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Circulating osteogenic Precursor cells

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Abstract

Circulating osteogenic precursor (COP) cells are blood-borne cells that express a variety of osteoblastic markers and are able to form bone in vivo. Strong evidence suggests that COP cells are derived from bone marrow and are of hematopoietic origin. The study of COP cells has been limited by several factors, including the difficulty in establishing long-term cultures and lack of a standardized protocol for their isolation and identification. However, experimental evidence supports that COP cells seed sites of injury and inflammation in response to homing signals and are involved in processes of pubertal growth, fracture, and diverse conditions of heterotopic bone formation. The role of COP cells in physiologic and pathophysiologic conditions of de novo bone formation suggests that they may serve as future targets for diagnostic measurements and therapeutic interventions.

Keywords

circulating osteogenic precursor cells; monocytes; heterotopic ossification; CXCR4; SDF-1

I. Introduction

Adult tissues undergo processes of growth and repair, but the sources and identities of the progenitor cells that contribute to these events are not well understood. The bone marrow has been recognized as a rich and supportive microenvironment for maintaining stem cell populations that contribute to hematopoietic as well as mesenchymal cell lineages. In addition, progenitor cells that can give rise to connective tissues, such as bone, likely reside within the connective tissues themselves. However, several studies have demonstrated that mesenchymal progenitor cells also circulate within the blood^{1–8} and that these circulating precursor cells may participate in connective tissue formation during both normal and pathological events.^{9,10}

Although circulating precursor cells that can be induced to osteogenic differentiation have been isolated and variously named and characterized, we will use circulating osteogenic precursor cells, or “COP cells,” as a general designation for circulating cells with osteogenic potential.

II. Identification of Circulating Osteogenic Precursor Cells

COP cells can be broadly defined as any population of cells within the blood that either have osteogenic potential or can be induced to differentiate along an osteoblast-like lineage. Several investigators have isolated such cells; however, each used different specific criteria to identify and characterize the populations of blood-borne cells with osteogenic capability. Although these studies have generated important information about circulating progenitor cells, the variable approaches and analyses used do not allow us to clearly define COP cells or to determine whether COP cells have a single cellular origin or arise from multiple progenitor lineages.

Among the blood-borne mesenchymal precursor cells (those that give rise to multiple cell fates, such as adipogenic, chondrogenic, and osteogenic lineages) that have been described,^{1-3,5-7,11-13} the more narrowly defined circulating populations of osteogenic cells have also been identified.^{9,10,14} In various studies, such COP cells have been referred to as blood mesenchymal precursor cells, circulating skeletal stem cells, monocyte-derived mesenchymal progenitors, osteogenic blood-derived adherent cells, circulating fibrocytes, and circulating osteoblast-lineage cells. Human umbilical cord blood as a source of COP cells has also been described.^{4,7,15} These osteoprogenitor cell populations share the common features of circulatory status, plastic adherence, expression of osteoblastic markers, and the ability to form a mineralized matrix *in vitro* or bone *in vivo* (Table 1). COP cells are recognized and characterized by expression of osteogenic markers such as type I collagen, osteocalcin, and alkaline phosphatase; bone formation or mineralization; as well as the differential expression of hematopoietic markers with time in culture (Table 1). Although some bone marrow osteoprogenitors can be derived as nonadherent cells,^{9,16} adherence seems to be a requirement for terminal differentiation (mineralization).⁹

At our current state of knowledge, it is unclear whether the circulating cells that are isolated and characterized on the basis of osteogenic markers also have the potential to differentiate along non-osteogenic mesenchymal lineages, or whether these cells are related to circulating mesenchymal stem cells (MSCs) that have been characterized on the basis of criteria for bone marrow-derived MSCs (plastic adherence; expression of CD105, CD73, and CD90; lack of expression of hematopoietic markers; and an ability to differentiate into osteoblasts, adipocytes, and chondrocytes *in vitro*).¹⁷ It does seem that monocyte-derived adherent cells from the blood are multi-potential and can acquire diverse mesenchymal cell fates.^{6,11,18} However, because circulating MSCs are usually characterized as being negative for hematopoietic markers, and since they are often isolated by negative selection of hematopoietic markers,^{5,12} comparisons of the cellular derivations of the variously described COP cells are difficult. Nevertheless, we can develop a framework, based on multi-lineage potentiality and expression of hematopoietic markers, to describe the spectrum of COP cells that have thus far been identified (Fig. 1).

