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What do we know about bone morphogenetic proteins and osteochondroprogenitors in inflammatory conditions?

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Abstract

Osteochondroprogenitors are crucial for embryonic bone development and postnatal processes such as bone repair in response to fracture injury, and their dysfunction may contribute to insufficient repair of structural damage in inflammatory arthritides. In the fracture healing, the early inflammatory phase is crucial for normal callus development and new bone formation. This process involves a complex interplay of many molecules and cell types, responsible for recruitment, expansion and differentiation of osteochondroprogenitor populations. In inflammatory arthritides, inflammation induces bone resorption and causes insufficient bone formation, which leads to local and systemic bone loss. While bone loss is a predominant feature in rheumatoid arthritis, inflammation also induces pathologic bone formation at enthesial sites in seronegative spondyloarthropaties. Bone morphogenetic proteins (BMP) are involved in cell proliferation, differentiation and apoptosis, and have fundamental roles in maintenance of postnatal bone homeostasis. They are crucial regulators of the osteochondroprogenitor pool and drive their proliferation, differentiation and lifespan during bone regeneration. In this review, we summarize the effects of inflammation on osteochondroprogenitor populations during fracture repair and in inflammatory arthritides, with special focus on inflammation-mediated modulation of BMP signaling. We also present data in which we describe a population of murine synovial osteochondroprogenitor cells, which are reduced in arthritis, and characterize their expression of genes involved in regulation of bone homeostasis, emphasizing the up-regulation of BMP pathways in early progenitor subset. Based on the presented data, it may be concluded that during an inflammatory response, innate immune cells induce osteochondroprogenitors by providing signals for their recruitment, by producing BMPs and other osteogenic factors for paracrine effects, and by secreting inflammatory cytokines that may positively regulate osteogenic pathways. On the other hand, inflammatory cells may secrete cytokines that interfere with osteogenic pathways, proapoptotic factors that reduce the pool of osteochondroprogenitor cells, as well as BMP and Wnt antagonists. The net effect is strongly context-dependent and influenced by the local milieu of cells, cytokines, and growth factors. Further elucidation of the interplay between inflammatory signals and BMP-mediated bone formation may provide valuable tools for therapeutic targeting.

Key words

osteochondroprogenitors, bone morphogenetic proteins, inflammation, inflammatory arthritis, fracture

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1. Introduction

Development of the skeletal system, bone growth, remodeling and regeneration after injury all depend on a pool of bone/cartilage progenitor cells and mechanisms regulating their differentiation. The bone morphogenetic protein (BMP) pathway is crucial for postnatal bone homeostasis and regeneration. Although the role of BMPs is well described in the regulation of osteochondroprogenitor proliferation, differentiation and apoptosis, data on specific changes under inflammatory conditions are still scarce. The inflammatory process has, paradoxically, a dual effect on bone tissue, with anabolic action described during fracture healing and enthesial ossifications, as well as catabolic and anti-anabolic action in rheumatoid arthritis (RA) and other immune-mediated osteoresorptive diseases. Understanding the interplay between the inflammatory response and BMP pathway modulation in osteochondroprogenitor cells promises insight into the pathogenesis of skeletal disorders such as non-unions, different forms of arthritis and osteoporosis, and aids to the development of new approaches for their treatment.

1.1. Definition of bone and cartilage progenitor populations

The concept of a postnatal multipotent self-renewing bone marrow stromal progenitor cell, able to differentiate into adipocytes, chondroblasts and osteoblasts emerged in 1988 (1) and the cell was termed mesenchymal stem cell shortly after its discovery (2). In 2006, the International Society for Cellular Therapy (ISCT) proposed a change in terminology into mesenchymal stromal cell (MSC), and defined minimal criteria for their definition based on their plastic-adherence in standard culture conditions, expression of surface markers, and their capability of trilineage differentiation *in vitro* (3). Since the establishment of these criteria, an overwhelming amount of new research has been published, characterizing the MSCs isolated from different tissues by various surface markers, urging scientists to call for additional, more stringent criteria (4). While cells from multiple anatomical locations that fit ISCT criteria are capable of *in vitro* trilineage differentiation after appropriate growth factor supplementation, not all of them inherently express osteo- or chondrogenic genes and pose this potency *in vivo* (5, 6). This prompted a further change in terminology from MSCs, now reserved for cells of ubiquitous tissue sources complying with ISCT criteria, to skeletal stem cells (SSCs), cells capable of self-renewal, present within the skeleton, with the ability to differentiate into osteoblasts, chondroblasts, bone marrow stroma, and adipocytes (5, 7). Recently, only chondrogenic and osteogenic differentiation have been proposed as sufficient

