stem cell research&therapy

REVIEW

The multi-differentiation potential of peripheral blood mononuclear cells

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Abstract

Peripheral blood is a large accessible source of adult stem cells for both basic research and clinical applications. Peripheral blood mononuclear cells (PBMCs) have been reported to contain a multitude of distinct multipotent progenitor cell populations and possess the potential to differentiate into blood cells, endothelial cells, hepatocytes, cardiomyogenic cells, smooth muscle cells, osteoblasts, osteoclasts, epithelial cells, neural cells, or myofibroblasts under appropriate conditions. Furthermore, transplantation of these PBMC-derived cells can regenerate tissues and restore function after injury. This mini-review summarizes the multi-differentiation potential of PBMCs reported in the past years, discusses the possible mechanisms for this multi-differentiation potential, and describes recent techniques for efficient PBMC isolation and purification.

Introduction

Stem cells (SCs) were first isolated in the 1960s from the peripheral blood of dogs and used to cure hematopoietic failure following irradiation [1,2]. The preponderance of evidence now suggests that many or most adult tissues contain SCs with the capacity for tissue-specific differentiation. In the decades since SCs were isolated, several distinct progenitor cell populations have been reported in the peripheral blood mononuclear cell (PBMC) fraction, including hematopoietic stem cells (HSCs) [3], endothelial progenitor cells (EPCs) [4], mesenchymal stem cells (MSCs) [5], osteoclast precursor cells [6], hematopoietic osteoclast precursor cells [7], and a population of circulating fibrocytes [8], suggesting that PBMCs may possess the potential to differentiate into a multitude of mature functional cell types in specific microenvironments. Indeed, recent studies have confirmed that PBMCs can differentiate along alternative lineages *in vitro* and *in vivo* depending on culture conditions or the site of transplantation [9-11].

Blood is the most convenient source from which to obtain SCs from patients, and can be frozen and stored for later use. Moreover, numerous frozen samples are already stored in blood banks. Not only do PBMCs contain many distinct progenitor cell types, they can be expanded in culture and reprogrammed to induced pluripotent stem cells (iPSCs) [12-14] with broad clinical applications in regenerative medicine. The known multi-differentiation potential of PBMCs indicates that these cells may be a source of many functional cell types for regenerative therapy.

Evidence for the multi-differentiation potential of PBMCs

Differentiation of PBMCs into blood cells

Tavassoli and colleagues [15] reported that autologously transplanted bone marrow (BM) survived in various extramedullary sites in the rat, rabbit, and dog, and that this ectopic BM completely reconstituted the hematopoietic system. However, a significant impediment to allogeneic BM grafting is the procurement of a sufficient quantity of BM from a single living donor for rapid restoration of hematopoietic function [1]. Recent studies indicate that HSCs with marrow re-population potential are present in the PBMC population. Thus, reconstruction of the hematopoietic system may be possible by harvesting large numbers of HSCs using safe and minimally invasive PBMC fractionation and selection [2,16-18]. Furthermore, these SCs may allow for long-term restoration of hematopoiesis [19]. Indeed, many recent studies [20-22] have demonstrated that PBMCs can both self-propagate and differentiate into mature blood cells *in vitro* and *in vivo*.

Goldberg and colleagues [21] cultured human PBMCs (hPBMCs) in a conditioned medium obtained from adherent PBMCs incubated with 2-mercaptoethanol and obtained colony forming unit-granulocyte-macrophage progenitors. The ^{3}H-thymidine-labeled proliferating cells appeared to be mononuclear cells as determined by autoradiographs. In addition, the granulocytic nature of these

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progenitor cells was also verified by the development of maturing progeny identified as promyelocytes, myelocytes, metamyelocytes, and both band- and polymorphonuclear-form granulocytes containing granules reactive to peroxidase stains and incorporating $Na_2{}^{35}SO_4$. Human PBMCs can also produce other hematopoietic colonies, including colony forming unit-granulocyte-erythroid-monocyte-macrophage-megakaryocyte, colony forming unit-monocyte-macrophage, blast forming unit-erythroid and colony forming unit-erythroid precursors during culture in a defined hematopoietic-conducive condition medium containing Iscove's modified Dulbecco's medium, fetal calf serum (FCS), horse serum, cortisol, and purified CR3/43 [23].

