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REVIEW

The origins and roles of osteoclasts in bone development, homeostasis and repair

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ABSTRACT

The mechanisms underlying bone development, repair and regeneration are reliant on the interplay and communication between osteoclasts and other surrounding cells. Osteoclasts are multinucleated monocyte lineage cells with resorptive abilities, forming the bone marrow cavity during development. This marrow cavity, essential to hematopoiesis and osteoclast-osteoblast interactions, provides a setting to investigate the origin of osteoclasts and their multi-faceted roles. This Review examines recent developments in the embryonic understanding of osteoclast origin, as well as interactions within the immune environment to regulate normal and pathological bone development, homeostasis and repair.

KEY WORDS: Osteoclast, Bone development, Hematopoietic stem cell, Yolk sac

Introduction

Interaction between different cell types is fundamental for development, repair and regeneration. In bone, recent data has uncovered that interactions between immune-regulated monocyte/macrophage lineage cells (osteoclasts) and mesenchymal cells that form the structural components of bone (osteoblasts) are crucial for normal bone homeostasis and its successful repair (Ambrosi et al., 2021; Kim et al., 2020; Cawley et al., 2020). Osteoclasts are multinucleated monocyte-lineage cells uniquely present in the bone, where they are required to form the bone marrow cavity, a site necessary for adult hematopoiesis (Sugiyama and Nagasawa, 2012; Aguila and Rowe, 2005). In addition, osteoclasts are essential for the establishment and maintenance of bone homeostasis, as well as repair after injury (Novack and Teitelbaum, 2008).

The multinucleated morphology of osteoclasts has generated much discussion about their origin, function and regulation since their discovery by Kolliker in 1873 (Nijweide et al., 1986). As a resident bone cell population, there was initially an assumed commonality between osteoclasts and osteoblasts in the early 20th century (Tonna, 1960; Young, 1962; Bloom et al., 1941). However, mounting evidence began to support a 'biphyletic origin' theory between the two bone cell types, and it was observed that there were morphological similarities between mature osteoclast and macrophage-derived cells (Hancox, 1949). Early rodent parabiosis and avian egg transplantation experiments provided evidence that osteoclasts shared a common hematopoietic origin with macrophages, explaining how osteoclasts could be recruited via blood (Jotereau and Douarin, 1978; Nijweide et al., 1986; Walker, 1973). Additional reciprocal rescue and induction analyses of osteopetrosis from spleen and bone marrow cell suspensions continued to delineate osteoclasts' hematopoietic origins (Walker, 1975a,b).

In recent years, our understanding of osteoclast biology faced a paradigm shift owing to advances in mouse genomics, intravital imaging and the emergence of single-cell genomics. Prior dogmas have been revisited and replaced by new concepts. Indeed, newly discovered notions of osteoclast origin and cell recycling have changed our interpretations of osteoclast cell fate, determination and longevity, and provide new insights into osteoclast formation and maintenance.

Here, we review these recent developments in understanding the origin and roles of osteoclasts. We describe the embryonic and adult origins of osteoclasts and their role in the regulation of normal and pathological bone development, homeostasis and repair. Finally, we discuss the immune functions of osteoclasts during homeostasis and disease.

Origins of the osteoclasts

The hematopoietic system is initially established in mammals by several sequential and overlapping waves during embryonic development (Hoeffel et al., 2015; Schulz et al., 2012; Sheng et al., 2015; Munro and Hughes, 2017) (Figs 1 and 2). The first wave of hematopoiesis in mammals (also termed 'primitive') starts in the blood islands of the yolk sac and gives rise to nucleated erythroblasts, megakaryocytes and macrophages (Moore and Metcalf, 1970; Palis et al., 1999; Hoeffel and Ginhoux, 2018; Tober et al., 2006; Naito et al., 1989; Takahashi et al., 1989). The yolk-sac hemogenic endothelium produces early erythromyeloid progenitors (EMPs) around embryonic day (E) 7-E7.5 in the yolk sac (Ginhoux et al., 2010; Italiani and Boraschi, 2017), which can become colony-stimulating factor 1 receptor $(CSF1R)^{+}$ yolk-sac macrophages by E8.5. These 'early' EMPs develop independently of the transcription factor MYB and give rise to primitive yolk-sac macrophages without passing through monocyte intermediates (Gomez Perdiguero et al., 2015; Hoeffel and Ginhoux, 2018). Around E8.25, the yolk-sac vascular system connects to the intra-embryonic circulation (McGrath et al., 2003) and yolk-sac macrophages can then colonize embryonic organs, such as the brain and liver (Kierdorf et al., 2013; Gomez Perdiguero et al., 2015). The second wave of hematopoiesis develops from E8.25, in which MYB-dependent 'late' EMPs emerge in the yolk sac and migrate into the fetal liver to produce fetal liver monocytes (Hoeffel et al., 2015; McGrath et al., 2015; Mass et al., 2016). Later in development, the final wave of hematopoiesis is initiated by hematopoietic stem cell (HSC) precursors, which emerge in the aorta-gonad-mesonephros

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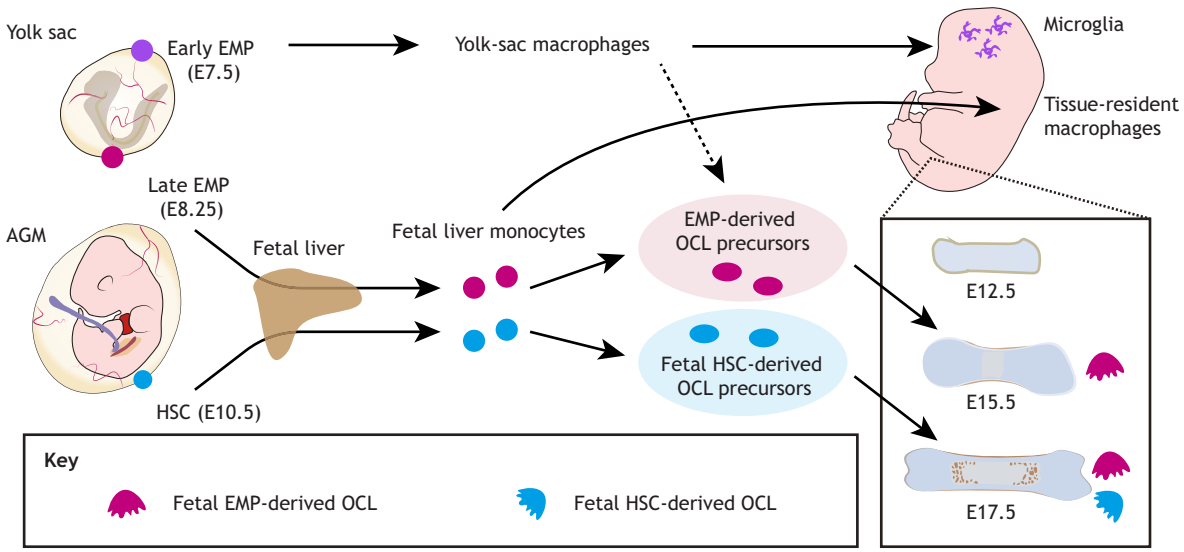