1 (8). The usage of transgenic strains for *in vivo* cell fate mapping is responsible for the
2 extensive characterization of skeletal progenitor populations in mice. SSCs have been
3 identified in bone marrow, periosteum, growth plates, metaphyses and in cranial sutures (9).
4 Murine mesenchymal progenitor populations express various combinations of surface
5 markers. Morikawa et al. characterized SSCs in perivascular regions in bone marrow as
6 CD45⁻TER119⁻Sca-1⁺CD140a⁺ cells (10). Ambrosi et al. showed that CD45⁻CD31⁻Sca-
7 1⁺CD24⁺ cells from the same compartment are able to differentiate into more committed
8 adipogenic and osteochondrogenic progenitors (CD45⁻CD31⁻Sca-1⁻CD140a⁺) (11). Recent
9 work by Chan et al. identified murine SSCs (mSSCs), contained within the CD45⁻Ter119⁻
10 Tie2⁻CD51⁺Thy⁻6C3⁻CD105⁻CD200⁺ subset of whole neonatal bone samples (12). The
11 authors proposed a hierarchical model of differentiation into bone, cartilage and bone marrow
12 stromal cells, where further stages of more committed progenitors are defined based on the
13 presence or absence of CD90, 6C3, CD105 and CD200 surface markers (12, 13) (Fig 1).
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15 At the gene expression level, bone marrow-residing SSCs were highlighted by the expression
16 of *Nes* (neuroectodermal stem cell marker, nestin) (14), *Acta2* (alpha smooth muscle actin,
17 α Sma) (15), *Mx1* (myxoma resistance 1) (16), *Hox11* (homeobox gene 11) (17) and *Prrxl1*
18 (paired related homeobox 1) (18). The majority of α SMA-labelled cells (15) exhibit the
19 surface markers SCA-1, CD51 and CD90, *Mx1*⁺ cells abundantly possess CD140a, CD105,
20 CD29, CD44 and CD133 (16), *Hox11*⁺ cells bear CD140a and CD51, whereas *Prrxl1*⁺ cells
21 express *Pdgfra*, *Grem1* (gremlin 1) and *Nes* (18). Simultaneous expression of *Mx1* and *Acta2*
22 selectively labels a subset of periosteal SSC, most of which are CD105⁺CD140a⁺, and
23 express *LepR* and *Grem1* (19). Substantial subset of growth plate SSCs are *PTHrP*⁺ (20). In
24 adult tissues, *LepR* (leptin receptor) expression was linked to a postnatal progenitor subset
25 residing in the bone marrow and on endosteal surfaces (21-23).
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27 Human SSCs (hSSC) have been characterized by multi-color panels, not overlapping with
28 murine markers (Fig 1). Sacchetti et al. described a population capable of self-renewal,
29 osteogenic differentiation and hematopoietic stem cell (HSC) niche support as CD146⁺
30 perivascular cells residing in bone marrow (24). Tormin et al. described perivascular
31 CD146⁺CD271⁺ progenitors and a distinct CD146^{-/low}CD271⁺ subset located in endosteal
32 regions (25). Li et al. identified a CD140a^{-/low}CD271⁺ population residing in the
33 perisinusoidal space in bone marrow, which highly expresses CD90, CD105, CD140b and
34 STRO-1 (26). Although they proposed CD140a as a marker of exclusion, a CD140a⁺CD51⁺
35 perivascular population was described as a pool of multipotent progenitors with trilineage
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1 potential by Pinho et al. (27). Chan et al. identified SSCs residing at different locations in the
2 human fetal and adult skeleton, especially enriched in the growth plate. Similar to the murine
3 model, they suggested a hierarchy of human skeletal progenitors, where hSSCs are
4 PDPN⁺CD73⁺CD164⁺CD146⁻, capable of osteochondrogenic differentiation, but like its
5 murine counterpart lack adipogenic potential (28). The multipotent hSSCs are CD146⁻, but
6 osteo/chondro-directed progenitors gain CD146 expression.
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8 While there is a great overlap of currently described populations, some progenitor subsets,
9 especially those at distinct skeletal locations, also differ by their surface marker or gene
10 expression, the ability to support the HSC niche, their osteochondroadipogenic or
11 osteochondrogenic potency, and the ability to form bone through endochondral or
12 intramembranous ossification, suggesting there is more than one type of SSC.
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22 **1.2. Signaling pathways driving differentiation of bone and cartilage progenitors**

