



**VICTORIA UNIVERSITY**  
MELBOURNE AUSTRALIA

*Circulating osteogenic precursor cells: Building bone from blood*

This is the Published version of the following publication

Feehan, Jack, Nurgali, Kulmira, Apostolopoulos, Vasso, Al Saedi, Ahmed and Duque, G (2019) Circulating osteogenic precursor cells: Building bone from blood. *EBioMedicine*, 39. pp. 603-611. ISSN 2352-3964

The publisher's official version can be found at  
<https://www.sciencedirect.com/science/article/pii/S2352396418305553?via%3Dihub>  
Note that access to this version may require subscription.

Downloaded from VU Research Repository <https://vuir.vu.edu.au/38111/>



## Review

## Circulating osteogenic precursor cells: Building bone from blood


 Jack Feehan<sup>a,b</sup>, Kulmira Nurgali<sup>a,c</sup>, Vasso Apostolopoulos<sup>c</sup>, Ahmed Al Saedi<sup>a,b</sup>, Gustavo Duque<sup>a,b,\*</sup>
<sup>a</sup> Australian Institute for Musculoskeletal Science (AIMSS), University of Melbourne and Western Health, St Albans, VIC, Australia

<sup>b</sup> Department of Medicine-Western Health, Melbourne Medical School, University of Melbourne, Melbourne, VIC, Australia

<sup>c</sup> Institute for Health and Sport, Victoria University, VIC, Australia

## ARTICLE INFO

## Article history:

Received 2 November 2018

Received in revised form 26 November 2018

Accepted 26 November 2018

Available online 3 December 2018

## Keywords:

COP cells

Circulating osteogenic precursor cells

Circulating mesenchymal stem cells

Circulating osteoprogenitors

Stem cells

## ABSTRACT

Circulating osteogenic precursor (COP) cells constitute a recently discovered population of circulating progenitor cells with the capacity to form not only bone but other mesenchymal tissues. There is a small, but growing body of literature exploring these cells, but with a great deal of disagreement and contradiction within it. This review explores the origins and biological characterization of these cells, including the identification strategies used to isolate these cells from the peripheral blood. It also examines the available knowledge on the *in vitro* and *in vivo* behaviour of these cells, in the areas of plastic adherence, differentiation capacity, proliferation, and cellular homing. We also review the implications for future use of COP cells in clinical practice, particularly in the area of regenerative medicine and the treatment and assessment of musculoskeletal disease.

© 2018 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## Contents

1. Introduction . . . . .	603
2. Search strategy and selection criteria . . . . .	604
3. Origins of the osteoblast – A historical perspective . . . . .	604
4. Characterization of COP cells . . . . .	605
5. Origins . . . . .	605
6. Marker expression . . . . .	605
6.1. COP cells with MSC markers . . . . .	605
6.2. COP cells with hematopoietic and vascular markers . . . . .	606
7. Plastic adherence . . . . .	607
8. Morphology . . . . .	607
9. Differentiation potential . . . . .	607
10. Proliferative capacity . . . . .	607
11. Cell homing . . . . .	608
12. COP cells and bone marrow MSCs: outstanding questions . . . . .	608
13. Conclusions . . . . .	609
Author contributions . . . . .	609
Declaration of interests . . . . .	609
Role of the funding source . . . . .	609
Acknowledgements . . . . .	610
References . . . . .	610

## 1. Introduction

Circulating osteogenic precursor (COP) cells are a relatively new entity in the field of stem cell and bone biology. They are defined as a population of fluid-phase, blood-borne cells with the capacity for osteogenesis, or differentiation into mesenchymal tissues. Such cells

\* Corresponding author at: Australian Institute for Musculoskeletal Science (AIMSS), Sunshine Hospital, 176 Furlong Road, St Albans, VIC 3021, Australia.  
 E-mail address: [gustavo.duque@unimelb.edu.au](mailto:gustavo.duque@unimelb.edu.au) (G. Duque).

have been identified by several groups, though a variety of terms have been used to reference them, including circulating osteoprogenitors, circulating mesenchymal stem cells (cMSCs), monocyte-derived mesenchymal precursors, and circulating skeletal stem cells, amongst others. However, there is little consensus between the studies regarding their characterization, with individual research groups using a range of different, and at times conflicting, cellular markers and criteria to define these cells. This contradiction and disagreement has diminished the applicability of the current research base and limited progress in the field. Herein, we analyze the literature describing COP cells to provide a clearer understanding of their characterization, as well as to compare them with the better-known bone marrow MSCs. We then identify areas for further research to allow a more comprehensive analysis of the biological identity and behavior of these cells.

## 2. Search strategy and selection criteria

The studies used in this review were identified through search of the MEDLINE and PubMed databases, using all or part of the search terms 'Circulating Osteogenic Precursor', 'Circulating Mesenchymal Stem cell', 'Circulating Osteoprogenitor', and 'Circulating Stem Cell', connected by the Boolean operator 'OR', identifying 1048 search items. Examination of paper titles and removal of duplicates yielded 72 included studies. Resulting abstracts were studied for suitability yielding a total of 37 included studies. Finally, reference lists of included the included studies

were searched for further possible inclusions, resulting in the inclusion of an additional two papers, bringing the total to 39 (Fig. 1).

## 3. Origins of the osteoblast – A historical perspective

Ossification and maintenance of the skeleton has long been known to involve a dichotomous relationship between bone forming osteoblasts and resorbing osteoclasts. Osteoblasts were identified on the external surface of bone, but it was unclear where they come from and how they are regenerated, as they are unable to undergo mitotic division. Osteoclasts were identified in the circulation in the 1870's [1], and thought to be osteoblasts that had fused with a neighboring chondrocyte. This connection sparked the idea of a circulating osteoblastic progenitor cell, as a means of answering the above question, however, despite repeated efforts these were never found. Little progress was made in finding an osteoblastic precursor until nearly a century later, when Urist [2] stimulated histiocytes into osteogenesis via autoinduction, and Tavassoli and Crosby [3] demonstrated that heterotopically transplanted bone marrow formed both blood and bone elements, identifying a source of progenitor cells of the mesenchymal and hematopoietic lineages. This work resulted in the pioneering research by Friedenstein et al., who isolated specific cells within the milieu of the bone marrow responsible for stromal tissues, though the current term "mesenchymal stem cell" was not coined until the early 1990's [4]. However, despite these discoveries in the 1960's and 70's,