Fig. 1. Schematic showing the developmental origin of osteoclasts. Early erythromyeloid progenitors (EMPs; purple lineage) appear around E7-E7.5 in the yolk sac and differentiate into yolk-sac macrophages that give rise to tissue-resident macrophage populations, such as brain microglia. Late EMPs (pink lineage) emerge in the yolk sac at around E8.25-E9 and migrate to the fetal liver to produce fetal liver monocytes. These EMP-derived monocytes/macrophages can give rise to embryonic osteoclasts (OCLs), forming the bone marrow cavity around E15.5, which is necessary for hematopoiesis during development. Fetal hematopoietic stem cells (HSCs; blue lineage) emerge at E10.5 in the aorta-gonad-mesonephros (AGM) region and also migrate to the fetal liver, where they give rise to OCL precursors.

region at E10.5. Subsequently, HSCs serve a central role in maintaining the hematopoietic system throughout the life of the organism (Medvinsky et al., 1993; Müller et al., 1994). HSCs mature and expand in the fetal liver, and later colonize the bone marrow for adult hematopoiesis. Bone marrow HSCs eventually establish circulating monocyte-derived macrophages. Initially, it was believed that the yolk-sac wave of hematopoiesis was a transient blood supply that was completely replaced by HSC-derived cells. However, accumulating evidence suggests that some adult macrophages are established during fetal development and maintained in postnatal tissues via proliferation (reviewed by Lee and Ginhoux, 2022; Epelman et al., 2014; Gomez Perdiguero et al., 2015). Both EMP-derived macrophages and HSC-derived monocytes can produce embryonic and postnatal osteoclasts (Jacome-Galarza et al., 2019; Yahara et al., 2020) (Fig. 1).

EMP-derived osteoclasts

A recent study using genetic lineage tracing demonstrated that EMP-derived osteoclasts are crucial for development of the normal fetal skeleton, and their absence impairs tooth eruption, skull formation and long bone formation (Jacome-Galarza et al., 2019). Genetic lineage-tracing experiments showed that EMP-derived osteoclasts are long-lived, surviving at least 6 months after birth, and are responsible for steady-state bone remodeling and fracture healing (Yahara et al., 2020). In addition, EMP-derived osteoclast precursors can migrate through the bloodstream to the site of bone injury and differentiate into mature osteoclasts (Yahara et al., 2020). EMP-derived osteoclast precursors are gradually replaced by HSC-derived, mononuclear, monocyte-progenitor cells (Jacome-Galarza et al., 2019). However, some monocyte-progenitor cells can fuse with long-lived EMP-derived osteoclasts, thus maintaining the population throughout adulthood (Jacome-Galarza et al., 2019).

HSC-derived osteoclasts

Many studies demonstrated that HSCs, and their myeloid progeny, provide osteoclast progenitor cells (Box 1; Fig. 2). In the classical model of hematopoiesis, bone marrow HSCs differentiate into multipotent progenitors (MPPs), resulting in lineage-restricted precursors (Kawamoto et al., 2010; Seita and Weissman, 2010). The precursors then give rise to oligopotent progenitors: common myeloid progenitors (CMPs) and common lymphoid progenitors (CLPs). CMPs produce megakaryocyte/erythrocyte progenitors (MEPs) and granulocyte/macrophage progenitors (GMPs) (Fig. 2). Furthermore, GMPs differentiate into a common macrophage/osteoclast/dendritic cell (DC) progenitor (MODP), with DCs functioning as an antigen-presenting cell (APC) population that assists in the adaptive immune system (Jacome-Galarza et al., 2013; Geissmann et al., 2008). These MODPs later give rise to mature osteoclasts through interaction with the receptor activator of the nuclear factor-kappa B (NF-κB) ligand (RANKL; also known as TNFSF11) and colony stimulating factor 1 [CSF1; also known as macrophage colony-stimulating factor (M-CSF)], which are essential for the continued differentiation of mature osteoclasts (Miyamoto et al., 2001; Cecchini et al., 1997).

Although bone marrow-derived HSCs and their descendants provide precursor cells for osteoclasts, there is controversy regarding whether differentiated monocytes/macrophages and DCs can give rise to osteoclasts. Udagawa and colleagues confirmed that osteoclasts originate from monocytes/macrophages (Udagawa et al., 1990). Arai and colleagues found that a KIT proto-oncogene receptor tyrosine kinase $(KIT)^+$ and $CSF1R^+$ population of mouse bone marrow mononuclear cells can be a source of early-stage osteoclast precursors (Arai et al., 1999). In human pediatric bone marrow, interleukin 3 receptor subunit alpha (IL3Rα)-expressing cells are the common osteoclast precursor (Xiao et al., 2015). Although monocyte/macrophage cells were thought to be the precursors of osteoclasts, some reports proposed that osteoclasts also arise through DCs (Rivollier et al., 2004; Speziani et al., 2007). Furthermore, a recent study using single-cell RNA sequencing (scRNA-seq) provided more detailed insights into the stepwise cell fate transition from the bone marrow-derived precursors to mature osteoclasts (Tsukasaki et al., 2020b). The differentiation trajectory