23 Proliferation, differentiation, and apoptosis of both chondrocytes and osteoblasts is regulated
24 by many genes, signaling pathways, as well as local and systemic factors. The major
25 pathways/molecules governing the fate of these cells are typically recognized as the Indian
26 hedgehog (Ihh), homologous wingless (wg)/integrated-1 (Wnt), BMPs, parathyroid hormone-
27 related protein (PTHrP), fibroblast-growth factors (FGFs), and Notch.
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33 Ihh is a protein from the evolutionary highly conserved Hedgehog family (Indian, Desert and
34 Sonic Hedgehog), produced by pre-hypertrophic and early hypertrophic chondrocytes. It
35 binds and thus, inactivates its cell-surface receptor Patched-1 (PTC-1), leading to the
36 activation (or rather, un-inhibition) of Smoothed (SMO, a seven-transmembrane protein)
37 which then transmits a signal to the cytoplasmic transcription factors Gli to regulate
38 chondrocyte proliferation and maturation during endochondral ossification (29).
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44 The Wnts are a large highly conserved family (of 19 members in both human and murine
45 genomes) (30). There are three pathways that are typically recognized for Wnt signaling (31):
46 1) the canonical Wnt/ β -catenin pathway (dominant in bone biology); 2) the non-canonical
47 Wnt-planar cell polarity pathway (Wnt/PCP); and 3) the Wnt-calcium pathway (Wnt/Ca²⁺).
48 Various agonistic (e.g. Norrin, RSPO) and antagonistic ligands (e.g. dickkopf1 – DKK1,
49 sclerostin, and Wise), as well as molecules that act as Wnt sequestrers (secreted frizzled-
50 related proteins, SFRPs and Wnt inhibitory factor 1, WIF1) influence both the canonical and
51 non-canonical signaling pathways.
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BMPs, another highly conserved group of proteins, have fundamental roles in embryonic development, postnatal bone homeostasis and fracture healing. BMPs bind as dimers to type I and type II serine-threonine kinase receptors. These tetrameric (oligomeric) receptor complexes can transduce signals to the canonical, Smad-dependent, signaling pathway and the non-canonical, Smad-independent pathway (p38-MAPK) (32). Of the 14 BMPs, only BMP-2, -4, -5, -6, -7, and -9 have a high osteogenic activity (33, 34). BMPs that exert osteogenic signals bind to three type I receptors (BMPRII/ALK3, BMPRII/ALK6, and type I activin receptor – AcvR1/ALK2). BMP-3 opposes osteogenic activities of other BMPs by transducing type IIB activin receptor (AcvRIIB)-Smad2/3 signaling (35). The canonical (Smad-dependent) signaling involves phosphorylation of R-Smads (Smad 1, 5 or 8) to create complexes with their partner Smad 4 and then they co-translocate to the nucleus to recruit RUNX2, which regulates gene expression of, e.g. Osterix (OSX) (36). Smads 6 and 7 (I-Smad) negatively regulate the canonical pathway by preventing R-Smad phosphorylation. The non-canonical (Smad-independent) signaling involves MKK-p38 or MKK-ERK1/2 signaling cascades (32). There are several subfamilies of BMP antagonists (DAN family; Twisted gastrulation; Chordin and Noggin), which directly associate with BMPs to block their receptor binding (37).

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PTHrP, secreted by proliferating chondrocytes (38), acts in bone by binding to the PTH receptor 1 (PTHR1) which also functions as a PTH receptor. PTHR1 is expressed at high levels in pre-hypertrophic and early hypertrophic chondrocytes (39), as well as in most tissues that express PTHrP (40). There, it interferes with locally produced Ihh keeping the chondrocytes of the growth plate in the proliferative pool (41, 42).