Dogs transplanted with GFP-transfected CD34- PBMCs (0.5×10^8 cells/kg) following 300 cGy total body irradiation exhibited peripheral white blood cell and platelet count recovery [24]. A BM biopsy after transplantation also showed GFP-expressing cells co-expressing osteocalcin, predominantly along the bone spicules ('bone lining cells'). There was also evidence that the engrafted CD34+ PBMC clones contributed long-term hematopoietic engraftment [25]. In another study, rhesus macaques received 500 cGy total body irradiation daily for two days, then were infused with autologous CD34+ PBMCs that had been expanded *in vitro* and infected with a retroviral vector gene (neo). Hematopoietic recovery was monitored by daily complete blood counts for up to one year post-transplantation. Animals infused with transduced CD34+ cells exhibited fastest recovery of T-cell numbers, especially naive T cells, compared to control irradiated animals. In a clinical study by Shadduck and colleagues [26] conducted from 1990 to 1997, more than 100 patients diagnosed with hemopoietic malignant tumors were treated by autologous CD34+ PBMC transplantation (with most patients receiving more than 4×10^6 CD34+ cells/kg). Patients receiving transplants typically showed faster neutrophil recovery (counts greater than 500 by day 10 to 11) and platelet recovery, and required fewer red cell and platelet transfusions than expected for control patients, suggesting that autologous PBMCs are a promising alternative to BM for HSCs for transplantation [27-37].

Differentiation of PBMCs into endothelial cells

Endothelial progenitor cells present in BM, peripheral blood and umbilical cord blood possess the capacity to directionally differentiate into mature endothelial cells (ECs) for revascularization at sites of ischemia, thus recapitulating an important developmental induction pathway for postnatal angiogenesis [38]. Putative EPCs were first isolated from human peripheral blood by magnetic bead selection and it was found that these cells could differentiate into ECs *in vitro* and incorporate into sites of active angiogenesis in animal models of ischemia [4]. There are two different types of EPCs circulating in adult human peripheral blood, early and late colonies. The late colonies appeared to be a better source for angiogenic therapy [39-43].

When hPBMCs were cultured on fibronectin- or gelatin-coated dishes in endothelial medium (EGM-2 or M-199), adherent clusters of cells appeared that formed cord-like or duct-like structures, and individual cells became spindle-shaped, reminiscent of cultured ECs. Most of these cells (85%) were positive for the EC markers CD31, CD34, Flk-1, and von Willebrand factor, expressed EC-typical genes like *Flt-1*, *Flk-1*, *ecNOS* and *tie-2*, and accumulated DiI-Ac-LDL [39,41,42].

Different from the culture mentioned above, Joensuu and colleagues [44] established a novel co-culture system of human BM-derived MSCs and PBMCs growing in monolayer cultures on bovine collagen. Co-culture resulted in the formation of PECAM-1- and endoglin-positive vessel-like structures, expressing vascular endothelial growth factor receptor 1 (VEGFR1). By contrast, no PECAM-1-, endoglin- or VEGFR1-expressing cells were found in MSC cultures without PBMCs [44], indicating that PBMC-derived factors are necessary for EC differentiation. This co-culture system is much more convenient than that using EC medium because no additional growth factors or extracellular matrix proteins are needed for EC differentiation.