Development (2022) 149, dev199908. doi:10.1242/dev.199908

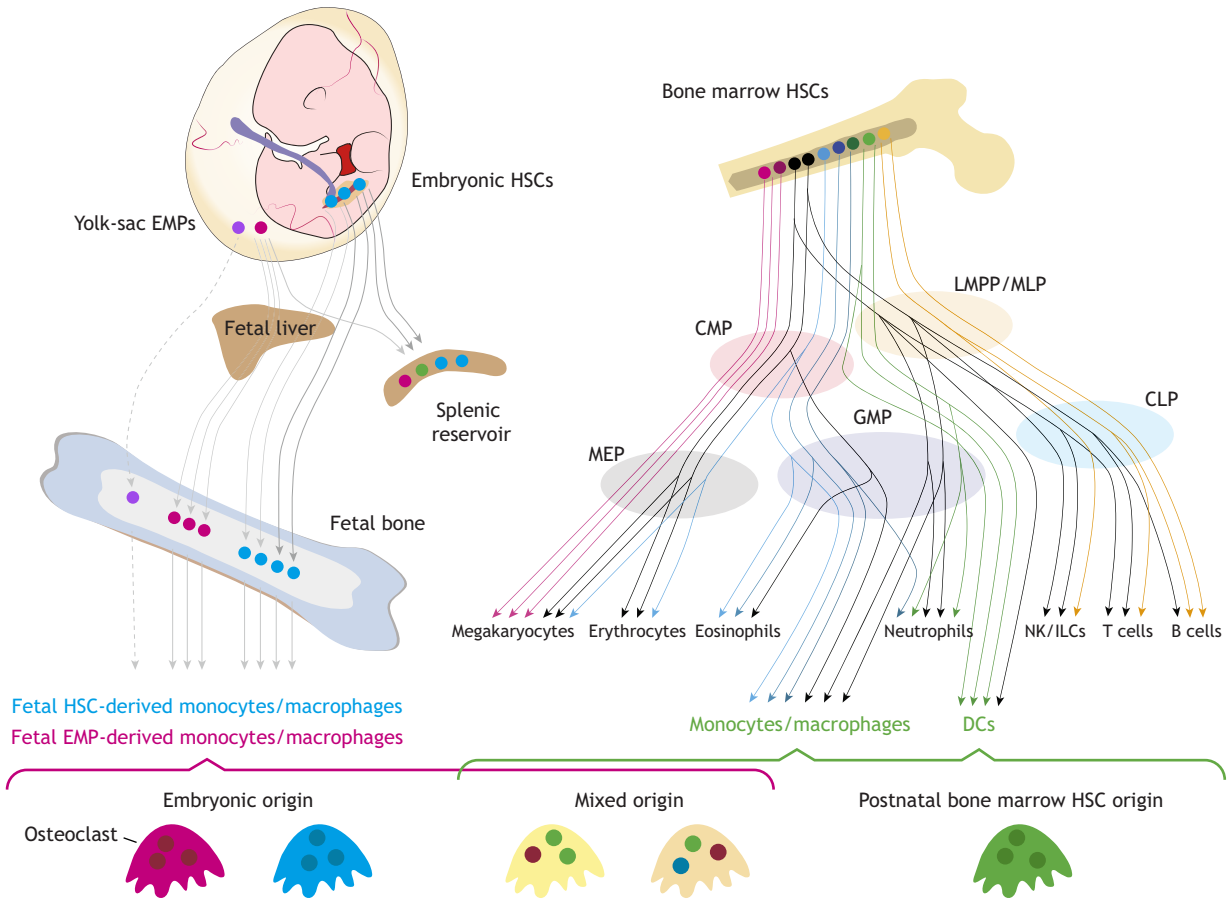

Fig. 2. Complexity and variety of osteoclasts with multiple developmental origins. Schematic showing the diversity of osteoclasts. Early and late embryonic yolk-sac erythromyeloid progenitors (EMPs; purple and pink lineages, respectively) and fetal hematopoietic stem cells (HSCs; blue) can produce fetal monocyte-derived osteoclast progenitors. Postnatal bone marrow HSCs form monocytes/macrophages and dendritic cells (DCs) through continuous differentiation processes, which give rise to osteoclasts (green) (schematic based on Laurenti and Göttgens, 2018). Postnatal bone marrow HSC-derived, fetal HSC-derived and embryonic late EMP-derived osteoclast precursors fuse to form a diverse and complex osteoclast diversity (yellow multinucleated cells). CLP, common myeloid progenitor; CMP, common myeloid progenitor; GMP, granulocyte-monocyte progenitors; ILCs, innate lymphoid cells; LMPP, lymphoid-primed multipotent progenitor; MEP, megakaryocyte-erythrocyte progenitors; MLP, multi-lymphoid progenitor; NK, natural killer cells.

of osteoclasts clearly shows that monocyte-like progenitor cells give rise to mature osteoclasts via CD11c (ITGAX)$^{+}$ DC populations (Fig. 2). In addition, ablation of RANK (TNFRSF11a) in CD11c^{+} cells suppressed osteoclast differentiation, suggesting that this CD11c^{+} DC-like precursor population is primed for osteoclast differentiation and maturation (Tsukasaki et al., 2020b).

Osteoclast multinucleation, differentiation and maturation

Cell-cell fusion of mononuclear osteoclast precursors and multinucleation are essential processes for osteoclast maturation and its bone resorption capacity (Fig. 3A). Osteoclast fusion is classically thought to consist of sequential steps: migration, recognition, intercellular adhesion and membrane fusion (Søe, 2020). Osteoclast precursor cells reside in the bone marrow cavity, adjacent to collagen fibers and vascular networks. Following cell-cell fusion with mature osteoclasts, single-nucleated precursors migrate from the bone marrow cavity to sites of resorption on the bone surface. Hobolt-Pedersen and colleagues proposed that osteoclast precursors fuse selectively, not randomly, to recognize their fusion partners (Hobolt-Pedersen et al., 2014). This selectivity is based on the intercellular heterogeneity, such as maturity, mobility and nuclearity (Hobolt-Pedersen et al., 2014; Søe, 2020; Søe et al., 2015). Additionally, osteoclast fusion factor, CD47, DC-specific transmembrane protein (DC-STAMP) and syncytin-1 are involved in the development of this heterogeneity (Søe, 2020). Mononuclear osteoclast precursors and their fusion partners need to move towards and adhere to each other for multinucleation to proceed. $\alpha_V\beta3$ integrin, which localizes to podosomes at the leading edge of osteoclasts, plays an important role in migration and formation of the sealing zone (Nakamura et al., 1999; McHugh et al., 2000). In mice deficient for the DC-STAMP, d2 isoform of vacuolar (H^{+}) ATPase (v-ATPase) V0 domain (ATP6v0d2) and osteoclast stimulatory transmembrane protein (OC-STAMP), only osteoclasts with a single nucleus were found, indicating that these proteins are essential for the multinucleation of osteoclasts (Yagi et al., 2005; Miyamoto et al., 2012; Lee et al., 2006). Impairment of osteoclast multinucleation reduced bone resorption activity resulting in osteopetrosis. CD44, CD47, syncytin-1, PIN1 (peptidyl-prolyl cis-trans isomerase NIMA-interacting 1) and tetraspanins (CD9, CD81) have also been reported to be crucial for osteoclast fusion and multinucleation (Sterling et al., 1998; Takeda et al., 2003; Cui et al., 2006; Søe et al., 2011; Islam et al., 2014; Møller et al., 2017). Thus, osteoclast fusion and multinucleation are not random processes, but are controlled through a strict mechanism by which fusion partners are selected based on the heterogeneity of osteoclast precursors.

Development (2022) 149, dev199908. doi:10.1242/dev.199908

Box 1. New insights into hematopoiesis

The classical model of hematopoiesis is based on a long-held dogma that HSCs are at the top of the hierarchy, capable of self-renewal and generation of all blood lineage cells. However, with the advancement of next-generation techniques, such as scRNA-seq combined with genetic lineage tracing, in the last few years this classical view of hematopoiesis has been questioned (Notta et al., 2016; Velten et al., 2017; Paul et al., 2015; Grün et al., 2016). For example, recent studies identified several hematopoietic cell populations and unexpected branching points of hematopoiesis, suggesting that hematopoiesis is better described as a continuous process rather than a discrete hierarchy (Velten et al., 2017; Laurenti and Göttgens, 2018; Cheng et al., 2020) (Fig. 2). HSCs and early progenitor cells are heterogeneous and contain distinct subpopulations with different differentiation potentials, suggesting that the lineages are determined at an early stage of hematopoiesis. Although most hematopoietic progenitors can develop into one mature cell type (i.e. uni-lineage potential), bi- and multi-lineage progenitors are rare, but present (Karamitros et al., 2018). GMPs, defined as a LIN-KIT^{+}, CD34^{high} and CD16/32^{high} lineage-primed progeny derived from CMPs, already contain early committed neutrophil progenitors, which increase extensively in the early stages of inflammation at the expense of monocyte differentiation (Kwok et al., 2020). Lymphoid-primed multipotent progenitors (LMPPs) can give rise to lymphoid and myeloid cells, including DCs (Fig. 2). A recent study showed that DC-lineage specification starts close to the HSC stage (Lee et al., 2017) and the transcription factor interferon regulatory factor 8 (IRF8) regulates chromatin at the LMPP stage to induce early commitment toward DCs (Kurotaki et al., 2019). Whether osteoclasts can be derived through alternative routes, such as LMPP-derived DCs, remains to be shown. Further study is necessary to elucidate when osteoclast-lineage specification starts and how their fate is determined.