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FGFs are a large family of evolutionary highly conserved proteins (43) that regulate both the endochondral as well as the intramembranous bone formation, acting through four receptors (FGFRs) (44). Their actions involve regulation of proliferation, migration, differentiation, as well as angiogenesis. FGF signaling starts with a dimerization (including two heparin sulfate chains) of two FGFs that bind to two FGFRs (45) leading to activation of the RAS/MAP kinase pathway that regulates cell proliferation and differentiation. In addition, FGFs utilize the PI3 kinase/AKT pathway (regulating cell survival) and the PLC γ pathway (regulating cell morphology and migration) (46-48).

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The Notch signaling pathway regulates bone development and regeneration (49-52). It is activated by binding of Notch ligands (Jagged 1 and 2, and Delta-like ligands 1, 3, and 4) to Notch receptors (1-4) on the neighboring cells. Ligand binding triggers a conformational change of the receptor, which leads to proteolytic cleavage of the Notch intracellular domain

1 (NICD) by the γ -secretase complex (53). Cleaved NICD enters the nucleus where it binds to
2 the recombination signal-binding protein for the Ig-j κ region (RBPj κ). NICD in complex with
3 RBPj κ removes co-repressors, binds mastermind-like (Maml), and activates transcription of
4 downstream target genes including the *Hes* and *Hey* family of transcriptional repressors (53).
5 In addition to the described RBPj κ -dependant canonical pathway, Notch also signals through
6 a RBPj κ -independent non-canonical pathway, which involves interactions with IKK α /NF- κ B
7 and Wnt/ β -catenin pathways (54). Notch actions in bone affect both osteoblasts and
8 osteoclasts, and are highly context-dependent. Activation of Notch signaling in immature
9 osteoblast lineage cells inhibits cell differentiation and causes cancellous bone osteopenia due
10 to impaired bone formation (55). However, stimulation of Notch pathway in osteocytes leads
11 to secretion of osteoprotegerin and thus, inhibits bone resorption (56).

12 In addition to major signaling pathways, several transcription factors are indispensable for
13 bone homeostasis. SOX9 (SRY-Box Transcription Factor 9) is a chondrocyte lineage-specific
14 transcription factor indispensable for early chondrocyte development (57), regulated by FGFs
15 (58). It is expressed until the pre-hypertrophic stage of chondrocyte maturation and blocks
16 osteoblastic differentiation of growth plate chondrocytes (59). It is regulated by several
17 osteogenic pathways (BMPs, PTH/PTHrP, insulin-like growth factor (IGF), and Notch) and
18 interacts with a multitude of transcription factors and regulatory proteins, influencing many
19 osteoblast-specific genes, such as bone sialoprotein (BSP) and osteocalcin (OC). OSX is a
20 downstream target of RUNX2 needed for early osteoblast differentiation (60), but also at
21 later stages of the osteoblast lineage (61). Besides RUNX2, OSX may be regulated by other
22 transcription factors, e.g. p53 (suppression of osteoblast differentiation) and NFATC1
23 (stimulation of osteoblast differentiation). *Ihh* pathways may also be involved in regulating
24 RUNX2 and OSX transcription and action. Furthermore, all of the described pathways – *Ihh*,
25 FGF, Wnt, PTH/PTHrP, Notch, and BMP are involved in active cross-talks between skeletal
26 cells – chondrocytes, osteoblasts, osteocytes and osteoclasts (31).

2. Effects of inflammation on osteochondroprogenitor populations during fracture repair

27 Fracture healing is a sequential process that requires a complex interaction between different
28 cell lineages, including immune cells, resident tissue cells, and osteochondroprogenitor cells.
29 Early after fracture, a hematoma forms and inflammatory cells infiltrate the fracture site,
30 producing cytokines/chemokines and modulating the microenvironment to promote healing.