Yeh and colleagues [45] transplanted human CD34+ PBMCs (about two and a half million per animal) into the left ventricle of female SCID mice after myocardial infarction (MI). Using human leukocyte antigen (HLA) as a marker for the donor cells, double staining of the blood vessels with anti-HLA and anti-VE-cadherin suggested that human CD34+ cells differentiated into mature ECs within 2 months. Rat PBMC-derived MSCs have similar morphological and antigenic characteristics to BM-derived MSCs [46]. Following intravenous injection of PBMC-derived MSCs harboring LacZ into rats subjected to permanent middle cerebral artery occlusion, abundant LacZ-positive cells were found in and around the ischemic lesion. Furthermore, these rats exhibited hemodynamic changes indicative of cerebral reperfusion as revealed by perfusion-weighted MR imaging and functional recovery as assessed by the treadmill stress test.

Pretreatment with granulocyte colony-stimulating factor can mobilize EPCs from the BM to peripheral blood [47-49]. Mobilized rabbit PBMC-derived MSCs promote active angiogenesis at ischemia sites, reduce the area of ischemic damage and improve myocardial function following carotid artery balloon catheterization [50]. In an open clinical trial, Ishida and colleagues [51] implanted autologous PBMCs mobilized with granulocyte colony-stimulating factor in six patients with severe

peripheral arterial disease and found significant improvements in limb ischemia as evidenced by improved ankle-brachial pressure index and reduced ischemic ulcers. The mean maximum walking distance of these patients also increased from 203 m to 559 m and these improvements were sustained for 24 weeks. In addition, significant improvement was seen in the physiological functioning subscale of the Short Form-36 health survey.

Differentiation of PBMCs into hepatocytes

Human PBMCs have the potential to differentiate into hepatocyte-like cells *in vitro* in the presence of hepatocyte growth factor or fibroblast growth factor (FGF)-4 [52]. During induction culture, cells gradually rounded but did not assume the polygonal shape of mature hepatocytes, which might suggest additional signals (present *in vivo*) are required for full differentiation [52].

However, a subtype of CD34+ SCs derived from hPBMCs may provide a ready source of hepatocyte precursors. These CD34+ SCs can be divided into two populations, adherent and non-adherent to plastic culture plates, with distinct morphologies, phenotypes, and gene expression patterns [53]. Only the adherent CD34+ population had the capacity to differentiate into functional hepatocytes. A clinical study by Korbling and colleagues [54] further demonstrated this point; hPBMCs contain a population of CD34+ SCs that can differentiate into liver cells after transplantation. Six female patients with hematologic cancers or breast cancer received PBMC-derived CD34+ SCs from a male donor, and biopsies were examined for the presence of donor-derived epithelial cells and hepatocytes using fluorescence *in situ* hybridization of interphase nuclei and immunohistochemical staining for cytokeratin, leukocyte common antigen CD45, and a hepatocyte-specific antigen. Host-donor chimeric tissues were detected in the skin, liver, and gastrointestinal tract by restriction-fragment-length polymorphism analysis, and XY-positive epithelial cells or hepatocytes accounted for 0 to 7% of the cells in histological sections of the biopsy as indicated by a DNA probe specific for the centromeres of the X and Y chromosomes. The XY-positive hepatocytes were large, with round nuclei, and contained abundant granular cytoplasm, consistent with functional hepatocytes [54]. The origin of this CD34+ SC subpopulation and the mechanisms guiding differentiation into hepatocytes are unknown. One possibility is that PBMCs contain multiple lineage-restricted SCs that can differentiate independently into functional cells within the corresponding mature tissue [54].

Differentiation of PBMCs into muscle cells

A distinct population of smooth muscle progenitor cells may also be present in the hPBMC population [55]. Growth factors such as transforming growth factor and platelet-derived growth factor BB participate in smooth muscle cell (SMC) differentiation [56-58]. Integrins and other surface adhesion molecules are essential for vascular SMC adhesion, matrix assembly, proliferation, and for homing to specific target sites, especially β1 integrin, the most abundant integrin expressed by proliferating SMCs *in vivo* [59-61]. Human PBMCs grown in platelet-derived growth factor BB-enriched medium differentiated into smooth muscle outgrowth cells that were positive for SMC-specific actin (SMA), myosin heavy chain, and calponin at both the mRNA and protein levels [55,62].