The earliest step in osteoclast formation begins with a mononuclear, monocytic progenitor cell expressing the E26 transformation-specific (ETS) domain transcription factor PU.1 (SPI1), which is indispensable for hematopoietic cell fate (Burda et al., 2010). PU.1 regulates the expression of CSF1R and RANK gene expression in myeloid progenitor cells, resulting in the establishment of osteoclast-specific transcriptional pattern driven by RANKL-RANK signaling (Tondravi et al., 1997; Kwon et al., 2005). In the bone microenvironment, osteoblasts, osteocytes and osteoclasts directly communicate with each other through either cell-to-cell interaction or paracrine signaling molecules (Furuya et al., 2018; Kim et al., 2020) (Fig. 3B). Osteoblasts secrete M-CSF (MacDonald et al., 1986; Fuller et al., 1993; Tanaka et al., 1993), RANKL (Kong et al., 1999; Lacey et al., 1998; Yasuda et al., 1998b) and Wnt gene family 5A (WNT5A) (Maeda et al., 2012) to promote osteoclast differentiation. In contrast, osteoprotegerin (OPG; TNFRSF11b), a decoy receptor of RANKL (Simonet et al., 1997; Tsuda et al., 1997; Yasuda et al., 1998a), semaphorin 3A (SEMA3A) (Hayashi et al., 2012) and WNT16 (Kobayashi et al., 2015) secreted by osteoblasts inhibit osteoclast differentiation. Osteoclast-derived factors that affect osteoblast differentiation and function include bone morphogenetic protein 6 (BMP6) (Pederson et al., 2008), collagen triple helix repeat containing 1 (CTHRC1) (Takeshita et al., 2013), sphingosine 1-phosphate (S1P) (Ryu et al., 2006; Pederson et al., 2008), SEMA4D (Negishi-Koga et al., 2011) and cardiotrophin-1 (CT-1; CTF1) (Walker et al., 2008). Osteocytes are multifunctional and dynamic cells that can integrate both mechanical cues (Qin et al., 2020) and hormonal signals (Wein, 2018) to modulate the development and function of osteoclasts and osteoblasts. Osteocytes are a prime source of RANKL, a major osteoclast differentiation facilitator (Nakashima et al., 2011; Xiong et al., 2011), and sclerostin (SOST), which inhibits preosteoblast differentiation via inhibitory effects on the WNT/β-catenin pathway (Winkler et al., 2003; Bezooijen et al., 2005). During osteoclast differentiation and maturation, osteoblasts and osteoclasts communicate with each other through direct cell-to-cell interaction allowing bidirectional signal transduction. Ephrin B2, which is expressed on the cell surface of osteoclasts, binds to the osteoblast surface molecule ephrin type-B receptor 4 (EPHB4). Reverse signaling through ephrin B2 into osteoclast precursors suppresses osteoclast differentiation by inhibiting the FOS/NFATc1 (nuclear factor of activated T cells c1) axis (Zhao et al., 2006). In contrast, forward signaling through EPHB4 into osteoblasts promotes osteoblast differentiation (Zhang et al., 2010). SEMA3A is produced by osteoblast lineage cells and works as a potent osteoprotective effector by simultaneously inhibiting bone resorption and promoting bone formation (Hayashi et al., 2012, 2019). By binding to neuropilin 1 (NRP1), SEMA3A inhibits RANKL-induced osteoclast function and promotes osteoblast differentiation via the WNT/β-catenin pathway (Hayashi et al., 2012). RANK-enriched vesicles secreted from osteoclasts can increase bone formation by promoting RANKL reverse signaling through activating runt-related transcription factor 2 (RUNX2) (Ikebuchi et al., 2018). This cell-cell interaction and the bone microenvironment promote osteoclast differentiation, eventually giving rise to mature osteoclasts. Mature osteoclasts drive bone resorption, a crucial component in the maintenance of bone homeostasis.

The role of osteoclasts in bone development

The embryonic skeleton begins its formation via intramembranous and endochondral ossification. Intramembranous ossification is characterized by mesenchymal cells directly differentiating into osteoblasts, as is the case for the skull, mandibles and clavicles (Berendsen and Olsen, 2015). Endochondral ossification begins with the condensation of mesenchymal cells to form a primordial bone template (Berendsen and Olsen, 2015) (Fig. 4A). The mesenchymal cells differentiate into chondrocytes, which then proliferate, increasing the size of the condensation (Berendsen and Olsen, 2015). The perichondrium covers the periphery of the primordial cartilage (Kronenberg, 2003). Chondrocytes differentiate into hypertrophic chondrocytes forming a hypertrophic zone that then mineralizes (Kronenberg, 2003). The surrounding perichondrium also differentiates and forms the osteogenic periosteum (Colnot et al., 2004). The epiphyses are at the ends of the bone and are composed entirely of growth plate cartilage and are separated by the primary ossification center with vascular invasion (Berendsen and Olsen, 2015). Subsequently, the hypertrophic chondrocytes undergo apoptosis or are directly converted to osteoblasts (Yang et al., 2014; Zhou et al., 2014b; Ono et al., 2014). Shortly after birth, a secondary ossification center appears in the epiphysis. The secondary ossification center is then vascularized and forms the articular cartilage and epiphyseal growth plate (Fig. 4A).

Osteoblast origins and differentiation

The origin of the cells that produce bone is an area of continued investigation; these cells can come from several sources, including skeletal stem cells (SSCs), vascular cells and growth plate chondrocytes that differentiate into osteoblasts. SSCs with multi-lineage differentiation potential were thought to be present in the bone marrow niche, identified based on combinations of cell markers, such as platelet-derived growth factor receptor alpha (PDGFRα)$^{+}$/stem cell

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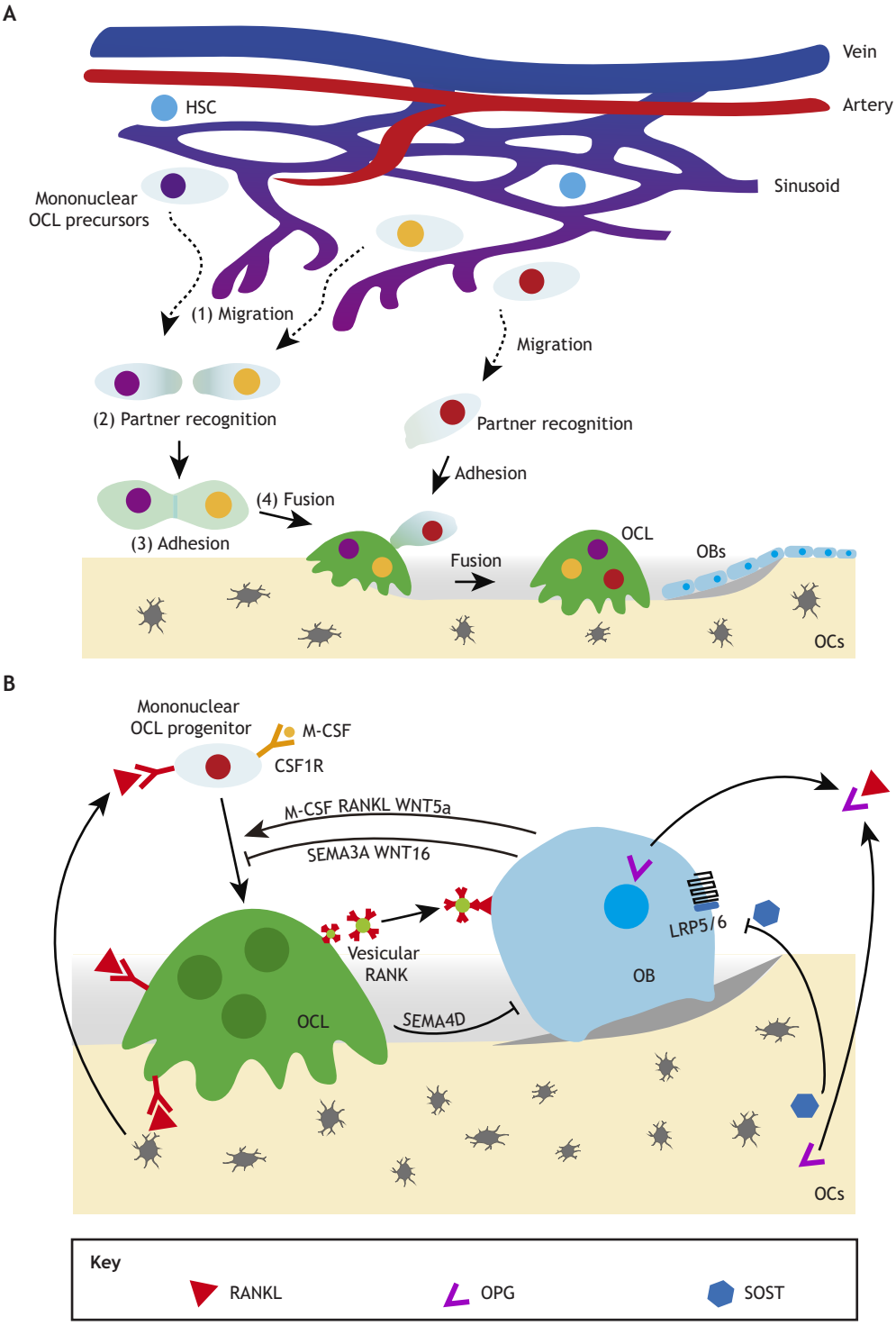