1 MSCs residing within the periosteal layer rapidly expand after the fracture, and differentiate
2 to chondrocytes and osteoblasts to form a fracture callus (62). As healing progresses, the soft
3 callus mineralizes and remodels until the original bone structure is restored (63).
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5 During a stabilized bone fracture without injury to the periosteum, bones heal by
6 intramembranous ossification. Instability and subsequent movements of the fragments at the
7 fracture site induce the activation of periosteal progenitors and formation of a
8 fibrocartilaginous callus, which is similar to endochondral ossification. The position of
9 osteochondroprogenitors alters their contribution to the fracture callus. In stabilized fractures,
10 periosteal progenitors are the greatest contributors to the outer callus, while bone marrow-
11 derived progenitors mostly contribute to callus formation at the proximal fracture area, as
12 well as on the endosteal surface and in the bone marrow. In open fractures, muscle-derived
13 progenitors (muscle satellite cells) also contribute to the regeneration process (64). Further
14 characterization of these progenitor populations with regard to their regulation of the BMP
15 pathways is required for better understanding their role in the fracture healing sequence.
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28 **2.1. Osteochondroprogenitor populations contributing to bone repair**

29 The key population responsible for fracture healing is a subset of non-hematopoietic
30 mesenchymal progenitors with tri-lineage potential (65). These cells reside in the
31 perivascular niche within the bone marrow (66, 67) and periosteum (62). Surface-marker
32 profiling identified an injury-induced CD45⁻Ter119⁻Tie2⁻CD51⁺CD90⁻6C3⁻CD105⁻
33 population, capable of self-renewal as well as *in vitro* and *in vivo* bone/cartilage/stromal
34 differentiation (68, 69). Compared to its uninjured counterpart, this callus-expanded
35 population specifically expresses the integrin subunit $\alpha 6$ (CD49f) and exhibits enhanced bone
36 formation, reduced apoptotic activity and increased *Ihh* signaling. In a tibial-fracture mouse
37 model, substantial proportion of non-hematopoietic callus progenitors expressed CD51 and
38 CD140b (70).
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48 In recent years, a variety of markers have been proposed to identify MSCs in their native
49 environment, utilizing visual transgenes and lineage tracing models, including *Prrx1* (71-73),
50 *Acta2*/ α SMA (15, 50, 74, 75), *Gli1* (76, 77), *Grem1* (78), *Osx/Sp7* (79, 80), and *Cxcl12* (79).
51 Contributions of *Nes*⁺ (81), *Sox9*⁺ (82), *Col2.3*⁺ (73), and transcortical progenitor channel
52 cells (83) to fracture regeneration were also suggested. Cells expressing the mesenchymal
53 marker *Prrx1* have been detected in the periosteum, perichondrium and, to a lesser extent,
54 endosteum and bone marrow (72). Sorted *Prrx1*⁺ cells differentiate into chondrocytes and
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osteoblasts, and their differentiation, induced by BMP-2, is superior to the *Prrx1*⁻ subset (72). Wang et al. used the fracture model in *Prrx1*CreERT2-GFP;*Rosa*-tdTomato mice to confirm numerous GFP⁺tdTomato⁺ cells (indicating active *Prrx1* expression) and GFP⁻tdTomato⁺ cells (indicating cell maturation) in the periosteum, cartilaginous and hard callus, contributing to fracture healing (73). Our group developed α SMACreERT2/Ai9-tdTomato mice to identify a rare population of perivascular α SMA-labeled cells in the periosteum and bone marrow, which expresses mesenchymal markers (Sca-1, CD140a, CD140b and LepR) and rapidly expands early after fracture (day 2), in parallel to periosteum thickening (15, 74). Six days after the fracture, α SMACre-labeled cells extensively contributed to the callus tissue (distal and proximal to the fracture site), and expressed chondrogenic (*Col2a1*, *Sox9*, *Aggrecan*) and osteogenic (*Bsp*, *Osx*) markers (50). Recently, *Gli1* was suggested as a postnatal osteochondroprogenitor marker (77), with tri-lineage potential *in vitro* (76). Lineage-traced *Gli1*⁺ cells from *Gli1*CreERT2/Ai9-tdTomato mice, expressing mesenchymal markers CD44, CD106, CD146, CD140a, CD140b, and α SMA, were present in the articular cartilage, metaphysis, and periosteum but not in bone marrow or endosteum (77). Upon fracture, around half of them gained the expression of Aggrecan and OC, and significantly contributed to the fracture callus. Cells expressing the BMP antagonist *Grem1* are considered osteochondroreticular (OCR) progenitors. Lineage-traced *Grem1*⁺ cells from *Grem1*CreERT2;R26-LSL-tdTomato mice were detected adjacent to the growth plate, trabecular bone, and bone marrow, and possessed self-renewing and tri-lineage potential (78). They contributed to callus formation, with around 28% of them becoming osteoblasts (Col2.3GFP⁺) and 14% chondrocytes (Sox9⁺) by day 7 after fracture. *Grem1*⁺ OCR cells are distinct from *Nes*⁺ cells, but some of them co-express α SMA. In comparison to *Grem1*⁻ cells, sorted *Grem1*⁺ cells overexpressed *BMP-2/5/6*, and BMP receptor *Acvr1*. Lineage traced *Osx*-expressing cells exhibited *LepR*, CD140a and CD140b, with a substantial fibroblast colony-forming activity and tri-lineage potential. In a semi-stabilized tibial fracture model, *Osx*⁺ cells were present within the cartilage tissue and around the pin insertion, whereas in a model of cortical bone regeneration, *Osx*⁺ osteocytes were detected in the healing bone 8 weeks after injury (79). CXCL12 (stromal cell-derived factor 1, SDF-1) is a chemokine expressed by a subset of quiescent osteoprogenitors (79). Using *Cxcl12*CreER/Ai9-tdTomato mice, Matsushita et al. observed a subset of CXCL12⁺ resting cells within the diaphyseal marrow space, which are readily induced by different injury stimuli (drill hole and bone

