pos ([60, 62])	–	–	–	–	–
	Thy-1 (CD90)	Pos ([66])	lPos ([87])	–	–	–	Pos[b]
	CD54	Pos ([63])	–	–	–	–	–
	CD166	Pos	–	–	–	–	–
	CD106(VCAM-1)	Pos ([53, 60])	–	–	–	–	Pos
	ICAM-1	Pos ([60])	–	–	–	–	–
	Vimentin	Pos ([66])	–	–	–	–	Pos[b]
Osteogenic	Osteonectin	Pos ([53])	–	Pos ([53])	Pos ([53])	–	–
	Osteopontin	Pos ([53])	–	Pos ([53])	Pos ([53])	–	–
	Bone sialoprotein	Neg ([53])	–	Pos ([53])	Pos ([53])	–	–
	Osteocalcin	lPos ([53])	–	–	–	Pos	Pos ([59])[b]
Adipogenic	CEBPα	lPos ([53])	–	–	–	–	–
	PPARγ2	Neg ([53])	–	Neg ([53])	–	–	–
Smooth muscle	α-Smooth muscle actin	Pos ([53])	–	Pos ([53])	Pos ([53])	–	–
	Smooth muscle	–	–	lPos ([53])	Pos ([53])	–	–
Skeletal muscle	MyoD	Neg ([53])	–	–	–	–	–
Fibroblastic	Type I collagen	Pos ([53, 60])	Pos ([58])	Pos ([53])	Pos ([53])	–	Pos ([59])[b]
	Type II collagen	–	–	–	–	–	Neg[b]
	Type III collagen	Pos ([53, 60])	–	lPos ([53])	Pos ([53])	–	–
	Type VI collagen	Pos ([60])	–	–	–	–	–
	Fibronectin	Pos ([60, 66])	–	Pos ([53])	Pos ([53])	–	–
	Fibrin	Pos ([66])	–	–	–	–	–
	CD44	Pos ([53, 62, 63])	–	–	–	–	Pos[b]
	β1 integrin subunit	Pos ([53])	–	–	–	–	–
Nerve	Neurofilament	Neg ([53])	–	–	–	–	–

— indicates not tested.
[a] Without serum.
[b] Data in our lab; single-colony strain.
Abbreviations: lPos, low expression, limited expression, dull expression, or indicates −/+ staining in literatures; Neg, negative expression by ICC and/or flow cytometry; Pos, positive expression by histochemical staining or by ICC and/or flow cytometry.

possible that there are circulating multipotent adult progenitor cells or pluripotent stem cells residing in the circulation by analogy with the findings in bone marrow [23], stringent evidence is still lacking. The multipotent differentiation ability of the PB CFU-Fs makes them a potential candidate for application in cell therapy and tissue engineering. The rabbit PB CFU-Fs combined with porous calcium phosphate resorbable substitutes have been demonstrated to enhance bone regeneration in the rabbit ulna critical-sized bone-defect model, suggesting allogeneic PB CFU-Fs may be a new source of circulating osteogenic cells for bone regeneration [54]. As the blood is more accessible than bone marrow, the advantages of using peripheral blood as a potential source of CFU-Fs are obvious. However the CFE of CFU-Fs is significantly lower in the PB than that of the BM, which is a major obstacle for their future characterization and clinical application.

Terminology

Until now, we deliberately used the term "PB CFU-Fs" instead of the term "PBMSCs". The purpose for doing so is to avoid potential confusion and misunderstanding as well as to make the delivery of information easier, because the terminology for this cell population has not yet been standardized. Since their discovery, dozens of names have been given to this cell population