Fig. 3. Osteoclast specification. (A) Osteoclast (OCL) fusion and maturation. The osteoclast fusion consists of sequential steps: (1) migration, (2) partner recognition, (3) adhesion and (4) fusion. Osteoclast progenitor cells reside in the bone marrow cavity proximal to vascular networks. Single-nucleated osteoclast precursors (shown with purple, orange and red nuclei) migrate from the bone marrow cavity to sites of resorption on the bone surface. Osteoclast progenitors selectively recognize their fusion partners, followed by cell-cell fusion and maturation. (B) Osteoclast-osteoblast-osteocyte interactions. Osteoblasts (OBs), osteocytes (OCs) and osteoclasts directly communicate each other through either cell-to-cell interaction or paracrine signaling molecules. Osteoblasts secrete M-CSF, RANKL and WNT5A to promote osteoclast differentiation. OPG, SEMA3A and WNT16 secreted by osteoblasts inhibit osteoclast differentiation. SEMA4D suppresses osteoblast differentiation. Osteocytes regulate the balance of bone formation and resorption by secreting RANKL/OPG. SOST from osteocytes interacts with LRP5/6 and suppresses preosteoblast differentiation via inhibitory effects on the WNT/β-catenin pathway. RANK-enriched vesicles secreted from osteoclasts can increase bone formation by triggering RANKL reverse signaling. HSC, hematopoietic stem cell.

antigen-1 (SCA-1; LY6A)$^{+}$/CD45 (PTPRC)$^{-}$TER119 (LY76)$^{-}$ cells (Morikawa et al., 2009), CD73 (NT5E)$^{+}$/CD31 (PECAM)$^{-}$ cells (Breitbach et al., 2018), CD271 (NGFR)$^{+}$/CD45^{-} cells (Álvarez-Viejo et al., 2015; Coutu et al., 2017), and leptin receptor (LEPR)$^{+}$ cells (Zhou et al., 2014a; Yue et al., 2016). However, recent *in vivo* lineage-tracing studies using mouse genetic models demonstrated that the growth plate and skeleton surrounding the periosteum containing SSCs plays an essential role in bone development and formation (Newton et al., 2019). During fetal and neonatal bone growth, chondroprogenitors in the resting zone are gradually consumed and recruited into the proliferative columns (Newton et al., 2019). Therefore, individual rows of proliferating and hypertrophic chondrocytes are not derived from a single resting chondrocyte but have multiple origins (Fig. 4B). However, after forming the secondary ossification center, chondroprogenitors start renewing and generate long columns from single clones (Newton et al., 2019). Parathyroid hormone-related peptide (PTHrP; PTHLH)$^{+}$ chondroprogenitors are present in the resting zone of the mouse growth plate, and give rise to columnar chondrocytes, osteoblasts and a subset of long-lived stem cells in the bone marrow niche (Mizuhashi et al., 2018) (Fig. 4C). Furthermore, recent studies also identified cathepsin K (CTSK)$^{+}$/CD200^{+} cells (Debnath et al., 2018; Han et al.,