The PBMC population can also differentiate into cells with the characteristics of cardiomyocytes under appropriate conditions. Human PBMCs in 5-azacytidine-containing medium manifest a spindle-like appearance and tend to form colonies of cells expressing myosin, sarcomeric actin, troponin T and desmin [63]. Unmobilized adult hPBMCs can also be induced to differentiate into cardiomyogenic progenitor cells under a defined cardiomyogenic-conducive condition of ES or long-term culture (LTC) medium containing 3.5 μg/ml purified CR3/4 [23]. In 'hanging drop' culture, colonies of these cells resembled beating embryoid bodies capable of synchronous rhythm. Moreover, these cells were able to differentiate into mature cardiomyocytes after implantation into the myocardium of non-irradiated, non-infarcted Rnu/Rnu nude rats [64].

Several *in vivo* studies have also suggested that PBMCs contribute to SMCs and cardiomyocytes after transplantation. Zhang and colleagues [65] found that hPBMCs could participate in the regeneration of the ischemic heart by differentiating into cardiomyocytes, vascular ECs, and SMCs after transplantation into mice and rabbit models of myocardial infarction. Their study also showed that mouse MHC-H2D was not expressed in the human HLA positive cells, suggesting no cell membrane fusion between transplanted cells and host cells. Badorff and colleagues [66] transplanted human PBMC-derived CD34+ cells into the infarcted myocardium of SCID mice. Using human HLA as a marker for the donor cells, double staining for HLA and SMA indicated that human CD34+ cells could differentiate into cells with mature cardiomyocyte morphology. Moreover, these cells integrated into the myocardium of the peri-infarct area and participated in the neovascularization after acute myocardial infarction. The frequency of PBMC-derived CD34+ cell differentiation was extremely low in uninjured hearts, however, indicating that differentiation or homing depends on signals released from injured heart tissue [45].

Differentiation of PBMCs into bone

Zvaifler and colleagues [5] first reported a precursor cell population within the normal human CD34- PBMC

fraction, termed peripheral blood-derived MSCs, that adhered to plastic and glass and proliferated logarithmically in DMEM medium supplemented with FCS. These cells could assume fibroblast-like and stromal morphologies, and this differentiation was not affected by eliminating CD34+, CD3+, or CD14+ cells from the original PBMC population. After the osteogenic supplements dexamethasone, ascorbic acid, and β-glycerophosphate were added to the culture, fibroblast formation was inhibited, and cells assumed the cuboidal shape of osteoblasts (OBs). Elutriated cells displayed the alkaline phosphatase and osteocalcin expression characteristic of OBs. A study by Wan and colleagues [67] showed that rabbit allogeneic MSCs derived from PBMCs and transplanted into porous calcium phosphate resorbable substitutes enhanced bone regeneration in the rabbit ulna 20-mm critical-sized bone defect model, as evidenced by serial radiography, peripheral quantitative computed tomography and histological examination.

Numerous studies have reported that osteoclasts (OCs) can be generated from colony forming unit-granulocyte-macrophage progenitors as well as from the PBMC population [68-77]. Costa-Rodrigues and colleagues [78] reported that the whole PBMC fraction and CD14+ PBMCs, but not CD14- PBMCs, survived in the absence of osteoclastogenic factors and exhibited spontaneous osteoclastogenesis as detected by cell type-specific protein and gene expression, TRAP activity, calcium phosphate resorption, and production of F-actin rings. Unlike CD14+ PBMCs, the total PBMC population was able to express macrophage colony-stimulating factor and RANKL (receptor activator of nuclear factor kappa-B ligand), as well as tumor necrosis factor-a, granulocyte-macrophage colony-stimulating factor, IL-1, IL-6, and IL-17, which are necessary for osteoclastogenesis [8,69]. While CD14- PBMC cultures exhibited limited cell survival and only a few typical OC features, this population may serve to positively modulate osteoclastogenesis [8,78]. In addition, OBs under hypoxic stress can signal the conversion of PBMCs to OCs, and OC formation was correlated with hypoxia inducible factor-1α-dependant upregulation of RANKL expression and VEGF release from OBs, which are critical for bone regeneration [79-85].