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or its subpopulation: fibrocytic cells [46], fibrocyte, fibrocytic colony [47], fibroblast, fibroblast colony, precursors for fibroblast colonies [52], fibroblast-like colonies, stellate colonies [57], fibroblastoid stromal cells [58], stromal cells [60], CD34−/low hematopoietic stem cell clones with mesenchymal stem cell characteristics [56], circulating skeletal stem cells [53], CD34−CD105+ mesenchymal cell lines [61], CD34-negative fibroblast-like cell lines, CD34-negative CD105-positive cell line [64], and mesenchymal stem cells [54, 59, 62, 63, 65]. Notwithstanding the multifarious names, the PB CFU-Fs is commonly adopted. However, "CFU-Fs" only emphasizes the morphological feature of the cells in the culture system in vitro. It does not deliver enough functional connotations and could not cover the state of the cells in vivo. A more biologically meaningful term is needed. "Fibrocyte" and "fibroblast" are apparently not appropriate. Fibroblast is defined as a stellate or spindle-shaped cell with cytoplasmic processes present in connective tissue, capable of forming collagen fibers. An inactive fibroblast is sometimes called a fibrocyte. Fibrocyte is also a designation for the leukocyte subpopulation circulating in PB and capable of entering sites of tissue injury rapidly. Circulating fibrocytes were first identified by Bucala et al. in 1994 [88] and were characterized by surface antigens of Type I collagen+/Type III collagen+/vimentin+/CD34+/CD45+/CD13+/D11b+/MHC class II+/CD86+ [88–91]. Although both circulating fibrocytes and PB CFU-Fs are described as "fibroblast-like," "spindle" and "elongated," the former are cells of fusiform [92] with a slightly plump body and two elongated thin ends, whereas the latter are more blanket-like, that is, stretched-out in shape, pale in color, and not having two sharp ends. As these two different cell types may simultaneity exist in the cultures, they need to be carefully discerned. The PB CFU-Fs isolated by using culture conditions similar to those defined for BM CFUFs showed the morphology, phenotype, and differentiation characteristics that are mainly apt to suggest that they are mesenchymal lineage cells. The name "mesenchymal stem cells," may be suitable for a subpopulation of the PB CFU-Fs after their mesenchymal stemness has been stringently proved. However, according to the position statement [32] on the nomenclature for MSC (for both mesenchymal stem cells and multipotent mesenchymal stromal cells), it is not suitable for applying to the PB CFU-Fs as a whole. Thus we adopt the nomenclature in that position statement [32] and use peripheral blood-derived multipotent mesenchymal stromal cells, that is, PBMSCs denoting the cells, which, to our knowledge to date, circulate in low numbers, share, most, although not all, of the surface markers with BMMSCs, are adherent, clonogenic and fibroblast-like, and contain a subpopulation capable of differentiating along and even beyond mesenchymal lineages. Cells termed circulating osteoblast-lineage cells [93] were recently isolated by flow cytometry with antibodies to osteocalcin and bone alkaline phosphatase, which were osteogenic both in vivo and in vitro [93]. This cell population may also fall in the category of PBMSCs. However, the circulating osteoblast-lineage cells were much larger in number than the PB CFU-Fs isolated by the plastic-adherence method.

Kuwana et al. [94] reported another blood-derived cell population termed "monocyte-derived mesenchymal progenitors (MOMP)." Exposure of these MOMP to certain inductive conditions resulted in the expression of genes and proteins specific for osteoblasts, skeletal myoblasts, chondrocytes, and adipocytes. However, the MOMP seemed to be morphologically similar to the circulating fibrocytes (based on images presented by the authors) and shared some surface markers with the latter, such as Type I collagen+/CD34+/CD45+. MOMP also expressed CD14. Interestingly, a cell population, which was very similar to the MOMP both in morphology and in phenotype (CD14+/CD34+/CD45+), was isolated from human peripheral blood by another independent research group, demonstrated to differentiate into mature macrophages, T lymphocytes, epithelial cells, endothelial cells, neuronal cells, and liver cells, and hence was termed "pluripotent stem cells (PSC)" [95]. As it has been documented more than once by independent research groups that PBMSCs are CD14−/CD45−/CD34−/low cell population, the MOMP/PSC and the PBMSCs are likely to be distinct cell types. There was also a paper published in 2000 [96] and commonly cited by other investigators as evidence for circulating mesenchymal stem cells [97]. This paper named a cell population "mesenchymal precursor cells" isolated from the blood of normal individuals. These cells had a phenotypic profile (CD105+/vimentin+/Type I collagen+/CD34−/CD45−) similar to the PBMSCs, yet took a morphology analogous to the circulating fibrocytes. Further investigations into the MOMP, the PSC and the so-called mesenchymal precursor cells are needed before we are able to accurately classify these cell populations. And we need to beware of any confusion and misunderstanding that may be caused by the names tagged with "mesenchymal."

Do PBMSCs Migrate from Bone Marrow?

With the suspected contamination during sample collection being excluded, how the PBMSCs enter blood circulation is still a mystery. A straightforward speculation is that they are migrants from bone marrow or other organs. Accumulating data showed that ex vivo expanded BMMSCs achieved engraftment in various normal and damaged tissues as well as homed to the bone marrow after systemic infusion [98–106]. However, the migration of the infused BMMSCs to extravascular tissues or homing to the bone marrow does not directly support the conjecture that the BMMSCs in situ could spontaneously leave the marrow cavity and enter the bloodstream, or migrate in response to systemic signals towards to tissues in need of repair. There was an observation made of mice in parabiosis that phenylhydrazine-induced hemolytic anemia resulted in a threefold increase in the PBMSCs, and partner-derived PBMSCs could be found in spleens and femoral bone marrow of both mice [58]. Real-time migration pattern of tail vein-injected BMMSCs in response to a tibia fracture revealed that the cells resided in the lungs for 1 day, moved to liver and brain on day 2, migrated to the fracture site by day 4, and remained there [107]. Intravenously infused rat PBMSCs homed to the bone marrow and migrated into the lesions of chronic rejection in the cardiac grafts in heart transplant recipients [59]. These data suggested that the mobility of the multipotent mesenchymal stromal cells (MSCs) between bloodstream and organs. But they still only provide one-directional evidence (bloodstream to bone marrow); when and why the PBMSCs in the phenylhydrazine-induced hemolytic anemia mice increased are still unclear.