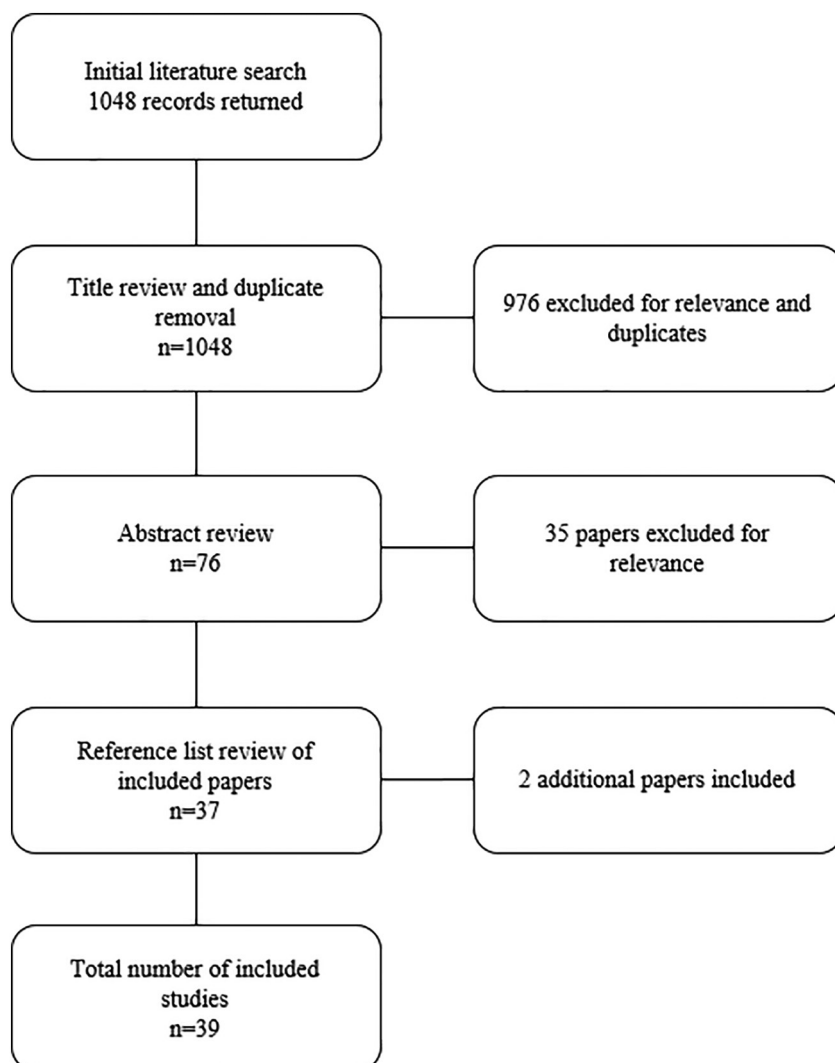


Fig. 1. Flow chart of paper inclusion and exclusion information.

some details on the origin of osteoblasts have puzzled scientists. While MSCs have been shown to differentiate into adult osteoblasts, it is unknown how they access sites of bone formation non-contiguous to bone marrow, rekindling the notion of a circulating osteoblastic precursor.

Circulating cells with some capacity for mesenchymal differentiation were identified many years earlier [5], however they were never shown to produce bone tissue. It was not until 1997 that studies identified circulating cells with osteoblastic characteristics in stem cell enriched blood taken from breast cancer patients [6]. These cells were soon demonstrated in healthy individuals at the turn of the 21st century, but could not be prompted to form bone *in vitro* or *in vivo*, though they did express several markers of osteogenesis and had hallmarks of the previously identified bone marrow MSCs [7]. Demonstration for bone formation capacity came soon in the pioneering work of Paolo Bianco and Pamela Robey demonstrating *in vivo* ossification shown after transplantation of the cells into immunocompromised mice, and coining the term circulating skeletal stem cell [8]. As the cells were similar in behavior, appearance and marker expression to the relatively well understood bone marrow MSCs, they logically came to be considered as a closely related surrogate population of cells. However, shortly after, similar cells which behaved and appeared similarly to bone marrow MSCs were identified, but unlike MSCs, expressed hematopoietic lineage markers [9,10]. This casts doubt on the origin of these cells – are they transitory bone marrow MSCs homing to sites of bone regeneration, or, are they of the hematopoietic line, as the other the major cell type involved in bone turnover, the osteoclast? Alternatively, are there two populations present in the circulation, and if so, what are their respective functions?

#### 4. Characterization of COP cells

COP cells are known to exist within the peripheral blood mononuclear cell (PBMC) fraction of the blood, estimated to represent approximately 0.42% of this population [11], and it appears that they circulate at a steady level throughout the lifespan in healthy individuals, increasing in times of accelerated bone growth [10,12], however their existence has been refuted by one study [13]. Due to their similarities, MSCs are commonly used as a comparison for COP cells. MSCs are typically classified as being (i) plastic adherent, (ii) capable of multilineage differentiation and logarithmic proliferation, (iii) expression of cell surface markers, CD105, CD73, CD90, and (iv) not expressing the hematopoietic markers CD34, CD45 and CD14 [14]. These qualities have been applied to characterize COP cells, however, despite these common criteria, there is still much contradiction between studies in regard to the expression of these markers. The characterization of COP cells varies widely in many aspects, including their origin, marker expression, plastic adherence, morphology, homing mechanism, differentiation and proliferative potential.

#### 5. Origins

Little definitive evidence exists regarding the specific cellular origin of COP cells. However, it is widely believed that the bone marrow is the likely origin. Several studies speculate that COP cells are bone marrow MSCs that have been stimulated to circulate by peripheral tissue demands [6,7,15–18]. This is largely due to their similarities in behavior and initial findings on cell surface marker expression. This has been supported by parabiotic mouse models involving transplantation of green fluorescence protein positive (GFP+) bone marrow into one paired animal and stimulation of bone formation in the other [19,20]. Once osteogenesis was initiated in the paired mouse, GFP+ cells were found at the site of bone formation, indicating a circulating osteogenic cell, though one study of similar methodology did not identify the circulating osteoprogenitors [13]. Despite this evidence that the bone marrow is the tissue of origin, the precise cellular lineage of COP cells remains unclear. It has been suggested that hematopoietic stem cells (HSCs) are

possible progenitors for osteoblasts [21,22]. This, combined with newer information on hematopoietic marker expression by COP cells, suggests that COP cells may be an intermediary between HSCs and osteoblasts. Pignolo and Kassem [23] proposed a model of both the hematopoietic and mesenchymal lineages beginning with an unknown common ancestor progenitor cell. It was proposed that this common ancestor gives rise to both HSCs and MSCs, before they both form their accepted terminal cells. The more recently discovered very small embryonic-like (VSEL) stem cells have been shown to have the capacity to regenerate tissue from all germ layers [24], and may be a candidate for this unknown common progenitor. It was also suggested that COP cells could fall directly under a number of ancestors including HSCs, mesangioblasts and MSCs, as a heterogeneous population of terminal cell types, providing a possible explanation for the contradictory findings in the literature [23].

#### 6. Marker expression

The identification of specific cell surface markers or expression of certain bone specific proteins has been a key point of contention in COP cell research. The majority of the studies target two main groups of markers to characterize COP cells: proteins related to bone formation, particularly osteocalcin (OCN), alkaline phosphatase (AP), and type one collagen (Col1); and markers of the hematopoietic/vascular endothelial line, largely centered on the pan-hematopoietic marker CD45, monocytic marker CD14, and the endothelial stem cell marker CD34. While there have been numerous other markers shown to be expressed, or not expressed by COP cells, one or more of these feature in most studies examining COP cells. Despite these commonalities, there is little consensus, and even outright contradiction, amongst authors regarding marker expression, particularly regarding the presence or absence of hematopoietic markers. Table 1 summarizes the range of marker combinations used to characterize COP cells.

##### 6.1. COP cells with MSC markers

The first characterizations of COP cells described a population of cells expressing the markers OCN, AP, and Col1, but not displaying CD45, CD34 and CD14 [6–8]. This pattern of expression fits within widely accepted criteria for the identification of bone marrow MSCs, which are typically described as being CD105, CD73, and CD90 positive, with co-expression of the markers of osteogenesis above, and lacking the CD45, CD34, and CD14 antigens [14]. Indeed, populations of circulating cells fitting exactly within these criteria have been identified in elderly hip fracture patients [16], and postmenopausal women with osteoporosis [25]. Another study described a population of CD44 and osteopontin (OPN) positive cells which also expressed Stro1, a marker believed to identify bone marrow stromal cells [26]. It was also proposed that these cells were bone marrow MSCs transiting to sites of bone formation via the C-X-C chemokine receptor 4 (CXCR4)/stromal cell derived factor 1 (SDF-1) pathway [15].