Differentiation of PBMCs into epithelial cells

The protein 14-3-3σ has been described as a highly specific marker for epithelial cells that may aid greatly in the study of the differentiation of PBMCs into epithelial cells [86,87]. Medina and colleagues [88] found that a medium containing 49% DMEM, 49% keratinocyte serum-free medium, and 2% FCS supported the differentiation of hPBMCs into epithelial-like cells and their long-term survival (more than 50 days). Human PBMCs started to express 14-3-3σ, keratin-5 and keratin-8 in culture, while dermal fibroblasts expressed matrix metalloproteinase (MMP)-1 after treatment with PBMC-conditioned medium [88].

It was also reported that CD34+ PBMCs accelerated neovascularization and epidermal healing in a model of chronic full-thickness skin wound in diabetic mice [89]. Circulating stem cells derived from CD34+ PBMCs could differentiate into mature epithelial cells of the skin, lung, and gastrointestinal tract following transplantation into sex-mismatched recipients [54]. Biopsy specimens from the skin and gastrointestinal tract of females after transplantation exhibited X and Y chromosome signals in cytokeratin-positive cells as revealed by fluorescence *in situ* hybridization. In epidermal tissue of the skin, donor-derived cells were located in the deep layer of Malpighi (the stratum spinosum of the stratum germinativum), close to the dermal-epidermal junction and the stratum granulosum. Moreover, in the glandular epithelium of the gastric cardia, cells containing the Y chromosome were found in the foveola or tubular pits of the superficial glandular layer, which is composed of mucus-containing cells lining the foveola [54].

Differentiation of PBMCs into neural cells

Several papers have demonstrated neural differentiation from hPBMCs under different *in vitro* microenvironments. Horschitz and colleagues [90] developed two protocols for neural differentiation from hPBMCs. Method A used all-trans retinoic acid, epidermal growth factor (EGF), and basic FGF (bFGF), while method B used EGF, FGF8b, sonic hedgehog, and ascorbic acid. Both culture methods yielded neuronal cells, as evidenced by changes in morphology and the expression of the neuronal markers microtubule-associated protein type 2, tau, and β-tubulin III. The second protocol resulted in predominantly dopaminergic neurons as indicated by the expression of the dopamine transporter. Abuljadayel [23] found neurons producing glutamate, GABA, tyrosine, dopamine, or serotonin as well as neurons accumulating taurine in hPBMC cultures in an ES medium, consisting of DMEM, FCS, L-glutamine, MEM non-essential amino acids, and β-2-mercapto-ethanol. In addition, Liu and colleagues [91] showed that normal hPBMCs could be induced to differentiate into neural-like cells in a defined neural stem cell medium mainly containing DMEM/F12, B27, bFGF, EGF, FCS, L-glutamine, and MEM non-essential amino acids, or when exposed to conditioned medium from rat retinal tissue cultures. Moreover, PBMCs pre-induced in a defined neural stem cell medium migrated into the retina of nude mouse after vitreous cavity transplantation [91].

Several precursor cell populations isolated from PBMCs can also differentiate into either neurons or glia

under appropriate conditions *in vitro* and *in vivo* [92-94]. Kim and colleagues [95] reported that MSCs isolated from PBMCs could be successfully induced to form neurospheres in the presence of EGF and bFGF and to differentiate into neural cells *in vitro*, suggesting a potential source for neural progenitors to treat central nervous system diseases. Kijima and colleagues [96] showed that human CD133+ PBMCs transplanted locally into a rat neural defect could induce vasculogenesis, providing an improved microenvironment for axonal regeneration; indeed, CD133+ cell implantation resulted in nerve reconstruction with abundant Schwann cells and myelinated axons. Moreover, the human *GAPDH* gene was detected in regenerated nerve tissue at eight weeks after transplantation. These studies strongly suggest that PBMCs have significant potential for establishing a nerve-regenerating microenvironment or a regeneration-conducive 'biological bridge'.