1 marrow ablation) to replenish the chondrocyte, osteoblast, and osteocyte pool within the
2 injured site.

3 Normal BMP expression is important for fracture healing. Global or conditional inactivation
4 of BMPs presents a range of phenotypes, from embryonically lethal, through phenotypes
5 characterized by various skeletal defects, to non-detectable phenotypes. Conditional deletion
6 of *Bmp-7* in *Prrx1*-expressing mesenchymal lineage cells did not exert noticeable effects on
7 the healing process (84), which was characterized by unaltered periosteal activation, bridging
8 of callus by day 10, and callus mineralization by day 20, comparable to the control
9 littermates. Conditional inactivation of *Bmp-2* in the limb mesenchyme (using
10 *Prrx1CreBMP-2* mice) results in spontaneous fractures with inability to heal (85).
11 Inactivation of *Bmp-2* in chondrocytes (using *Col2Cre*) increases the proportion of cartilage
12 within the callus, but it's inefficient mineralization eventually results in weaker bones (86). In
13 contrast, inactivation of *Bmp-2* in osteoblasts (using *Col1Cre* mice), does not affect the
14 fracture healing, suggesting that *Bmp-2* expression in osteoblasts is not crucial for callus
15 formation. Global *Gdf5* and *Bmp-14* (87) knockout mice have similar fracture phenotypes,
16 characterized by decreased chondrogenesis, delayed fracture healing, and more prominent
17 callus infiltration with inflammatory cells.
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32 **2.2 Effects of fracture-associated inflammation on BMP signaling**