The CXCR4/SDF-1 axis is a potent chemotactic pathway, and this study proposed the CXCR4 receptor as a further means of identification of COP cells. Peripheral tissues factors have been shown to cause mobilization of COP cells with markers similar so MSCs. The first time these cells were described was in response to growth factor mobilization, by the intravenous administration of granulocyte colony stimulating factor (G-CSF) or granulocyte-monocyte colony stimulating factor (GM-CSF). These factors are used to mobilize granulocytes and HSCs from the bone marrow for cancer therapies, and were shown to cause the release of COP CD45-/CD34-/CD14- COP cells [6]. The inflammatory mediator substance P has also demonstrated this capacity, stimulating the release of a population of CD29+/CD45-/CD11b- stromal-like cells in the peripheral blood of rabbits after its intravenous administration [17]. Chronic hypoxia has also shown to cause COP cell mobilization with CD44+/CD54+/CD73+/CD90+/CD45- cells mobilized into the

**Table 1**  
Summary of marker strategies used to identify COP cells.

Study	Hematopoietic Markers	Other Markers	Study	Hematopoietic Markers	Other Markers
Kuznetsov et al., (2001) [8]	CD45- CD34- CD14-	CD44+ OCN+ Col1+ Stro1- OPN+ ON+	Egan et al. (2011 & 2018) [33,34]	CD45+	OCN+, Col1+
Zvaifler et al., (2000) [7]	CD45-, CD34-, CD14-	CD105+ OCN+ AP+ Col1+	Otsuru et al., (2017) [36]	CD45+	Nil
Fernandez et al., (1997) [6] Undale et al., (2010) [25]	CD45-, CD34-, CD45-, CD14-	Col1+ AP+	Suda et al., (2009) [35] Fadini et al., (2011) [47]	CD45+ CD45+ CD34- CD14+	Col1+ OCN+ AP+
Otsuru et al., (2008) [15]	CD45-	CD44+ OPN+ Stro1+ CXCR4+	Eghbali-Fatourehchi et al., (2007) [12] Pirro et al. (2010, 2011 & 2012) [38–40] Rattazzi et al., (2016) [41] Manavalan et al., (2012) [44] Rubin et al. (2011 & 2012) [43,45]	CD34+ CD34+	OCN+ AP+
Alm et al., (2010) [16]	CD45- CD34- CD14-	CD105+, CD73+, CD90+		CD34+	OCN+ CD146+
Dalle Carbonare et al., (2009) [30]	CD45+, CD34+, CD14+	Nil	D'Amelio et al., (2010) [27] Eghbali-Fatourehchi et al., (2005) [10] Pal et al. (2010 & 2011) [43–44]	Nil Nil Nil	OCN+ AP+ OCN+ GFP+
Kuwana et al., (2003) [9] Matsumoto et al., (2006) [78]	CD45+, CD34+, CD14+ CD45+, CD34+	AP+ Col1+ CD31+, CD133+, CD105+	Boban et al., (2010) [13] Kumagai et al., (2008) [20] Otsuru et al., (2007) [19] Kuznetsov et al., (2007) [46]	Nil Nil Nil Nil	OPN+, BSP+, ON+ Nil
Ritz et al., (2014) [61]	CD45+, CD34+	CD31+, CD133+			
Al Saedi et al., (2017) [32] Gunawardene et al. (2015 & 2017) [11,31]	CD45+	OCN+	Wan et al., (2006) [48]	Nil	Nil

OCN: Osteocalcin, AP: Alkaline Phosphatase, Col1: Type 1 collagen, CXCR4: C-X-C chemokine receptor type 4, OPN: Osteopontin, BSP: Bone sialoprotein, ON: Osteonectin.

peripheral circulation in a rat model [18]. These studies suggest these cells are involved in processes of tissue injury, inflammation and healing, being released from the bone marrow and circulating to sites of repair, however much more research is required to validate this.

Taken together, this evidence appears to describe cells closely related to bone marrow MSCs which have been stimulated to mobilize into the peripheral circulation, providing a logical explanation for the genesis of these cells. However, a number of studies have noted circulating osteogenic cells which do not conform to the typical MSC identification criteria, expressing a range of hematopoietic markers raising questions regarding the identity, behaviors and functions of COP cells.

Although the majority of research on COP cells has utilized a hematological or vascular marker of some kind either by inclusion or exclusion, a handful of studies have used only the expression of proteins involved in bone formation as the criterion for the identification and isolation of COP cells. As such, OCN+ and AP+ cell behaviors have been studied in times of pathologically or physiologically increased bone formation, including puberty and fracture [10,27]. Others have simply used OCN expression by PBMCs alone to classify COP cells and their relationship to vascular calcification [28,29]. Whilst this approach has the benefit of simplicity, the lack of characterization of additional marker expression makes the relationships of the cells studied unclear.

## 6.2. COP cells with hematopoietic and vascular markers

The first cells to be identified as COP cells with hematopoietic markers were coined monocyte-derived mesenchymal precursors. They were shown to be CD45+/CD34+/CD14+/Col1+ cells and capable of multilineage mesenchymal differentiation and expansive proliferation [9]. In another study, a similar CD45+/CD34+ (low) population was identified as COP cells, due to their expression of the genes coding for osteoprotegerin (OPG) and receptor activator of nuclear factor kappa-B ligand (RANKL), both important markers of bone formation. The cells identified in this study were heterogenous in their expression of CD14 with a small proportion of the cells expressing the marker, and the rest not [30]. More recently, the co-expression of CD45, OCN and/or Col1 have been used to identify and characterize COP cells. Expression of

CD45+/OCN+ alongside the exclusion of CD3+ (T lymphocyte) and CD19+ (B lymphocyte) cells, has been used to study COP cells in a series of experiments on their behavior in frail older adults [11,31,32]. Addition of the marker Col1 to the above criteria has also been used to isolate COP cells in studies showing the role of COP cells in heterotopic ossification [33] and vascular calcification [34]. Furthermore, the role of CD45+/Col1+ COP cells have been studied in the hereditary condition Fibrodysplasia Ossificans Progressiva (FOP) [35]. This growing body of evidence clearly shows the presence of COP cells with hematopoietic markers, though the range of expression patterns studied makes it difficult to identify whether there is one, or many populations present.

Moreover, the biological behavior and functional significance of these cells is still unknown. Indeed, a recent study using a novel triple transgenic mouse model has shown that while CD45+ COP cells were recruited to sites of bone formation, they did not contribute to ossification via differentiation into osteoblasts [36]. They used mice with three gene modifications – CD45-Cre, Z/RED and Col2.3GFP, which allowed them to identify cells which had expressed CD45 at any time, even if it was not currently present. While they showed the presence of these CD45+ cells, and that their capacity to home to a site of bone morphogenic protein-2 (BMP2) mediated heterogenous ossification (HO), they did not differentiate into osteoblasts. The parallels between the expression of the hematopoietic lineage COP cells and the myeloid lineage osteoclast precursors coupled with this finding raises interesting questions about their biological role. Their expression of markers of bone formation implies an osteogenic role, even if it is not to differentiate osteoblasts. It could be possible that they instead regulate the balance between osteoclastic resorption and osteoblastic activity, directing the formation of bone. Thus, while hematopoietic cells with osteogenic potential seem to exist in the circulation and be implicated in states of bone formation or loss, their exact role is yet to be fully determined.