Differentiation of PBMCs into myofibroblasts

While studying acute cellular responses in a model of wound repair, Fahey and colleagues [97] observed large numbers of adherent, spindle-shaped cells that resembled fibroblasts and secreted a unique profile of cytokines, growth factors, and chemokines. The appearance of these cells was attributed to recruitment from surrounding subcutaneous tissue and suggests that a cell population arising from the PBMCs may participate in wound healing and connective tissue formation. Subsequent studies have provided direct evidence that myofibroblasts may be derived from PBMCs [98,99].

A novel population of PBMCs with fibroblast properties, termed 'fibrocytes', was characterized by its distinctive CD45+/CD34+/CD14- phenotype [8]. Fibrocytes from human male PBMCs rapidly and specifically entered sites of tissue injury in female mice after transplantation [8]. Mori and colleagues [100] also reported a CD45+/CD34+/CD14- fibrocyte population in the PBMC fraction and demonstrated invasion of male PBMC-derived fibrocytes into wounds after transplantation in female mice previously exposed to total body irradiation. These results strongly suggest that circulating fibrocytes contribute to the myofibroblast population in the wound as well as to the marked increase in the number of cells expressing α-SMA in the granulation tissue [94]. Then Hu and colleagues [7,101] subsequently reported a CD14- cell population from PBMCs with both mesenchymal and hematopoietic features, designated peripheral blood multipotential mesenchymal progenitors. After transplant into a skin wound model, these progenitors differentiated into fibroblasts and participated in both extracellular matrix secretion and epidermal regeneration. Different from the work described above, Abe and colleagues [102] reported a CD14+ PBMC population with the capacity to differentiate into a-SMA1-positive, transforming growth factor-b1-responsive fibrocytes with characteristics similar to wound-healing myofibroblasts, including transient presence within the wound and production of numerous inflammatory cytokines and growth factors.

Transformation of PBMCs into iPSCs

Takahashi and colleagues [103] were the first to successfully reprogram adult human fibroblasts into a pluripotent state. Subsequent studies were able to obtain iPSCs from primary fetal tissues (lung, skin), neonatal fibroblasts, adult fibroblasts, and MSCs by constitutively expressing four transcription factors, Oct3/4, Sox2, Klf4, and c-Myc [104-106].

Loh and colleagues [13] first reported the derivation of iPSCs from multiple human PBMCs harvested by routine venipuncture. The resultant human iPSCs were similar to human embryonic stem cells in morphology, proliferation, surface antigens, gene expression patterns, epigenetic status of pluripotent cell-specific genes, and telomerase activity. Furthermore, like embryonic stem cells, iPSCs can differentiate into cell types from all three germ layers *in vitro* and in teratomas. The ectopic expression of Oct4, Sox2, Klf4, and c-Myc in GMP (clinical)-grade PBMCs and hematopoietic progenitor cells resulted in their rapid transition to iPSCs [107]. Human PBMCs can be isolated with minimal risk to the donor and obtained in sufficient numbers to enable reprogramming under GMP conditions, making them an ideal somatic cell source for clinical-grade patient-specific iPSCs.

Possible mechanisms for the multi-differentiation potential of PBMCs

To date, many papers on the multi-differentiation potential of PBMCs have been published, but the biological mechanisms of this potential are poorly understood. Several hypotheses have been proposed.