The following two experiments may provide some direct evidence for PBMSCs' origin under pathological conditions. In the first experiment, labeled BMMSCs were injected into the femurs of osteogenesis imperfecta mice, and they were later detected in the contralateral femurs, lung, and liver besides the local bone cavity [108]. The other study [109] tested the hypothesis that following a bone fracture there is systemic recruitment of bone-forming cells to a fracture site by using a rabbit ulnar osteotomy model. In this study, labeled BMMSCs were reimplanted into the remote tibial bone marrow cavity 48 h after the osteotomy, and the labeled cells were detected in the callus of the ulnar fracture site after 3 weeks. Inert beads were also used in the experiment to rule out the possibility of passive leakage of the labeled cells into peripheral circulation. We know that hematopoietic stem cells do circulate.

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Whether or not BMMSCs naturally migrate into the circulation is an important question to address the existence/origin of PBMSCs. But this argument will only be meaningful when the view holds up that the marrow stromal cells and hematopoietic cells are of separate origin.

Are There Common Precursors in Adults for Mesenchymal and Hematopoietic Lineage Cells?

Whether there is a common hematopoietic-mesenchymal stem cell in adults has been a long-time debate. Historical views considered the hematopoietic and mesenchymal cells to be two histogenetically independent cell lines. This was grounded on the failure of detecting both recipient BMMSCs in heterotopic bone marrow transplants [110, 111] and donor BMMSCs after systemic bone marrow transplantation [7, 111–114]. These observations were challenged by data from other research groups, which showed that the BMMSCs of donor origin enhanced hematopoietic recovery and engrafted in marrow sinuses after bone marrow transplantation [115–117]. However, the evidence of donor-originated BMMSCs in recipient bone marrow is not necessarily in support of the common hematopoietic-mesenchymal stem cell idea because it could be an outcome of the donor BMMSCs competing with the host BMMSCs, as bone marrow contains both hematopoietic cells and BMMSCs, and they are inseparable.

In recent years, studies on the extensive plasticity of cells from bone marrow emerged continuously. Single bone marrow-derived stem cells had multiorgan, multilineage engraftment including hematopoietic lineage epithelial cells of the liver, lung, gut, and skin on transplantation either into irradiated hosts [118] or into a non-irradiated host [23]. Numerous reports also described bone marrow stromal cells turning into neural cells [119, 120], cardiac and skeletal muscle [121–123], hepatocytes [124–126], epithelia and endothelia in lung [127], and epithelia of the gastrointestinal tract [128]. And similar cross-lineage differentiation was also demonstrated on the peripheral blood-derived hematopoietic stem cells into hepatocytes and epithelia [129]. The versatile behavior of both the bone marrow stromal cells and the circulating hematopoietic stem cells seems to make a common precursor conceivable for hematopoietic and mesenchymal stromal cells. In addition, several CD34− cell populations, such as CD34−/c-Kit+/Sca-1+, CD34−/Lin−/CD38−, and CD34−/Lin− subsets, were identified and demonstrated to differentiate into CD34+ progenitors, initiate multilineage hematopoiesis, and reconstitute the lymphohematopoietic system [130–132], suggesting that the CD34− cell population may contain more primitive cells. Singer et al. described adherent cells from bone marrow contain cells with hematopoietic as well as stroma-like features [133, 134], whereas Dominici et al. reported that plastic nonadherent population from bone marrow can generate both functional osteoblasts/osteocytes and hematopoietic cells [135]. Are we very close to a common hematopoietic-mesenchymal precursor? Some investigators argued that all these versatile stem cells are in fact different subpopulations of tissue-committed stem cells [136].