Following the finding that the CD34+ fraction of the bone marrow more reliably produced osteoblasts [37], expression of this endothelial stem cell marker in conjunction with osteogenic markers has been employed to isolate COP cells. One study found that there are two distinct populations amongst OCN+ cells: (i) CD34+ population, and (ii)



CD34<sup>+</sup> population. The CD34<sup>+</sup> cells are smaller and less granular, whilst the CD34<sup>−</sup> cells are large and dense. They proposed that they are not different populations, but that the change in expression occurs as the cells mature and differentiate towards their terminal tissue, losing expression of CD34 as they lose their ‘stem’ capacity [12]. They also found that the proportions of CD34<sup>+</sup>/− cells changed as a function of ages, with less CD34<sup>−</sup> cells present in older populations. Co-expression of CD34 and OCN or AP has also been used to identify these cells in osteoporotic women, exploring the association between COP cell numbers and rates of bone loss [38], arterial stiffness [39], thyroid hormones [40] and the effect of the hypercholesterolemia medication atorvastatin [41]. Cells with a CD34<sup>+</sup>/OCN<sup>+</sup> phenotype in rates with artificially induced tibial fractures. They described these cells as peaking in number at three weeks post fracture, proposing them to have a role in the transition between the cartilaginous and mineralized callus [42]. Several studies also used the endothelial and mesenchymal stem cell marker CD146 to isolate COP cells to study their behavior in diabetic patients, seeking to provide a rationale for the decrease in bone mass in type 2 diabetes mellitus [43–45]. The addition of markers classically associated with vascular endothelium further complicates the landscape of COP cells. The interaction between the bone, blood, and vascular lineages remains unclear, however there seems to be some association between the three in COP cell biology.

While there is a wide variance in the characterizations of COP cells, some unifying themes can be identified. The literature appears to describe at least two distinct populations of COP cells, one with a very similar pattern of marker expression to bone marrow MSCs, and at least one other displaying surface receptors associated with the hematopoietic/vascular lineages. These populations require further characterization before they are fully understood. Additionally, the inconsistencies within the literature should be explored, and each identified population further studied for their functionality to determine their biological behavior and roles within normal physiology, as well as in pathological conditions.

## 7. Plastic adherence

Plastic adherence is one of the central characteristics used to identify bone marrow MSCs. By commonly agreed on definition, MSCs must adhere to plastic in culture – a characteristic often shown to be shared by COP cells [6–9,16,25,30,35,40,46–48]. However, the discovery of a non-adherent cell population in the marrow expressing bone specific markers [49,50], which has significantly greater capacity for regenerating bone [21] has prompted the examination of the non-adherent PBMC fraction as well. Three other studies used this same methodology when analyzing COP cell behavior [15,25,43]. These differences in adherence raise further questions regarding the nature of these cells and their relationship to bone marrow MSCs, as more inconsistencies between their biological behavior arise. It is currently unclear whether the differences between adherent behavior correlates to different patterns of observed marker expression, as well its functional significance.

## 8. Morphology

COP cell morphology has been documented in a number of studies. They are typically described as small round cells initially, but become elongated, “spindle shaped” or fibroblast-like cells over 3–7 days in general growth media [7,9,16,30]. Some studies have also reported a second population of flat or polygonal COP cells [8,48], though in one study it was noted that these cells displayed a nearly identical panel of markers to their fibroblastic counterparts [8]. These spindle and flat morphologies have been demonstrated in bone marrow MSCs, being shown to represent different stages of differentiation, with the flat cells being further differentiated than their spindle shaped counterparts [51]. The flat cell morphology has not been identified in hematopoietic COP cells,

though their morphology has only been described in a single study [9]. Upon osteogenic differentiation, both hematopoietic and MSC-like COP cells take on the characteristic rounded, cuboidal or “cobble stone” appearance of osteoblasts [7,9], consistent with the morphological behavior of bone marrow MSCs. It is also yet unknown how the morphologies of these cells change in relation to external factors, such as physiological stress, aging or time in passage. It has been demonstrated that with increased number of passages, bone marrow MSCs become broader and flatter, with more numerous podia [52]. Additionally, they appear to show decreased affinity for the spindle morphology with increasing lifespan, with a correlating decrease in differentiation and proliferation capacity [53]. It is yet unknown whether these patterns of aging are seen also in COP cells, or how they may affect the cells functionally.

## 9. Differentiation potential

Differentiation into different mesenchymal tissues by COP cells has been established in a number of studies. As might be expected, numerous studies have shown osteoblastic differentiation potential of COP cells, both *in vitro* and *in vivo*. Osteoblastic differentiation *in vitro* has been demonstrated via a number of means including: staining of calcified nodules after culture [10,15,16,25,40,43,47,48], upregulation of genes related to bone formation [30,43], and increased secretion of factors associated with osteogenesis [7,9,19]. Several studies have also shown that these cells are osteogenic when injected or implanted on pellets into mice, forming trabecular bone and some hematopoietic elements [8,15,35,46]. Evidence demonstrating multilineage capacity is less frequent, but some studies have been able to demonstrate COP cells forming tissues other than bone, showing them capable of *in vitro* adipogenesis and chondrogenesis in humans [16–18,48] and guinea pigs [46]. Interestingly, these authors were all experimenting on MSC-like cells, with evidence demonstrating the same stem capacity in hematopoietic COP cells scarcer. Only one study has shown multilineage differentiation in COP cells with hematopoietic markers, demonstrating the *in vitro* formation of fat, muscle, and cartilage, in addition to bone [9]. These authors provide support to the “stemness” of COP cells, however further investigation is necessary to understand the full extent of their capacity for differentiation, particularly if and how it varies between the different subcategories of identified COP cells by marker expression. It is yet unknown how the differentiation of COP cells compares with their bone marrow or adipose tissue counterparts either *in vitro* or *in vivo* in terms of the rate, extent and functionality. It has also yet to be demonstrated how their differentiation is affected by external variables such as patient age, culture conditions and number of passages. Bone marrow MSCs are known to undergo changes in differentiation behavior throughout the lifespan, seemingly more inclined to undergo adipogenesis than bone or muscle formation with increasing age [54,55]. Additionally, bone marrow MSCs have been shown to have the capacity to form tissues outside the traditional mesenchymal lineages, such as neural cells [56], a capacity yet to be investigated in COP cells. This information is essential in the evaluation of COP cells as a potential target for diagnostic or therapeutic intervention.