One possible biological mechanism is that multiple lineage-restricted progenitor cells circulate in the blood and can differentiate independently into their corresponding mature tissues [54]. Indeed, many phenotypically distinct progenitor cells have been described in the PBMC population in both laboratory animals and humans [108]. The existence of HSCs was suggested as early as the mid-20th century [109], then subsequent studies found that relatively few HSCs circulate in the peripheral blood of healthy donors and participate in the restoration of hematopoietic function [3,108]. Endothelial progenitors were isolated from hPBMCs by magnetic bead selection in 1997 and can differentiate into ECs [4]. Mesenchymal stem cells with the capacity for self-renewal and the potential to differentiate into bone,

cartilage, fat, tendon, muscle, nerve, and other specified tissues [109-111] were also found in peripheral blood and maintained in culture [50,112-114]. OC precursor cells, hematopoietic OC precursor cells, and a population of blood-derived, plastic-adherent HSC clones with MSC characteristics were also isolated from PBMCs [7,18,102]. In addition, circulating fibrocytes, a distinct mesenchymal cell type, were demonstrated in cultures derived from the PBMC fraction of whole blood [8,102]. Circulating fibrocytes could possess either monocyte-like or fibroblast-like characteristics, and were shown to have multilineage differentiation capacity (including the potential to differentiate into bone, fat, muscle and other tissue types) under appropriate conditions *in vitro* [9,11,100]. Injury and neoplasia are believed to be responsible for homing these progenitor cells to injury areas where they participate in tissue repair [100].

Cell retrodifferentiation, the reversal of the differentiation program and genomic reprogramming of differentiated adult cells to generate progenitor or stem cells, is also proposed to be one possible biological mechanism for this multi-differentiation potential of PBMCs [23,115-118]. The local microenvironment, including solid support structures and signaling matrix components, as well as tissue-specific cytokines, and growth factors are responsible for the cell retrodifferentiation [9].

Cell fusion may also underlie some instances of multidifferentiation. OCs formed by the fusion of mononuclear precursors of the monocyte/macrophage lineage present in the peripheral circulation have been demonstrated *in vitro*, although there is no conclusive evidence to suggest that OCs can also be formed from the fusion of PBMCs [119-120]. It is possible, however, that PBMCs undergo nuclear division but not cytokinesis [73]. Cell fusion may also underlie the tissue-specific differentiation after transplantation *in vivo* [121]. However, fusion alone may not underlie all the multi-differentiation potential of PBMCs by using the multi- and high-specific techniques [45,54,96].

It is also possible that PBMC-derived SCs committed to differentiation along a particular pathway can switch to another lineage under the influence of signals within the local microenvironment [54]. To maintain viability and retain the ability to proliferate, stem cells need cell-to-cell contact and a microenvironment that provides an adequate supply of the necessary growth factors, which act in an autocrine or paracrine fashion [24]. Direct cell-cell interactions may play a more important role than paracrine effects in inhibiting cell differentiation [24].

It is clear now that both robust, sustained, multilineage engraftment and functional activity representative of multiple phenotypic characteristics of the converted cells are required to show full differentiation [9]. But most of the studies on the differentiation potential of PBMCs are still based on visible morphological changes of the cultured cells and engrafted cells, and on immunohistochemistry, which is insufficient to conclude the occurrence of differentiation [39,42,44,52,90]. Besides, in most of the published studies, the data indicate that differentiation occurs very infrequently [12-14,54]. To enrich the foundation of PBMCs in research and regenerative therapy, scientists need to make more effort to understand the biological mechanism of this multi-differentiation potential.

Methods for the isolation and purification of PBMCs

Unleashing the full potential of PBMCs for regenerative medicine requires reliable methods for isolation and purification, techniques that have been gradually refined and improved over the past several decades.

At present, the most common methods for isolating PBMCs from peripheral blood are apheresis and density gradient centrifugation, with apheresis used more often to isolate PBMCs from patients for clinical use [122]. Large numbers of PBMCs can be isolated safely and conveniently from peripheral blood using an automated apheresis separator. Multiple studies have confirmed the presence of large quantities of pluripotential stem cells in cytapheresis products collected from healthy donors [20,122-126]. These cells can be successfully stored for later use in liquid nitrogen using DMSO as a cryoprotectant. Density gradient centrifugation [4,6-8,97,98], especially over ficoll-paque, is more widely used to isolate PBMCs from whole heparinized peripheral blood (including peripheral blood already stored in blood banks) for further use. Occasionally, the PBMC fraction isolated by density gradient centrifugation is then mixed with a red blood cell lysis solution (such as 0.83% NH_4Cl solution) to destroy the mixed red blood cells for further PBMC purification [92,45].