33 BMPs are involved in cell proliferation, differentiation, and apoptosis (88), so their effects on
34 osteochondroprogenitor cells during regeneration are crucial. The expression of BMP-2,
35 BMP-4, Noggin, and Chordin is evident in healing tissues, confirming the central role of
36 BMP signaling in progenitor recruitment and endochondral ossification during bone repair.
37 Therefore, BMPs have therapeutic potential to enhance the process of fracture healing (89).
38 Although treatments with BMP-2 and -7 have been FDA-approved, treatments with BMPs
39 are accompanied with undesirable side effects due to the supraphysiological dosages used.
40 Understanding the interactions between different signaling pathways during fracture healing
41 is important to optimize the treatment dosage of BMPs (90).
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51 Fracture injury affects the bone as well as the periosteum layer, blood vessels, and the
52 surrounding soft tissue. The inflammatory phase is initiated when disruption of the
53 vasculature leads to a hematoma formation around the fracture site, which becomes infiltrated
54 by inflammatory cells (91). Within 24 hours, neutrophils predominate within the fracture
55 hematoma, providing chemotactic signals for subsequent macrophage homing. In the next
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2 several days, the fracture hematoma becomes intrinsically osteogenic and angiogenic, due to
3 recruitment of MSCs and accumulation of osteogenic/angiogenic factors. Finally, T
4 lymphocytes are recruited into the fracture hematoma and subsequent granulation tissue.
5 Highly regulated inflammatory responses, as well as the release of cytokines and growth
6 factors, are crucial for fracture healing. Infiltrating macrophages and neutrophils secrete
7 cytokines (IL-1, IL-6, TNF- α), chemokines (CCL2, CCL4, CCL5, CXCL12), and several
8 growth factors (BMP-2, BMP-7, TGF- β , PDGF etc.). In the absence of inflammatory
9 cytokines, such as IL-6 and TNF- α (using IL-6 or TNF- α knockout mice), fracture healing is
10 delayed (92, 93). Administration of an anti-IL-6 antibody in the early phase after fracture
11 reduces systemic inflammation, recruitment of immune cells, and bone regeneration, thus
12 impairing fracture healing (94). It was further observed that classic signaling (membrane IL-
13 6R/membrane gp130) and not trans-signaling (soluble IL-6R/membrane gp130) is essential
14 for bone repair. Mice that lack the proinflammatory molecule PTX3 showed a delayed
15 sequence of callus mineralization (70). NOD/scid-IL2R γ_c^{null} mice with impaired innate and
16 adaptive immune responses have an increased cartilaginous area suggesting defective
17 endochondral ossification (95). Nevertheless, RAG1 $^{-/-}$ mice deficient for T and B
18 lymphocytes showed improved callus mineralization (96). Although this study implies a
19 negative effect of the adaptive immune system on fracture healing, it seems that activated T
20 lymphocytes support osteogenic differentiation (97). Despite the importance of initial acute
21 inflammatory response for fracture healing, prolonged and uncontrolled inflammation leads
22 to poor fracture regeneration, mainly due to overproduction of inflammatory and apoptotic
23 factors as well as tissue destruction and insufficient vascularization (98, 99).

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40 The inflammatory phase of fracture healing is necessary to recruit mesenchymal progenitors
41 by chemotactic signals from the fracture site as well as to activate osteoprogenitor subsets
42 within the periosteal layer (Fig1). Both major subtypes of macrophages – inflammatory M1
43 and alternative M2 are able to produce BMP-2 (100, 101). In addition, cytokines (TNF- α ,
44 IFN- γ , IL-17, TGF- β) produced by T lymphocytes enhance BMP-2 production in MSCs (97).
45 BMPs released by immune and osteoprogenitor cells induce osteogenic differentiation in an
46 autocrine and paracrine manner (91, 102). In the mouse fracture model, BMPs are expressed
47 during the initial phase of callus formation and increase up to 3 weeks post fracture (103).
48 The highest expression of BMP-2 and GDF8 is observed right after injury, during the
49 inflammatory phase, in parallel to the high concentration of proinflammatory cytokines.
50 GDF5 and TGF- β are expressed during chondrocyte expansion and maturation, which is
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1 followed by increased expression of BMP-3a, -4, -7, and -8 along the reparatory phase of
2 fracture healing (104). Secreted BMPs contribute to bone regeneration by promoting
3 angiogenesis (105), progenitor cell recruitment (106) and osteoblast differentiation (98). In
4 human callus tissue, active osteoblasts abundantly express BMP-7 and BMP-3 as well as
5 BMPR-IA, -IB, and -II. Expression of phosphorylated R-Smads confirmed active BMP signal
6 in both osteoblast and cartilage callus cells (107). Noggin is released by immune cells
7 (macrophages and neutrophils) and, along with other BMP antagonists in the callus (Gremlin,
8 Chordin, Smad-6/7, and BAMBI), may contribute to impaired fracture repair (108, 109).
9 PDGF signaling is active in periosteal and callus cells and has the ability to modulate the
10 BMP-2 response during periosteal cell differentiation (110). BMP-induced VEGF release by
11 osteoblasts stimulates vascularization and further delivery of progenitor cells (91). Clearly,
12 complex signaling cascades of many cytokines and growth factors within the callus tissue
13 determine the net shape of the healing process, whose precise interactive network is yet to be
14 fully determined.
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28 **3. Effects of inflammation on osteochondroprogenitor populations in arthritis**