## 10. Proliferative capacity

High capacity for proliferation is one of the hallmarks of a stem cell, as it allows for generation of a large number of cells from a relative few, enabling the maintenance of constant cellular turnover throughout the life span. Proliferation is commonly assessed by calculation of population doublings over time or with immunofluorescent measures such as the bromodeoxyuridine (BrdU) assay, giving an indication of how rapidly, and expansively, a cell type can multiply [57,58]. Despite the important role proliferation plays in the definition of a stem cell, only few studies have assessed this quality in COP cells. COP cells have been shown to have a similar capacity for proliferation as bone marrow

MSCs [16], with a population doubling time of 2.5 days, with  $5 \times 10^5$  cells becoming  $6.7 \times 10^7$  in 17 days [7]. Another study was able to passage COP cells more than 6 times, and generate millions of additional cells over that time, though no specific measurements were reported [46]. The rate of proliferation over time has also been assessed in COP cells. Using the BrdU assay one study showed that at first passage approximately 50% of the COP cells were dividing, but declined to 5% after five passages [9]. Of these four studies, three were assessing COP cells which were similar in expression to MSCs [7,16,46], with only one study examining COP cells with hematopoietic markers [9]. The functional effect of proliferation has yet to be explored in COP cells. For example, it is known that as the number of passages increase, the ability of bone marrow MSCs to differentiate into their terminal cell types is diminished as the cells become senescent [59]. It has also been demonstrated that the secretome of MSCs changes depending on number of passages, with later passage cells having increased inflammatory activity, cancer cell genesis and migration [59]. It is also important to note that proliferation *in vitro* is tightly regulated, with stem cells multiplying in response to peripheral demand, and entering a quiescent state when the need has been met. This contrasts with a dedicated progenitor cell, which is able to multiply a certain number of times in order to regenerate local cells [60]. While it appears that the MSC-like COP cells exhibit behaviour more akin to stem cells, it is yet unclear how their hematopoietic counterparts behave. These behaviors must be explored in COP cells in order for them to safely and effectively become a viable option for medical use.

## 11. Cell homing

A key piece of information required for utilization of *ex vivo* cellular therapies is the mechanism of cell homing, as it allows for locally targeted therapies and reduces the risk of unintended effects in distant tissues. Both hematopoietic and MSC-like COP cells have been shown to be mobilized by a range of peripheral tissue states and factors including fracture [16,20,27,61], thyroid hormones [43], substance P [17], and hypoxia [18]. The mechanisms by which they home to sites of tissue formation or repair still require much investigation, although it is clear that the CXCR4/SDF-1 plays a role in the process. A number of studies have suggested this by identifying the CXCR4 surface membrane receptor on these cells [15,35], by selectively inhibiting this receptor [15,47] or demonstrating expression of the gene coding for the SDF-1 protein [7]. Additionally, COP cells have been shown to be mobilized by several growth factors used medically to stimulate HSC migration in cancer patients [6]. That the CXCR4/SDF1 axis is also involved in the trafficking of the HSC and leukocyte populations [62] is yet another intriguing parallel between cells of the hematopoietic and mesenchymal lineages worthy of investigation. Despite this evidence supporting the CXCR4/SDF-1 axis as a homing mechanism for these cells, there are numerous other avenues requiring investigation. It is not yet known how COP cells respond to other common chemotactic factors related to tissue injury, growth or development. MSCs are known to migrate in response to a vast range of factors including platelet derived growth factor AB (PDGF-AB), insulin-like growth factor 1 (IGF-1), and macrophage derived chemokine (MDC) [63]. Additionally, the changes in cell homing with increasing number passages must be evaluated, as bone marrow MSCs are known to undergo a decrease in homing ability with increasing expansions [64]. Full evaluation of how these cells target tissues is vital to progress them to medical therapeutics, as well as to provide insights into their physiological behaviors.

## 12. COP cells and bone marrow MSCs: outstanding questions

The similarity between COP cells and bone marrow MSCs is clear, and as such they provide the most likely comparison population on which to base future research. They appear to have similar morphologies and capacity for differentiation into mesenchymal tissues,

however their patterns of surface marker expression seem to vary. There are, however, many properties of MSCs that are unexplored in COP cells. Perhaps most noteworthy of these are the effects and relationships with the human immune system. Bone marrow MSCs are known to have a strong immunomodulatory effects in humans, with suppressive effects on a range of immune cells, including T, B and natural killer cells and enhancement of macrophage activity *via* delay of apoptosis [65]. Additionally, it has been shown that MSCs possess the capability of immune cell evasion, allowing them to be administered to non-host organisms without risk of immune response and rejection [66]. This is mediated by a number of mechanisms, including lack of the major histocompatibility complex class II (MHCII) and related co-stimulatory molecules [67], local immunosuppressive mechanisms inhibiting immune cell activation *via* prostaglandin and interleukin suppression [68], and direct modulation of lymphocyte and dendritic cell function [69,70].

These immune effects are vital in the application of MSC therapeutics, however no studies have evaluated these functions in COP cells. It is also known that MSCs have a strong paracrine function in humans, enabling them to have physiological or therapeutic effects in a diverse range of tissues and diseases [71]. For example, paracrine effects of MSCs have been credited for a large percentage of the cardioprotective benefit they confer in post-myocardial infarction patients [72]. It is also demonstrated that the secretory and functional activity of MSCs changes when the cells are placed under stress. For example, culture of MSCs in an oxidative environment results in increased secretion of antioxidant factors, providing evidence of their ability to respond to local tissue demands [73].

The secretome of COP cells is currently completely unexplored, but research in this area may identify mechanisms of therapeutic action thus far unidentified, as well as potentially broadening the scope of conditions in which they have use. It is currently unknown whether COP cells secrete any factors, or whether this behavior changes according to local conditions. The evaluation and use of MSC therapies has also been limited by the so called “pulmonary first pass effect” in which significant numbers of intravenously injected MSCs become trapped in the lungs before reaching the target tissue [74]. Local therapeutic application of COP cells has been investigated in an animal model with positive results on fracture healing [48], however the fates of the injected cells has not been assessed, so whether they are subject to this effect is unknown.

While the literature surrounding COP cells is still sparse, some preliminary conclusions can be drawn regarding their characterization. Firstly, it is apparent that there are at least two major populations present – a cell population that is very similar in most areas to a bone marrow MSC, and another discrete population with similar behavior, but expressing hematopoietic markers (Fig. 1). The MSC-like COP cells appear to be rarer [7,8,46] and they may only circulate in response to peripheral factors associated with tissue damage and repair, such as substance P [17], hypoxia [18], or SDF-1 [15]. These cells express a very similar panel of surface markers to bone marrow MSCs, do not express hematopoietic markers such as CD45, 34, or 14, and are similar, if not identical, to MSCs in behavior and appearance. The second population has been more extensively researched but is also possibly less well understood due to the divergent methods of identification and analysis. It is currently unclear as to whether this group is made up of one cell type or multiple, as researchers have not used consistent strategies for identification and isolation. It appears that the population expresses some combination of CD45, CD34, and CD14, as well as the markers of bone formation OCN, Col1, and AP. This pattern of expression is unique compared to other known circulating adherent cell types such as endothelial stem cells (CD34+/CD45-) [75], and macrophages (CD34-) [76] though is similar to that of fibrocytes. While the behavior of fibrocytes and COP cells is distinct, more research is required to identify the relationships and roles of these two cell types, particularly given the ability of fibrocytes to differentiate into osteoblasts *in vitro* [77].