Development (2022) 149, dev199908. doi:10.1242/dev.199908

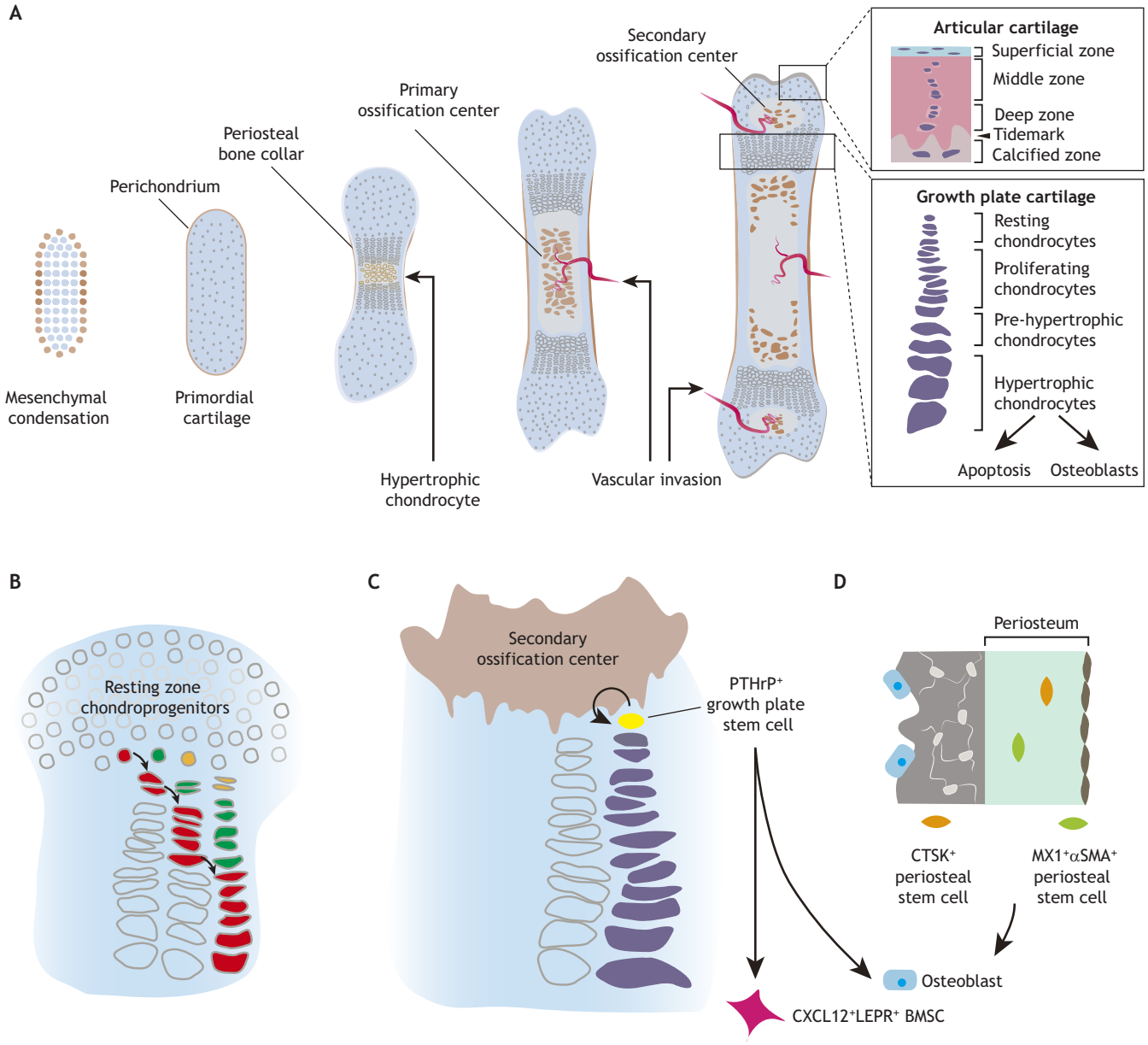

Fig. 4. Endochondral ossification and the skeletal stem cell niche. (A) Schematic of endochondral ossification and long bone development. Mesenchymal cells condensate and form primordial cartilage. Chondrocytes start to proliferate and then differentiate into hypertrophic chondrocytes with the mineralization. The diaphysis is separated by the primary ossification center with vascular invasion. Hypertrophic chondrocytes undergo apoptosis or are directly converted to osteoblasts. The secondary ossification center appears in the epiphysis, which is vascularized and forms the articular cartilage and epiphyseal growth plate. (B) The round-shaped resting-zone chondroprogenitors in the epiphysis are recruited into the proliferative columns during fetal bone development, leading to their gradual consumption. (C) After forming the secondary ossification center, PTHrP$^+$ chondroprogenitors (yellow) are present in the resting zone; these cells start renewing and generate long columns from single clones. PTHrP$^+$ skeletal stem cells can give rise to CXCL12$^+$ LEPR$^+$ bone marrow stromal cells (BMSCs; pink) and osteoblasts (blue). (D) CTSK$^+$ (orange) and MX1$^+$αSMA$^+$ (green) skeletal stem cells in the periosteum, which can give rise to osteogenic cells.

2019) and MX dynamin-like GTPase 1 (MX1)$^+$/α-smooth muscle actin (αSMA; ACTA2)$^+$ cells (Ortinau et al., 2019) in the periosteum, which can give rise to bone-forming osteoblasts and participate in the healing of fractures (Fig. 4D). Thus, bone contains multiple stem cell niches with different physiological functions, which can change with development, growth and disease.

The role of osteoclasts in establishing the bone cavity

HSCs home and migrate from the fetal liver to vascularized regions of bone marrow to initiate definitive hematopoiesis as early as E16.5 (Coşkun et al., 2014). However, osteoclasts are a crucial cell population that create a space for hematopoiesis before bone marrow is established. Osteoclast precursors initially appear at the mesenchyme surrounding the bone rudiments (Taniguchi et al., 2007; Blavier and Delaisse, 1995) and differentiate into TRAP (ACP5)$^+$ cells at E15.5 (Jacome-Galarza et al., 2019). Fate-mapping experiments demonstrated the presence of EMP-derived TRAP$^+$ multinucleated osteoclasts in the bone marrow cavity at E15.5 (Jacome-Galarza et al., 2019). Furthermore, TRAP$^+$ multinucleated osteoclasts were also observed at E16.5 in mouse embryos in which HSC-derived osteoclastic progenitors had been eliminated. These findings suggest that osteoclasts arising in the primary

Development (2022) 149, dev199908. doi:10.1242/dev.199908

ossification center during early osteogenesis are derived from EMPs, rather than HSCs (Jacome-Galarza et al., 2019). During this short period, EMP-derived embryonic osteoclasts play an important role in generation of the bone marrow cavity, facilitating the migration of hematopoietic stem cells and myeloid cells (Fig. 1).

Osteoclasts are thought to undergo apoptosis when bone resorption is finished (Jansen et al., 2012; Yagi et al., 2005; Miyamoto et al., 2012). The survival time of mouse osteoclasts is thought to be less than 6 weeks (Marks and Seifert, 1985) and the lifespan of activated osteoclasts is estimated to be 2-3 weeks (Manolagas, 2000; Weinstein and Manolagas, 2000). However, a recent study suggested that individual osteoclasts can be long-lived and acquire a new nucleus every 4-8 weeks from circulating HSC-derived blood cells (Jacome-Galarza et al., 2019) (Fig. 5A). Thus, it would take 6 months to renew five nuclei in an individual osteoclast. Furthermore, data from intravital imaging revealed an unexpected cell fate of mature osteoclasts that divide into smaller, motile cells termed 'osteomorphs' (McDonald et al., 2021). Osteomorphs can fuse with neighboring osteoclasts, or in some cases with each other, and are 'recycled' into mature bone-resorbing osteoclasts under the control of RANKL signaling (Fig. 5B). An accumulation of osteomorphs caused by RANKL inhibition was observed in some patients who discontinue osteoclast inhibitory therapy (denosumab treatment). This recycling system of osteoclasts and osteomorphs may contribute to energy efficiency and rapid osteoclast regeneration.

The role of osteoclasts in bone homeostasis and repair

Bone is a dynamic organ that is continuously resorbed by osteoclasts and subsequently rebuilt with new bone by osteoblasts throughout life. This remodeling activity is a tightly controlled process to maintain serum elements, such as calcium, and the mechanical strength of the skeleton. It is regulated by various hormones, cytokines, chemokines, extracellular vesicles and biomechanical stimuli (Hattner et al., 1965; Takayanagi, 2007; Li et al., 2016; Deng et al., 2015; Ikebuchi et al., 2018). These sites of remodeling activity occur asynchronously at basic multicellular units (BMUs), and the BMUs in cortical and trabecular bone differ because of their structure (Hattner et al., 1965).

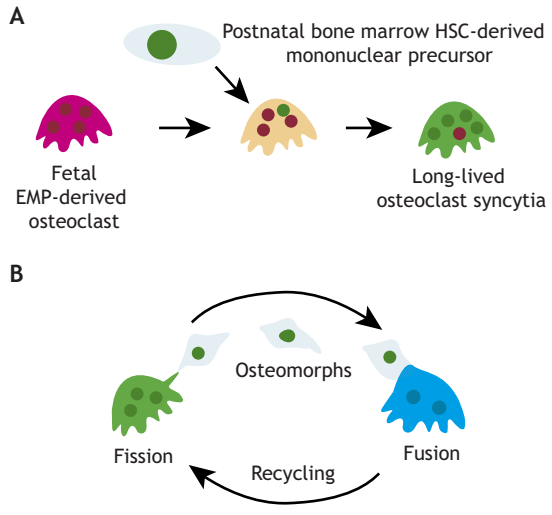

Fig. 5. Osteoclast maintenance and recycling. (A) Postnatal maintenance of osteoclasts in the long-lived syncytia occurs through the sequential acquisition of new nuclei from hematopoietic stem cell (HSC)-derived precursors in the blood. (B) Osteoclasts divide into smaller, more motile daughter cells called osteomorphs, which are recycled by fusing to form functional osteoclasts.

Bone remodeling

The bone remodeling cycle consists of overlapping phases: initiation, resorption, reversal, formation and termination (Fig. 6). The entire process is achieved by the coordinated actions of osteoclasts, osteoblasts and other osteoblast-lineage cells, such as bone-lining cells and osteocytes. Biological and functional differences between EMP-derived osteoclasts and HSC-derived osteoclasts in bone homeostasis need to be further explored.