III. Cell and Tissue Origins

Evidence of bone marrow-derived osteogenic cells has been provided by a mouse model of ectopic bone formation: following bone marrow ablation and subsequent green fluorescent protein (GFP)-transgenic bone marrow cell-transplantation, GFP-positive osteoblastic cells were detected in the newly generated ectopic bone.¹⁹ In a recent study, we demonstrated that human COP cells are derived from bone marrow by detecting COP cell markers in donor-derived circulating blood cells from female patients who received sexmismatched bone marrow transplants.¹⁰ We further found that virtually all blood-derived adherent cells come from bone marrow, and greater than 87% of these cells display markers consistent with their identity as COP cells.

Adherent blood-derived cells with osteo-blast-like features having variable expression of the hematopoietic markers CD14, CD34, and CD45 have been reported.^{2,3,6} Approximately 37% of osteocalcin-positive circulating osteoblast-lineage cells isolated by FACS are CD34 positive.¹⁴ A subset of bone marrow MSCs may be derived from a CD45^{med,low} population; therefore, CD45 expression, at least at low levels, may not be unique to hematopoietic cells.²⁰ Suda et al. found that early passage COP cells *in vitro* express levels of CD45 comparable to those expressed by the major hematopoietic lineages,¹⁰ but then lose expression of CD45 and other hematopoietic markers, such as CD14 and CD34, with time in culture. That mesenchymal progenitors express hematopoietic markers is consistent with reports demonstrating a common precursor for both hematopoietic stem cells and for cells that give rise to osteoblast-like cells.^{21,22}

In early experiments on the origin of cells responsible for extraskeletal bone formation, a parabiosis model was used to demonstrate that osteo-inductive cells can be derived from blood-borne mononuclear cells.²³ Among the mononuclear hematopoietic lineages, monocyte-derived mesenchymal progenitors (MOMPs) have also been reported as a CD14⁺/CD34⁺/CD45⁺ population that can differentiate into osteoblast-like cells with concomitant loss of hematopoietic markers.⁶ Taken together, these results suggest that hematopoietic markers are present in early COP cell cultures and then are subsequently lost. When examined early in culture, the weight of evidence suggests that COP cells are derived from the monocyte lineage. Therefore, monocytes may be precursors of cells that play roles not only in bone resorption (by differentiation into osteoclasts, either directly, or after their well-described differentiation into macrophages),²⁴ but also in bone formation.

Spontaneous osteoblast-like differentiation has also been observed in circulating CD14⁺ cells after selection by adherence and clonal expansion (Fig. 2),¹⁰ and adherent CD14⁺ mononuclear cells derived from whole blood can be induced also to differentiate into macrophages, T-lymphocytes, hepatocytes, and epithelial-, neuronal-, and endothelial-like cells,¹⁸ as well as fibrocytes.^{25–31} Given shared morphological characteristics, phenotypic markers, and common methods of isolation, some COP cells may share identity with cells that have been described as circulating fibrocytes. Circulating fibrocytes were originally described as type I collagen-producing cells of hematopoietic origin that contribute to wound healing and various fibrosing disorders. More recently, fibrocytes were shown to undergo osteogenic and chondrogenic differentiation.¹¹ Fibrocytes, as well as other COP cells of monocyte origin, lose expression of hematopoietic markers with time in culture, after exposure to specific serum components, and under certain conditions *in vivo* (Table 2).^{6,10,28,32–37}

IV. Physiologic and Pathophysiologic Roles

Although the physiologic functions of COP cells remain undetermined, a possible role for these osteogenic cells includes bone formation during development or fracture healing.^{9,38} For example, Eghbali-Fatourehchi et al. reported that osteocalcin-positive circulating osteoblast-lineage cells are more abundant during pubertal growth and in patients postfracture.⁹

Increasing evidence supports the participation of COP cells in conditions of pathologic bone formation, such as heterotopic ossification (HO) that occurs following hip arthroplasty, end-stage aortic valvular disease (R.J. Pignolo, unpublished data, 2010), and rare genetic syndromes of extraskeletal bone formation.¹⁰ Contributions of COP cells to HO have also been demonstrated in animal models of ectopic bone formation.^{10,19,39}

Fibrodysplasia ossificans progressiva (FOP) is a rare genetic disorder caused by mutations in ACVR1, a bone morphogenetic protein (BMP) type I receptor, and is characterized by

congenital malformation of the great toes and progressive heterotopic ossification.^{40–42} Blood samples from patients with FOP with active episodes of HO contain significantly higher numbers of clonally derived COP cell colonies than patients with stable disease or unaffected individuals.¹⁰ Histopathologic studies of FOP lesions reveal monocyte and lymphocyte infiltration into skeletal muscle, followed by widespread myocyte degeneration and then fibroproliferation, chondrogenesis, and osteogenesis.^{43,44} A bone marrow-derived subpopulation of mononuclear adherent cells expressing hematopoietic and osteogenic markers is present in early fibroproliferative lesions in patients with FOP, and has been shown to nucleate heterotopic bone formation when implanted in mice.¹⁰