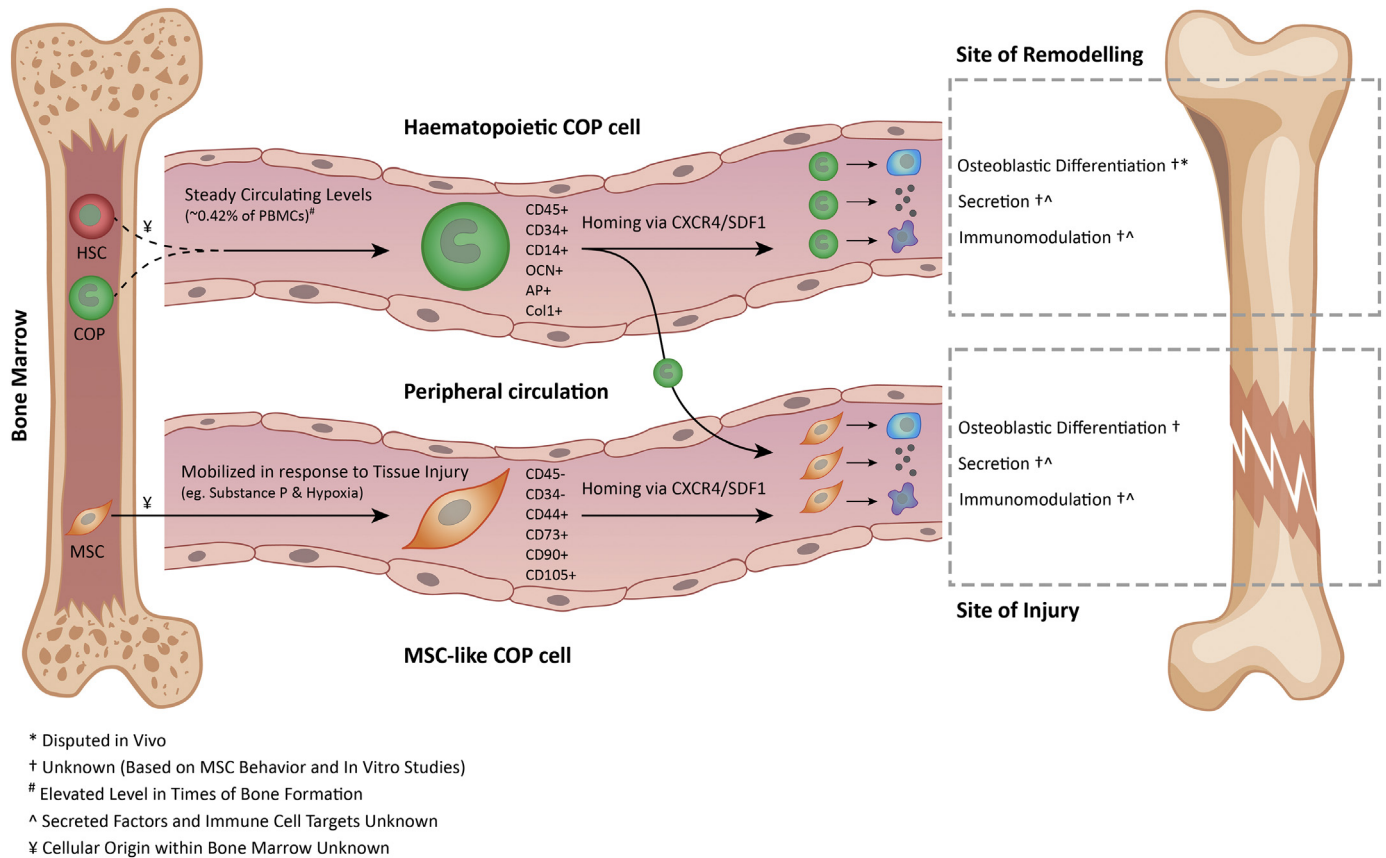


Fig. 2. Summary of known and unknown information in COP cells biology.

Preliminary evidence on the COP cells of hematopoietic lineage suggests they behave similarly to MSCs, however, confirmation and exploration of their proliferative and differentiation capacity is required, alongside careful characterization of their marker expression. Hematopoietic COP cells have been associated with several states of physiological or pathological bone formation, such as puberty and fracture [10], osteoporosis [38–40], and frailty [31,32], indicating that these cells may be a diagnostic or therapeutic target for use in managing these conditions. It has frequently been identified that the isolation and extraction of COP cells is a difficult task due to their poorly understood expression of markers, and their low frequencies [23]. While MSC like COP cells are consistently shown to be rare, and likely circulate only in response to peripheral injury or demand [8,16], hematopoietic COP cells have been shown to circulate in greater numbers [12] and at a steady state throughout the lifespan [11] perhaps providing a greater reservoir to draw upon for culture. There is also minimal evidence demonstrating that they can be sufficiently expanded and manipulated once cultured, providing further hurdles to their use in therapeutic settings, particularly in the case of hematopoietic COP cells. While much remains unknown, it is clear that cells of the blood, vascular and bone lineages are more physiologically connected than once thought, and further study must be done to explore the interplay between these tissues.

### 13. Conclusions

COP cells have profound and exciting implications for future use, particularly in the area of regenerative medicine and the treatment and assessment of musculoskeletal disease. An easily accessible reservoir of stem cells in adult patients would overcome many hurdles in the therapeutic or diagnostic application of these cells. Removal of blood carries significant advantages over bone marrow biopsy in risk and patient comfort, making stem cell therapies a more feasible

treatment strategy. However, before they can be applied in medical settings, significantly further understanding of their biological nature, morphological, and functional properties is required. Future research must therefore, further examine the different cell populations identified in the literature, fully characterizing both, as well as determining their respective physiological roles, behaviors, and interactions. Once this is achieved, it will provide a foundation upon which future research on the role of COP cells in pathology can be based, providing consistency amongst researchers as to the precise identity of the populations being studied.

### Author contributions

Jack Feehan performed the literature search, prepared the manuscript and figures. Gustavo Duque, Vasso Apostolopoulos and Kulmira Nurgali edited the review and supervised the preparation of the manuscript. Ahmed Al Saedi edited the review and assisted with the literature search.

### Declaration of interests

Mr. Jack Feehan has nothing to disclose.  
Dr. Kulmira Nurgali has nothing to disclose.  
Dr. Vasso Apostolopoulos has nothing to disclose.  
Dr. Ahmed Al Saedi has nothing to disclose.  
Dr. Gustavo Duque has nothing to disclose.

### Role of the funding source

No specific funding was received specifically for the preparation of this literature review.



## Acknowledgements

The authors would like to thank the University of Melbourne and the Australian Government Research Training Program Scholarship program for the financial support of Mr. Jack Feehan. The authors would also like to acknowledge Ms. Melanie Thewlis for the artistic production of Fig. 2.