The following observations directly made on the interleukin (IL)-6-mediated CFU-Fs from both BM and PB held great interest. Huss et al. [26, 56, 137–139] found that round cells developed as clusters from the CD34− adherent fibroblastic cell layer during culture especially when the adherent cells reached 80%–90% confluency. The round cells expressed specific hematopoietic markers such as CD34, HLA-DR, c-kit, myeloid antigen DM5, MHC class II antigens, and so on, in different degrees. Although adherent cells were negative or had very low expression levels for these markers [137]. In addition, the round cell would reattach and proliferate in an adherent fashion if increasing numbers of cells detached [137]. Similar phenomena were also observed by Rogers and Berman [140]. After they treated the hematopoietic cell-eliminated stromal layer of murine cultures with tumor necrosis factor-α, cells showed bursts of hematopoietic activity. In vivo study indicated that IL-6-mediated CFU-Fs differentiated into osteocalcin-positive bone-lining cells [56] besides achieving the hematopoietic reconstruction [87].

Thus the “stem cell cycle” was postulated by Huss [27, 141]. In his stem cell cycle model, CD34− fibroblast-like cells “contain hematopoietic pluripotency and a certain number of those cells circulate in the peripheral blood” [141]; “they can still return to their setting environment within the marrow stroma” [141], becoming the quiescent stem cells; the quiescent stem cells, once activated, could differentiate into both hematopoietic stem cells and mesenchymal stem cells. The quiescent stem cell in this proposal is obviously a common hematopoietic mesenchymal stem cell. Although this is a fascinating paradigm, the idea of a common hematopoietic-mesenchymal stem cell still remains controversial. Neither sufficient mesenchymal characteristic were proved in those IL-6-mediated CFU-Fs nor were abundant hematopoietic characteristics demonstrated in the classic media-developed CFU-Fs. Furthermore, undetected hematopoietic stem cell contamination of mesenchymal stromal cells cannot be ruled out in those results on which the stem cell cycle proposal was based. More experimental data are needed to confirm if there is a common precursor in adult for mesenchymal and hematopoietic lineage cells.

Multipotent Mesenchymal Stromal Cells from Other Sources

Besides the BM and PB, other organs and tissues in adults were also shown to be sources of MSCs, including pleural cavity, spleen, thymus, peritoneal cavity, lymph node, adipose tissue, muscle, brain, and exfoliated deciduous teeth [1, 8, 57, 86, 142–145]. Although Wexler et al. [73] failed to identify MSCs in umbilical cord blood (UCB) from full-term deliveries, the isolation of MSCs from UCB was successful in other laboratories [62, 146–149]. It was found that human first-trimester fetal blood contained more MSCs than blood from the second and third trimesters [147]. Naruse et al. [149] isolated MSCs not only from the UCB but also from the entire circulating blood of fetal rat. It was reported that the embryonal circulating MSCs can be retrovirally transduced with 99% efficiency without selection [148]. A cell therapy using embryonal circulating MSCs has also been demonstrated [150].

MSCs from other sources shared many characteristics with BMMSCs or PBMSCs but still showed some differences from them or between each other in phenotype, proliferation, and differentiation abilities. In addition, the experimental results from different laboratories are far from consistent. For example, adipose tissue-derived MSCs did not express CD106 [151]; Mareschi et al. [152] failed to confirm the adipocytic, osteogenic, and chondrocytic differentiation ability of MSCs from the UCB using similar inductive conditions for BMMSCs.

At the current stage of investigation, neither those additional organs/tissues nor the adult peripheral blood can serve as a reliable source for MSCs. The bone marrow is the richest and most reliable reservoir for MSCs. But the exploration of the existence of MSCs in other sources is important for understanding mesenchymal cell biology. The transit of MSCs in the embryonal circulation and their distribution in other organs and tissues in adulthood may help answer many unsolved questions such as the origin and destination of the PBMSCs and their relationship with BMMSCs.

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Summary

Our knowledge on the PBMSCs is still very limited. It is unclear where they come from and where they go to. They cannot be easily isolated. PBMSCs, like BMMSCs, contain heterogeneous cell populations even after the immunodepletion or immunoselection treatments. Given the findings that PBMSCs possess the ability of multidifferentiation, they might be considered a new cell source for cell-based therapy purposes. However, there are two major obstacles for studying the PBMSCs. Firstly, the number of PBMSCs is very low, especially in an adult human. Poor yield of the PBMSCs may be due to the current methods of cell isolation, purification, and culture conditions, which have not been optimized. Secondly, as few markers for MSCs have been so far ascertained, both immunoselected and unselected PBMSCs showed a wide diversity in their phenotypes, gene expression profiles and biological behaviors. Lack of phenotypic markers makes the identification and study of PBMSCs difficult. The presence of the PBMSCs in the adult peripheral blood, although at low frequency, might relate to many interesting, but controversial subjects in the field of adult stem cell biology, such as systemic migration of BMMSCs, the existence of common hematopoietic-mesenchymal precursors, and so on.

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Disclosures

The authors indicate no potential conflicts of interest.

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