Nonhereditary forms of heterotopic ossification occur in association with several common medical conditions or procedures.⁴⁵ Heterotopic bone formation is a frequent complication of total hip arthroplasties for age-related degenerative joint disease and can cause adverse postoperative outcomes.^{46–50} In the setting of degenerative calcification, ectopic bone formation occurs in up to 13% of aortic valve stenosis.⁵¹ As in other forms of HO, the osteogenic lesion in vascular disease develops in the setting of injury and inflammation,⁵¹ suggesting that the formation of bone in late stage lesions may share similar precipitating events. Likewise, circulating endothelial progenitor cells expressing an osteogenic phenotype are increased in patients with coronary atherosclerosis, in which abnormal endothelial function and structural coronary artery disease may be associated with calcification.⁵² Cells similar to COP cells can also be found in the inflammatory joint fluids and synovium of patients with rheumatoid arthritis, suggesting a possible role for these cells in degenerative bone and joint deformities.⁵³

In postmenopausal women, changes in gene expression in circulating alkaline phosphatase positive cells may reflect rates of bone loss.⁵⁴ Another study suggests that circulating MSCs in patients with osteoporosis are increased but undergo abnormal osteogenic differentiation with downregulation of RUNX2 and COL1A1.⁵⁵

V. Homing of COP Cells

As noted above, COP cells may be more abundant in those individuals who are predisposed to pathological ossification or in those who are undergoing normal physiologic growth or repair of bone tissue. These cells may be directed toward an osteogenic fate when triggered or recruited by inflammatory signals, such as during fracture repair or heterotopic ossification, or when induced by signals released from a hypoxic microenvironment, such as from soft tissue injury or by formation of hypertrophic chondrocytes during long bone growth.

In the case of heterotopic ossification, ectopic bone formation can be precipitated by soft tissue injury in skeletal muscle, causing the presumptive release of inflammatory cytokines and migratory factors (Fig. 3). Inflammatory signals seem to be necessary for heterotopic ossification induced by bone morphogenetic protein (BMP) signaling, and a recent study has shown that cells of the monocyte lineage were required for triggering the extraskeletal bone formation following injury.⁵⁶

Stromal cell-derived factor-1 (SDF-1) and BMP may be important signaling molecules in the inflammatory microenvironment, especially given their roles in hypoxic tissue injury and chemotaxis.^{37,57,58} SDF-1 is induced by hypoxic tissue injury and attracts cells expressing the SDF-1 cognate receptor CXCR4. BMP may serve dual roles in bone formation as well as in chemoattraction of inflammatory cells.⁵⁹ These factors may recruit COP cells from bone marrow, with homing of COP cells to sites of injury occurring through CXCR4 (Fig. 3), as occurs in fibrocyte localization in lesions of pulmonary fibrosis.³⁷ In a mouse model of BMP2-induced ectopic bone formation, bone marrow-derived osteoblast progenitor cells

expressed CXCR4 and migrated to regions of bone formation by SDF-1 chemoattraction.^{19,39} BMP stimulation of brown adipocytes may promote the early steps of heterotopic ossification by lowering oxygen tension in adjacent tissue,⁶⁰ creating a microenvironment for chondrogenesis as well as inducing SDF-1-mediated homing of COP cells that may then ossify the cartilage template.

Circulating fibrocytes, which account for as much as 10% of the inflammatory cell infiltrate at sites of acute injury,²⁸ mediate wound healing by replacement of the same tissue in the correct place, albeit sometimes with excessive fibrous deposition (e.g., scar or keloid formation, fibrosis). However, in the case of extraskeletal bone formation, the cellular link between injury and repair may be compromised, with the transformation of damaged tissue into another (normal) tissue, but at an inappropriate location.⁶¹

VI. Current Challenges and Future Directions

A key obstacle to understanding the characteristics and functions of COP cells is the difficulty associated with their isolation and expansion in culture. Long-lived COP cell strains are infrequent. The majority of clonally expanded COP cell strains do not survive past the fourth passage *in vitro* and 60% to 70% of COP cell isolates cannot be successfully propagated after the first passage (R.J. Pignolo, unpublished data, 2010). Therefore, COP cell analysis is limited to clonally derived outgrowths that survive the first passage, longer-lived strains when they can be successfully derived, or freshly derived peripheral blood mononuclear cells in short-term experiments that do not require clonal expansion (e.g., flow-cytometry-based characterizations).