Initiation phase

Initiation of bone remodeling occurs when necessary, such as in the event of injury or old age. In the initiation phase, mechanical loading and microdamage are sensed by osteocytes through their extensive network of dendritic processes, which leads to the release of paracrine factors that increase local angiogenesis and recruit osteoclast precursors (Dallas et al., 2013; Nakashima et al., 2011; Xiong et al., 2011; Cabahug-Zuckerman et al., 2016).

Resorption phase

In the resorption phase, osteoclastogenesis is stimulated by RANKL, M-CSF and ligands for immunoglobulin-like receptors, which are produced by osteoblast-lineage cells, including osteocytes. The cytoskeleton of the osteoclast is realigned and a sealing zone is formed, enhancing the secretory surface. After completing bone resorption, osteoclasts undergo apoptosis and the bone resorption phase is terminated, ensuring that excess resorption does not occur.

Reversal phase

In the early reversal phase, scattered osteoclasts on the bone surface release secreted factors, matrix-released factors and extra vesicles (Lassen et al., 2017; Sims and Martin, 2020). These osteoclasts also make direct cell-cell contacts, allowing a signal to recruit the osteoblast lineage on the bone surface. Mature osteoclasts initiate bone resorption when they are not in contact with osteoblasts, whereas mature osteoclasts in contact with mature osteoblasts do not resorb the bone in the reversal phase (Furuya et al., 2018). Osteoblasts, especially for the decorin $(DCN)^{high}$ subset, directly suppress osteoclast production by producing OPG, which plays a role in terminating the resorption phase (Cawley et al., 2020; Tsukasaki et al., 2020a). The number of osteoblast-lineage cells increases, reaching sufficient mass to promote their bone formation activity (Lassen et al., 2017).

Formation and termination phase

The formation phase is distinct by the complete replacement of osteoclast with osteoblastic cells. In the formation phase, the resorption lacuna is synthesized to a new bone matrix and then mineralized to fill the resorption lacuna. During this process, osteoblasts deposit a new bone matrix called the osteoid, which gradually mineralizes and terminally differentiates into osteocytes (Dallas and Bonewald, 2010). Osteocytes play a key role in signaling the termination of the remodeling cycle through the secretion of antagonists to osteogenesis, such as SOST (van Bezooijen et al., 2004). The resting bone environment is maintained until the next wave of the remodeling cycle is initiated.

Bone fracture repair

Bone fractures are common during the lifetime of an organism, and effective repair is crucial for survival. During fracture repair, the commonly occurring secondary bone healing begins with an inflammatory response, followed by the recruitment of various

Development (2022) 149, dev199908. doi:10.1242/dev.199908

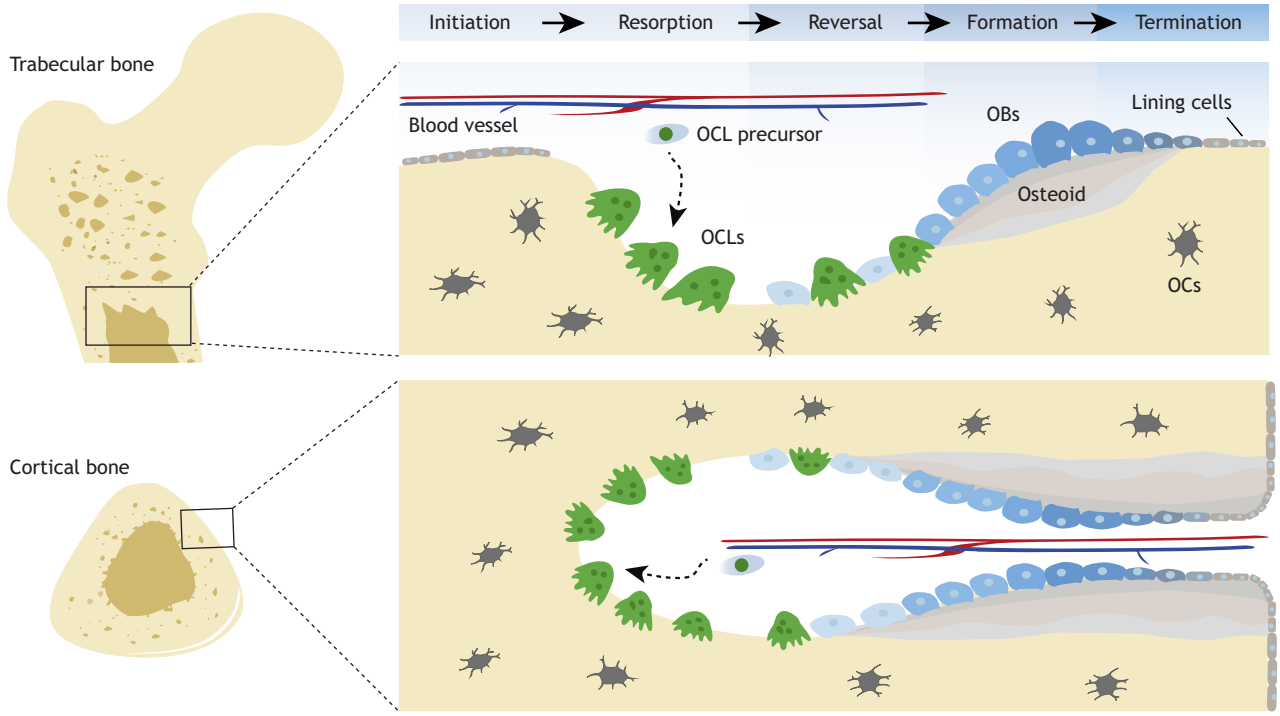

Fig. 6. Schematic of trabecular (top) and cortical (bottom) bone remodeling. The bone remodeling cycle consists of overlapping phases: initiation, resorption, reversal, formation and termination. The lining cells and osteocytes (OCs) release local factors that attract osteoclast precursors from the perivascular and bone marrow niches to the remodeling compartment. Osteoclasts (OCLs) initiate bone resorption, followed by the breakdown and removal of old bone. Osteoclasts then begin to interact directly or indirectly with osteoblasts (OBs), which deposit osteoid and new lamellar bone. Osteoblasts trapped in the bone matrix differentiate into osteocytes, whereas others die or turn into quiescent lining cells on the bone surface. The resting bone environment is maintained until the next wave of remodeling cycle is initiated.

immune and mesenchymal cells at the fracture site. In the initial phase, bleeding from the fracture site causes hematomas, which further develop into vascularized and innervated granulation tissue (Loi et al., 2016; Hu et al., 2017). A temporary scaffold created by the hematoma is characterized by hypoxia and low pH, enabling the invasion of hematopoietic cells, such as neutrophils, lymphocytes and macrophages (Claes et al., 2012). The influx of various cytokine-secreting immune cells evokes acute inflammation (Salhotra et al., 2020). Subsequently, mesenchymal cells are recruited at the fracture site by factors, such as PDGF, transforming growth factor beta (TGFβ) and CXCL12 (Dimitriou et al., 2005; Kitaori et al., 2009; Granero-Moltó et al., 2009). Secreted factors, including BMP-4, vascular endothelial growth factor (VEGF), interleukin (IL) 17A (IL17A), IL6, tumor necrosis factor alpha (TNFα) and CCL2, are also released at the fracture site, promoting osteogenic differentiation of the mesenchymal cells (Peng et al., 2002; Ono et al., 2016; Ono and Takayanagi, 2017; Yang et al., 2007; Wallace et al., 2011; Ishikawa et al., 2014; Xing et al., 2010). At the callus periphery and inside cortical area where bone is repaired by intramembranous ossification, mesenchymal progenitor cells differentiate directly into osteoblasts, whereas mesenchymal progenitor cells that accumulate around damaged bone differentiate into fibroblasts, osteoblasts and mainly chondroblasts through endochondral ossification (Granero-Moltó et al., 2009; Debnath et al., 2018; Duchamp de Lageneste et al., 2018). In endochondral ossification, these cells synthesize the cartilage matrix to form a soft callus, which is then replaced by woven bone. Subsequently, woven bone is transformed into a hard callus, which is further remodeled by osteoclasts and osteoblasts to restore its original shape and function. This remodeling process is crucial for effective and adequate bone repair, and perturbations in osteoclast-mediated bone and cartilage resorption may negatively impact fracture repair (Lin and O'Connor, 2017; Flick et al., 2003; Vi et al., 2015; Yahara et al., 2021).