After isolating the PBMCs from peripheral blood, specific progenitor cells (for example, HSCs, MSCs and EPCs) can be purified. Magnetic bead activated cell sorting (MACS) is widely used for purifying a specific progenitor cell population from PBMCs according to cellular phenotype; for example, CD34 microbeads are used to purify CD34+ HSCs, CD133 microbeads are used to purify CD133+ cells and EPCs, and CD14 microbeads are used to isolate CD14+ monocytes [5,19,86,38,127]. However, both the quantity and activity of these cells may be decreased after purification by MACS, so gentler substrate adhesion and negative selection protocols are becoming more common. In earlier studies, T-cell depletion was used to remove nonadherent cells by a non-immune rosette sedimentation technique [15]. Alternatively, Kim and colleagues [95] selected the MSC population from PBMCs and eliminated the nonadherent

cells by simply replacing the medium 48 hours after cell seeding. Human PBMC-derived stem/precursor cells were also purified using a negative selection human progenitor cell enrichment kit with CD41 depletion [87,128]. Yang and colleagues [62] established a method for directionally generating late EPCs and HSCs from hPBMCs by adhesion onto gelatin- and fibronectin-coated culture plates.

Conclusion

PBMCs have been studied for more than 50 years. hPBMCs contain a multitude of distinct multipotent progenitor cell populations and posses the potential to differentiate into almost all cells of the three embryonic layers, including blood cells, ECs, hepatocytes, cardiomyogenic cells, muscle cells, OBs, OCs, epithelial cells, neural cells and myofibroblasts. Moreover, PBMCs can be reprogrammed to iPSCs, further expanding the phenotypic conversion potential of these cells. Techniques for isolation and purification of specific PBMC populations are gradually improving, and it is now possible to obtain pure populations from the PBMC fraction.

PBMCs may be superior to other cell sources for cell-based therapy. In contrast to BM or other sources of multipotent cells, the isolation of peripheral blood is minimally invasive, and does not require general anesthesia. In addition, autologous PBMC transplantation does not require long-term immunosuppressive therapy and can be applied with no ethical limitations [30,104,129,130]. These advantages will accelerate the transition from cell therapies for animal models to clinical applications.

Human peripheral blood HSC transplantation is now a well established curative treatment for patients with various hematologic malignancies [131], and techniques that exploit the multi-differentiation potential of PBMCs could lead to the isolation of cells for the treatment of presently incurable non-hematologic diseases as well [132-135]. However, many preclinical studies are still needed to better characterize the PBMC population and understand the biological mechanism of their multi-differentiation, and to further define their differentiation capacity, transplantability and biosafety.

Abbreviations

bFGF, basic fibroblast growth factor; BM, bone marrow; DMEM, Dulbecco's modified Eagle's medium; EC, endothelial cell; EGF, epidermal growth factor; EPC, endothelial progenitor cell; FCS, fetal calf serum; FGF, fibroblast growth factor; GFP, green fluorescent protein; HLA, human leukocyte antigen; hPBMC, human peripheral blood mononuclear cell; HSC, hematopoietic stem cell; IL, interleukin; iPSC, induced pluripotent stem cell; MACS, magnetic bead activated cell sorting; MSC, mesenchymal stem cell; OB, osteoblast; OC, osteoclast; PBMC, peripheral blood mononuclear cell; RANKL, receptor activator of nuclear factor kappa-B ligand; SC, stem cell; SMA, SMC-specific actin; SMC, smooth muscle cell; VEGF, vascular endothelial growth factor.

Competing interests

The authors declare that they have no competing interests.

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