Although circulating osteogenic progenitor cells can be defined by shared features of circulatory status, adherence in culture, osteoblastic markers, and osteogenic potential, no standard criteria based on non-osteoblastic markers have been defined. One of the major discrepancies among reports that describe COP cells is the presence or absence of hematopoietic markers. This variability is likely explained by the loss of hematopoietic markers with time in culture; therefore, the window during which these markers could be detected is early and narrow. Suda et al. confirmed that hematopoietic markers in at least one of the identified populations of COP cells are no longer detected with time in culture, with CD14 preferentially lost first and followed by CD45 and CD34.¹⁰

COP cells are associated with heterotopic ossification, fracture healing, and pubertal growth spurts. More vigorous studies are needed not only to link peripheral blood levels of COP cells to states of *de novo* bone formation, whether pathologic or physiologic, but also to confirm the presence of COP cells in tissue sites of new ossification (such as fracture hematoma or callus, vascular lesion, or areas of posttraumatic HO formation). The ability to detect an increase in numbers of COP cells in blood may be reduced by the uncertainty of when COP cells are mobilized in response to an inductive event, especially in chronic conditions (e.g., vascular disease) or when the timing of injury and inflammation is uncertain.

At the cellular and molecular level, whether COP cells are committed to their osteogenic fate upon leaving the bone marrow or whether their fate is determined once they home to a conducive local tissue environment remains unknown. It is also unclear whether COP cells represent a committed subpopulation of circulating MSCs or if they are a distinct lineage. The signaling pathways that regulate COP cell fate are also not understood.

A possible mechanism for the homing of COP cells to regions of new bone formation through SDF-1 chemoattraction, as described above, is suggested by an animal model of BMP2-induced ectopic bone formation.³⁹ However, better physiologic and pathophysiologic

models are needed to elucidate the mechanism(s) by which COP cells may contribute to *de novo* ossification.

The future characterization of COP cells holds promise for their potential use as a diagnostic tool (higher circulating levels associated with a disease state, lower levels with resolution), an autologous cell replacement therapy, and a target cell population for gene therapy in pathologic ectopic bone formation.

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Figure 1.

Characteristics of various isolated COP cell populations based on multi-lineage potential and expression of hematopoietic markers. Circulating osteoprogenitors have been identified using several different criteria and the overlap among these populations is undetermined. Blood mesenchymal precursor cells and umbilical cord blood mesenchymal stem cells have similar multi-lineage potential. BdACs, blood-derived adherent cells.

Figure 2. Clonal outgrowth of blood-derived adherent cells in primary culture. Expanded COP cell clones express osteogenic and hematopoietic markers and can form bone in vivo as previously described.¹⁰

Figure 3. Possible mechanism for COP cell homing to soft tissue sites of injury and inflammation that precedes heterotopic ossification.

Table 1
Hematopoietic and Osteoblastic Markers in Circulating Osteogenic Precursor Cells

Name	Hematopoietic markers	Osteoblast markers	In vivo bone formation	Reference
Postnatal				
Blood mesenchymal precursor cells	CD 14-, CD 34-, CD 45-	Col I+, AP+	ND	2
Circulating skeletal stem cells	CD 14-, CD 34-, CD 45-	Col I+, ON+, OPN+/-, OC +/-, AP-	Yes	3
Monocyte-derived mesenchymal progenitors	CD 14+, CD 34+, CD 45+	Col I+, AP+, OC+	ND	6
Osteogenic blood-derived adherent cells/ circulating fibrocytes	CD 14-, CD 34+, CD 45+	Col I+, OC+, AP+	Yes	10
Circulating osteoblast-lineage cells	CD 34+	Col I+, OC+, AP+	Yes	9, 14
Perinatal				
Human umbilical cord blood stromal cells	ND	Col I+, OC+, AP+	Yes	4
Umbilical cord blood mesenchymal stem cells	CD 14-, CD34-, CD 45-	Col I+, OC+	ND	7

Abbreviations: Col I, Type I collagen; ON, osteonectin; OPN, osteopontin; AP, alkaline phosphatase; ND, not done.

Table 2
Loss of Hematopoietic Markers in Circulating Osteogenic Precursor Cells

Marker	Conditions	Reference
CD 14	TGF- β 1/chondrocyte differentiation; DEX/osteoblast differentiation; adipocyte differentiation	6
	TNF- α	36
CD 34	DEX/osteoblast differentiation	6
	Extended time in culture	10, 28, 37
	Invasive ductal carcinoma	35
	Neoplastic pancreatic lesions	34
	Scleroderma	33
	Hypertrophic scars and keloids	32
CD 45	TGF- β 1/chondrocyte differentiation; DEX/osteoblast differentiation; adipocyte differentiation	6
	Extended time in culture	37

Abbreviations: TGF- β 1, transforming growth factor-beta 1; TNF- α , tumor necrosis factor alpha; DEX, dexamethasone.