Osteoclasts can be divided into two subtypes based on their activation phase during fracture repair. Early-induced osteoclasts, which are present before callus formation, have high mobility and a low resorption profile. Late-induced osteoclasts have strong adhesion ability with a high bone resorption profile (Takeyama et al., 2014; Schell et al., 2006). Furthermore, increasing evidence suggests that, although bone marrow cells are a major source of osteoclast precursors in homeostasis, circulating monocytes could be a source of osteoclasts in pathogenic conditions, including during fracture repair (Novak et al., 2020; Kotani et al., 2013). EMP-derived osteoclast precursors could migrate through the bloodstream from the spleen to the fracture site (Yahara et al., 2020), although the mechanism of splenic cell mobilization to the fracture site remains to be identified. However, several studies have shown that reservoirs of macrophages and monocytes play an essential role in tissue inflammation and repair (Hoyer et al., 2019; Kotani et al., 2013).

Immune functions of osteoclasts

A principal function of skeletal bone marrow is to provide a specialized HSC niche to maintain postnatal hematopoiesis, which is crucial for regulating the development of immune cells and, thus, immune responses. Bone cells and the immune system are closely related through cellular and molecular interactions in the bone marrow microenvironment (Tsukasaki and Takayanagi, 2019; Takayanagi, 2007). It follows that osteoclasts are associated with

Development (2022) 149, dev199908. doi:10.1242/dev.199908

many pathological conditions, including osteoporosis, rheumatoid arthritis (RA), chronic inflammation and cancer. Furthermore, similar to other members of the monocytic lineage, osteoclasts exhibit a wide range of phenotypic and functional heterogeneity involved in anti/pro-inflammatory effects and antigen presentation, depending on their environment.

Immune disease

Osteoclasts are activated in immune diseases. The interaction between osteoclasts and the immune system is observed in the autoimmune disease RA, a form of inflammatory polyarthritis that can lead to joint destruction, deformity and loss of function. $LY6C^{high}$ (Charles et al., 2012) and fragment crystallizable (Fc) γ receptor IV^{+} inflammatory monocyte cells are the primary sources of osteoclasts in RA (Seeling et al., 2013). The arthritis-committed osteoclast precursors express $CX3CR1^{high}LY6C^{int}F4/80$ $(ADGRE1)^{+}I\text{-}A^{+}/I\text{-}E^{+}$ in mouse RA synovium (Hasegawa et al., 2019). Osteoclast differentiation is accelerated by T cell activation and their differentiation into type 17 helper T (Th17) cells. Th17 cells then stimulate synovial inflammation and produce proinflammatory mediators, such as IL1 and TNFα from synovial fibroblasts, macrophages and chondrocytes. Furthermore, IL17A induces RANKL expression in synovial fibroblasts and osteoblasts, which induces osteoclast differentiation and bone destruction associated with RA. Thus, Th17 cells have been proposed as an osteoclastogenesis-inducer in pathogenic arthritis.

APCs

Osteoclasts also play a role as APCs (Rivollier et al., 2004; Li et al., 2010; Ibáñez et al., 2016; Grassi et al., 2011). Human osteoclasts derived from monocytes express major histocompatibility complex (MHC) class I and II, CD80, CD86 and CD40, and have the potential to present allogeneic cells, resulting in the activation of both $CD4^{+}$ and $CD8^{+}$ (cytotoxic) T cells (Li et al., 2010). Furthermore, T cell receptors (TCRs) on $CD8^{+}$ and $CD4^{+}$ T cells can initiate T-cell activation and trigger TCR signaling by binding to MHC class I and II complexes, respectively (Li et al., 2010). Thus, osteoclasts can enfold soluble antigens and present them on their cell surfaces, demonstrating that osteoclasts can function as APCs.

Immunosuppressive roles

T regulatory (Treg) lymphocytes are a subpopulation of $CD4^{+}$ T lymphocytes, which can cause immunosuppression, maintain peripheral tolerance and prevent chronic inflammation. The suppressive effects of Tregs, which express the forkhead box protein P3 (FOXP3) and CD25 (IL2RA) (Fontenot et al., 2003; Hori et al., 2003), are due to modulation of the effector functions of inflammatory cells (such as T cells, B cells, neutrophils and macrophages) (Alvarez et al., 2020). Tregs inhibit osteoclastogenesis through direct interaction with osteoclast precursors via cytotoxic T-lymphocyte-associated protein 4 (CTLA4) (Zaiss et al., 2007). Enhancing the activity of Treg cells improved the clinical signs of RA and suppressed local and systemic bone destruction in the TNF-mediated arthritis model (Zaiss et al., 2010). In contrast, $CX3CR1^{-}$ inflammatory osteoclasts have significantly higher bone resorption capacity *in vitro* than the $CX3CR1^{+}$ fraction (Madel et al., 2020). Both subsets can function as APCs, but the T-cell activation capacity is higher in the $CX3CR1^{-}$ inflammatory osteoclast subset. Both can induce TNFα-producing $CD4^{+}$ T cells, resulting in accelerated inflammation; however, the $CX3CR1^{+}$ osteoclast subset expresses high levels of co-suppressor molecules, such as the programmed death-ligand 1 (PD-L1; CD274), Galectin-9 and herpes virus entry mediator (HVEM; TNFRSF14). These factors are the major immune checkpoint molecules involved in immunosuppression by autoimmune diseases and tumors (Madel et al., 2020). Thus, a subset of osteoclasts are thought to act as immunosuppressive cells that emerge in response to inflammatory signals and regulate inflammation. However, much remains to be clarified to understand the role of osteoclasts as immune cells in all aspects of biology.

Conclusions

EMP-derived macrophage and osteoclast precursor populations persist during adult life and produce long-lived cells that can self-renew locally. Cell populations established during embryonic development behave differently from the HSC lineage, having distinct roles in tissue homeostasis and repair. However, the principal mechanisms causing the differences between the HSC- and EMP-derived macrophages and osteoclasts remain to be elucidated. Further delineation of the role of this embryonic cell population will determine its function in bone homeostasis. As there are insufficient therapeutic agents for the treatment of disorders caused by osteoclasts, differences in the regulation of osteoclast precursors from different origins could be exploited to develop optimal methods to target osteoclasts therapeutically. Developing a new framework for osteoclast biology along with technological advancements could thus not only allow us to improve our understanding of osteoclast biology, but also help in the development of novel treatments for bone disease.

Competing interests

The authors declare no competing or financial interests.

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