## References

- [1] Geddes A. The origin of the osteoblast and of the osteoclast. *J Anat Physiol* 1913;47 (Pt 2):159.
- [2] Urist MR. Bone: formation by autoinduction. *Science* 1965;150(3698):893–9.
- [3] Tavassoli M, Crosby WH. Transplantation of marrow to extramedullary sites. *Science* 1968;161(3836):54–6.
- [4] Caplan AL. Mesenchymal stem cells. *J Orthop Res* 1991;9(5):641–50.
- [5] Maximow AA. Cultures of blood leucocytes. From lymphocyte and monocyte to connective tissue. *Gustav Fischer* 1928;5:189–178.
- [6] Fernandez M, Simon V, Herrera G, Cao C, Favero H, Minguell J. Detection of stromal cells in peripheral blood progenitor cell collections from breast cancer patients. *Bone Marrow Transplant* 1997;20(4):265.
- [7] Zvaifler NJ, Marinova-Mutafchieva L, Adams G, et al. Mesenchymal precursor cells in the blood of normal individuals. *Arthritis Res Ther* 2000;2(6):477.
- [8] Kuznetsov SA, Mankani MH, Gronthos S, Satomura K, Bianco P, Robey PG. Circulating skeletal stem cells. *J Cell Biol* 2001;153(5):1133–40.
- [9] Kuwana M, Okazaki Y, Kodama H, et al. Human circulating CD14+ monocytes as a source of progenitors that exhibit mesenchymal cell differentiation. *J Leukoc Biol* 2003;74(5):833–45.
- [10] Eghbali-Fatourehchi GZ, Lamsam J, Fraser D, Nagel D, Riggs BL, Khosla S. Circulating osteoblast-lineage cells in humans. *N Engl J Med* 2005;352(19):1959–66.
- [11] Gunawardene P, Al Saedi A, Singh L, et al. Age, gender, and percentage of circulating osteoprogenitor (COP) cells: the COP Study. *Exp Gerontol* 2017;96:68–72.
- [12] Eghbali-Fatourehchi GZ, Modder UI, Charatcharoenwithaya N, et al. Characterization of circulating osteoblast lineage cells in humans. *Bone* 2007;40(5):1370–7.
- [13] Boban I, Barisic-Dujmovic T, Clark SH. Parabiosis model does not show presence of circulating osteoprogenitor cells. *Genesis* 2010;48(3):171–82.
- [14] Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006;8(4):315–7.
- [15] Otsuru S, Tamai K, Yamazaki T, Yoshikawa H, Kaneda Y. Circulating bone marrow-derived osteoblast progenitor cells are recruited to the bone-forming site by the CXCR4/stromal cell-derived factor-1 pathway. *Stem Cells* 2008;26(1):223–34.
- [16] Alm JJ, Koivu HM, Heino TJ, Hentunen TA, Laitinen S, Aro HT. Circulating plastic adherent mesenchymal stem cells in aged hip fracture patients. *J Orthop Res* 2010;28(12):1634–42.
- [17] Hong HS, Lee J, Lee E, et al. A new role of substance P as an injury-inducible messenger for mobilization of CD29(+) stromal-like cells. *Nat Med* 2009;15(4):425–35.
- [18] Rochefort GY, Delorme B, Lopez A, et al. Multipotential mesenchymal stem cells are mobilized into peripheral blood by hypoxia. *Stem Cells* 2006;24(10):2202–8.
- [19] Otsuru S, Tamai K, Yamazaki T, Yoshikawa H, Kaneda Y. Bone marrow-derived osteoblast progenitor cells in circulating blood contribute to ectopic bone formation in mice. *Biochem Biophys Res Commun* 2007;354(2):453–8.
- [20] Kumagai K, Vasanji A, Drazba JA, Butler RS, Muschler GF. Circulating cells with osteogenic potential are physiologically mobilized into the fracture healing site in the parabiotic mice model. *J Orthop Res* 2008;26(2):165–75.
- [21] Dominici M, Pritchard C, Garlits JE, Hofmann TJ, Persons DA, Horwitz EM. Hematopoietic cells and osteoblasts are derived from a common marrow progenitor after bone marrow transplantation. *Proc Natl Acad Sci U S A* 2004;101(32):11761–6.
- [22] Olmsted-Davis EA, Gugala Z, Camargo F, et al. Primitive adult hematopoietic stem cells can function as osteoblast precursors. *Proc Natl Acad Sci* 2003;100(26):15877–82.
- [23] Pignolo RJ, Kassem M. Circulating osteogenic cells: implications for injury, repair, and regeneration. *J Bone Miner Res* 2011;26(8):1685–93.
- [24] Ratajczak MZ, Zuba-Surma EK, Wysoczynski M, Ratajczak J, Kucia M. Very small embryonic-like stem cells: characterization, developmental origin, and biological significance. *Exp Hematol* 2008;36(6):742–51.
- [25] Undale A, Srinivasan B, Drake M, et al. Circulating osteogenic cells: characterization and relationship to rates of bone loss in postmenopausal women. *Bone* 2010;47(1):83–92.
- [26] Simmons PJ, Torok-Storb B. Identification of stromal cell precursors in human bone marrow by a novel monoclonal antibody, STRO-1. *Blood* 1991;78(1):55–62.
- [27] D'Amelio P, Cristofaro MA, Grimaldi A, et al. The role of circulating bone cell precursors in fracture healing. *Calcif Tissue Int* 2010;86(6):463–9.
- [28] Pal SN, Clancy P, Golledge J. Circulating Concentrations of Stem-Cell-Mobilizing Cytokines are Associated with Levels of Osteoprogenitor Cells and Aortic Calcification Severity. *Circ J* 2011;75(5):1227–34.
- [29] Pal SN, Rush C, Parr A, Van Campenhout A, Golledge J. Osteocalcin positive mononuclear cells are associated with the severity of aortic calcification. *Atherosclerosis* 2010;210(1):88–93.
- [30] Dalle Carbonare L, Valenti MT, Zanatta M, Donatelli L, Lo Cascio V. Circulating mesenchymal stem cells with abnormal osteogenic differentiation in patients with osteoporosis. *Arthritis Rheum* 2009;60(11):3356–65.
- [31] Gunawardene P, Bermeo S, Vidal C, et al. Association between circulating osteogenic progenitor cells and disability and frailty in older persons: the Nepean Osteoporosis and Frailty Study. *J Gerontol Ser A: Biomed Sci Med Sci* 2015;71(9):1124–30.
- [32] Al Saedi A, Gunawardene P, Bermeo S, et al. Lamin A expression in circulating osteoprogenitors as a potential biomarker for frailty: the Nepean Osteoporosis and Frailty (NOF) study. *Exp Gerontol* 2017;102:69–75.
- [33] Egan KP, Duque G, Keenan MA, Pignolo RJ. Circulating osteogenic precursor cells in non-hereditary heterotopic ossification. *Bone* 2018;109:61–4.
- [34] Egan KP, Kim JH, Mohler 3rd ER, Pignolo RJ. Role for circulating osteogenic precursor cells in aortic valvular disease. *Arterioscler Thromb Vasc Biol* 2011;31(12):2965–71.
- [35] Suda RK, Billings PC, Egan KP, et al. Circulating osteogenic precursor cells in heterotopic bone formation. *Stem Cells* 2009;27(9):2209–19.
- [36] Otsuru S, Overholt KM, Olson TS, et al. Hematopoietic derived cells do not contribute to osteogenesis as osteoblasts. *Bone* 2017;94:1–9.
- [37] Chen JL, Hunt P, McElvain M, Black T, Kaufman S, Choi EH. Osteoblast precursor cells are found in CD34+ cells from human bone marrow. *Stem Cells* 1997;15(5):368–77.
- [38] Pirro M, Leli C, Fabbriani G, et al. Association between circulating osteoprogenitor cell numbers and bone mineral density in postmenopausal osteoporosis. *Osteoporos Int* 2010;21(2):297–306.
- [39] Pirro M, Schillaci G, Mannarino MR, et al. Circulating immature osteoprogenitor cells and arterial stiffening in postmenopausal osteoporosis. *Nutr Metab Cardiovasc Dis* 2011;21(9):636–42.
- [40] Pirro M, Manfredelli MR, Scarponi AM, et al. Association between thyroid hormone levels, the number of circulating osteoprogenitor cells, and bone mineral density in euthyroid postmenopausal women. *Metabolism* 2012;61(4):569–76.
- [41] Rattazzi M, Faggini E, Buso R, et al. Atorvastatin Reduces Circulating Osteoprogenitor Cells and T-Cell RANKL Expression in Osteoporotic Women: Implications for the Bone-Vascular Axis. *Cardiovasc Ther* 2016;34(1):13–20.
- [42] Kelly RR, McDonald LT, Pellegrini VD, Cray JJ, Larue AC. Identification of circulating murine CD34+OCN+ cells. *Cytotherapy*.
- [43] Rubin MR, Manavalan JS, Dempster DW, et al. Parathyroid hormone stimulates circulating osteogenic cells in hypoparathyroidism. *J Clin Endocrinol Metab* 2011;96(1):176–86.
- [44] Manavalan JS, Cremers S, Dempster DW, et al. Circulating osteogenic precursor cells in type 2 diabetes mellitus. *J Clin Endocrinol Metab* 2012;97(9):3240–50.
- [45] Rubin MR, Manavalan JS, Agarwal S, et al. Effects of rosiglitazone vs metformin on circulating osteoclast and osteogenic precursor cells in postmenopausal women with type 2 diabetes mellitus. *J Clin Endocrinol Metab* 2014;99(10):E1933–42.
- [46] Kuznetsov SA, Mankani MH, Leet AI, Ziran N, Gronthos S, Robey PG. Circulating connective tissue precursors: extreme rarity in humans and chondrogenic potential in Guinea pigs. *Stem Cells* 2007;25(7):1830–9.
- [47] Fadini GP, Albiero M, Menegazzo L, et al. Widespread increase in myeloid calcifying cells contributes to ectopic vascular calcification in type 2 diabetes. *Circ Res* 2011;108(9):1112–21 [CIRCRESAHA. 110.234088].
- [48] Wan C, He Q, Li G. Allogenic peripheral blood derived mesenchymal stem cells (MSCs) enhance bone regeneration in rabbit ulna critical-sized bone defect model. *J Orthop Res* 2006;24(4):610–8.
- [49] Long MW, Robinson J, Ashcraft E, Mann KG. Regulation of human bone marrow-derived osteoprogenitor cells by osteogenic growth factors. *J Clin Invest* 1995;95(2):881–7.
- [50] Long MW, Williams JL, Mann KG. Expression of human bone-related proteins in the hematopoietic microenvironment. *J Clin Invest* 1990;86(5):1387–95.
- [51] Staveland R, Robinson AM, Miller S, Boyd R, Sakka S, Nurgali K. Human adult stem cells derived from adipose tissue and bone marrow attenuate enteric neuropathy in the Guinea-pig model of acute colitis. *Stem Cell Res Ther* 2015;6(1):244.
- [52] Mauney JR, Kaplan DL, Volloch V. Matrix-mediated retention of osteogenic differentiation potential by human adult bone marrow stromal cells during ex vivo expansion. *Biomaterials* 2004;25(16):3233–43.
- [53] Baxter MA, Wynn RF, Jowitt SN, Wraith JE, Fairbairn LJ, Bellantuono I. Study of telomere length reveals rapid aging of human marrow stromal cells following in vitro expansion. *Stem Cells* 2004;22(5):675–82.
- [54] Singh L, Brennan TA, Russell E, et al. Aging alters bone-fat reciprocity by shifting in vivo mesenchymal precursor cell fate towards an adipogenic lineage. *Bone* 2016;85:29–36.
- [55] Sethe S, Scutt A, Stolzinger A. Aging of mesenchymal stem cells. *Ageing Res Rev* 2006;5(1):91–116.
- [56] Scuteri A, Miloso M, Foudah D, Orciani M, Cavaletti G, Tredici G. Mesenchymal stem cells neuronal differentiation ability: a real perspective for nervous system repair? *Curr Stem Cell Res Ther* 2011;6(2):82–92.
- [57] Cc-W Yu, Woods AL, Levison DA. The assessment of cellular proliferation by immunohistochemistry: a review of currently available methods and their applications. *Histochem J* 1992;24(3):121–31.
- [58] Lawless C, Wang C, Jurk D, Merz A, von Zglinicki T, Passos JF. Quantitative assessment of markers for cell senescence. *Exp Gerontol* 2010;45(10):772–8.
- [59] Turinetti V, Vitale E, Giachino C. Senescence in Human Mesenchymal Stem Cells: Functional changes and Implications in Stem Cell-based Therapy. *Int J Mol Sci* 2016;17(7):1164.
- [60] Seaberg RM, van der Kooy D. Stem and progenitor cells: the premature desertion of rigorous definitions. *Trends Neurosci* 2003;26(3):125–31.
- [61] Ritz U, Spies V, Mehling I, Gruszka D, Rommens PM, Hofmann A. Mobilization of CD34+—progenitor cells in patients with severe trauma. *PLoS One* 2014;9(5):e97369.
- [62] Kucia M, Jankowski K, Reza R, et al. CXCR4–SDF-1 signalling, locomotion, chemotaxis and adhesion. *J Mol Histol* 2004;35(3):233–45.

- [63] Eggenhofer E, Luk F, Dahlke MH, Hoogduijn MJ. The life and fate of mesenchymal stem cells. *Front Immunol* 2014;5:148.
- [64] Sohni A, Verfaillie CM. Mesenchymal stem cells migration homing and tracking. *Stem Cells Int* 2013;2013:130763.
- [65] Ghannam S, Bouffi C, Djouad F, Jorgensen C, Noël D. Immunosuppression by mesenchymal stem cells: mechanisms and clinical applications. *Stem Cell Res Ther* 2010;1(1):2.
- [66] Ryan JM, Barry FP, Murphy JM, Mahon BP. Mesenchymal stem cells avoid allogeneic rejection. *J Inflamm* 2005;2(1):8.
- [67] Le Blanc K, Tammik C, Rosendahl K, Zetterberg E, Ringdén O. HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. *Exp Hematol* 2003;31(10):890–6.
- [68] Ankrum JA, Ong JF, Karp JM. Mesenchymal stem cells: immune evasive, not immune privileged. *Nat Biotechnol* 2014;32(3):252.
- [69] Zhang W, Ge W, Li C, et al. Effects of mesenchymal stem cells on differentiation, maturation, and function of human monocyte-derived dendritic cells. *Stem Cells Dev* 2004;13(3):263–71.
- [70] Krampera M, Glennie S, Dyson J, et al. Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. *Blood* 2003;101(9):3722–9.
- [71] Gnechi M, Zhang Z, Ni A, Dzau VJ. Paracrine mechanisms in adult stem cell signaling and therapy. *Circ Res* 2008;103(11):1204–19.
- [72] Gnechi M, He H, Noiseux N, et al. Evidence supporting paracrine hypothesis for Akt-modified mesenchymal stem cell-mediated cardiac protection and functional improvement. *FASEB J* 2006;20(6):661–9.
- [73] Lanza C, Morando S, Voci A, et al. Neuroprotective mesenchymal stem cells are endowed with a potent antioxidant effect in vivo. *J Neurochem* 2009;110(5):1674–84.
- [74] Fischer UM, Harting MT, Jimenez F, et al. Pulmonary passage is a major obstacle for intravenous stem cell delivery: the pulmonary first-pass effect. *Stem Cells Dev* 2009;18(5):683–92.
- [75] Urbich C, Dimmeler S. Endothelial progenitor cells: characterization and role in vascular biology. *Circ Res* 2004;95(4):343–53.
- [76] Woltman AM, de Fijter JW, Kamerling SW, et al. Rapamycin induces apoptosis in monocyte- and CD34-derived dendritic cells but not in monocytes and macrophages. *Blood* 2001;98(1):174–80.
- [77] Choi YH, Burdick MD, Strieter RM. Human circulating fibrocytes have the capacity to differentiate osteoblasts and chondrocytes. *Int J Biochem Cell Biol* 2010;42(5):662–71.
- [78] Matsumoto T, Kawamoto A, Kuroda R, et al. Therapeutic potential of vasculogenesis and osteogenesis promoted by peripheral blood CD34-positive cells for functional bone healing. *Am J Pathol* 2006;169(4):1440–57.