29 The inflammatory milieu is often associated with alterations of skeletal remodeling, affecting
30 both osteoclasts and osteoblasts. Among the most common diseases accompanied by
31 inflammation-induced bone disorders are inflammatory rheumatic diseases. They are
32 generally divided into: 1) a seropositive RA, characterized by production of autoantibodies
33 such as rheumatoid factor and/or anti-citrullinated protein antibodies; and 2) seronegative
34 spondyloarthritis (SpA), including ankylosing spondylitis (AS), psoriatic arthritis (PsA),
35 reactive arthritis, arthritis associated with inflammatory bowel disease, and juvenile SpA. In
36 RA, bone damage typically occurs at joint margins, where the inflamed synovium produces
37 focal erosions of cortical bone. Progression of bone erosions causes a loss of the subchondral
38 bone and contributes to destruction of the articular cartilage. Finally, patients exhibit
39 systemic bone loss in the form of osteopenia or osteoporosis involving the axial and
40 appendicular skeleton remote from the synovial inflammation. Patients with SpA may
41 develop erosions of articular bone as well as erosions of sacroiliac joints. In addition,
42 inflammation affects the spine at enthesial sites in the form of concurrent bone erosion and
43 induced bone formation. Disease progression may eventually lead to bony fusion (ankylosis)
44 of sacroiliac joints and syndesmophyte formation between vertebral bodies (111-118). In this
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1 review, we specifically discuss the effects of rheumatic diseases on osteoblast lineage cells,
2 while the osteoclastogenic effects are extensively reviewed elsewhere (111, 112, 119, 120).
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5 **3.1. Osteochondroprogenitor populations within the synovial compartment**

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7 Progenitor populations from periarticular tissues such as synovia, cartilage or subchondral
8 bone have been suggested to participate in bone regeneration during arthritis. However, apart
9 from murine and human bone-residing progenitors described before, most of the cells from
10 other locations have not been stringently assessed for their self-renewing capacity and *in vivo*
11 trilineage potential (8, 121). De Bari et al. first isolated potential progenitors from the human
12 synovial membrane termed “SM-derived MSCs” and confirmed their ability to proliferate
13 and maintain multilineage potential *in vitro* (122). These cells were also identified in the
14 synovial fluid from arthritic patients (123). According to Sakaguchi et al., synovial MSCs
15 had superior chondrogenic and osteogenic potential in comparison to other sources (124).
16 Futami et al. isolated and characterized a similar population from the mouse infrapatellar fat
17 pad synovia (125). These cells express several markers defined by ISCT criteria (122, 124,
18 125). Developmentally, synovial cells originate from the embryonic mesenchymal joint
19 interzone (JI) cells, which contribute to joint structural elements including the articular
20 cartilage, synovial lining and intra-articular ligaments (126). Roelofs et al. have recently
21 shown that *Gdf5*-expressing JI cells persist in the adult murine synovium, subchondral bone,
22 bone marrow and articular cartilage, and proliferate upon cartilage injury (127). These cells
23 have the ability to form synovial lining cells, chondrocytes and adipocytes, but were not
24 osteogenic, supporting the existence of a distinct osteochondroprogenitor subset within the
25 synovial tissue. However, the developmental origin of such synovia-derived
26 osteochondroprogenitors with *in vitro* multilineage potential has not been precisely
27 determined. The relationship of described MSC populations to fibroblast-like synoviocytes
28 (FLSs), which rapidly proliferate in RA, is also not clear, as their phenotypes largely overlap
29 (128). Similarly as in the synovia, progenitor cells have been reported in normal and
30 osteoarthritic (OA) human articular cartilage (129), but their progenitor properties have not
31 been completely proven.
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34 During joint inflammation, we and other groups have observed increased cellularity and
35 proliferation of stromal cells derived from affected joints, with the ability to differentiate
36 towards osteogenic or chondrogenic lineages (130, 131). This population is a heterogeneous
37 mixture and includes highly proliferative hypertrophic FLSs, requiring further
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1 characterization by surface marker profiling (CD29, CD140b, Sca-1, CD44, CD90.1, CD105,
2 CD51, and CD200). We observed that cells released from collagenase-treated synovial joints
3 showed apparent heterogeneity in the expression of mesenchymal markers amongst control
4 and arthritic mice, reflecting their distinct differentiation and activation status (131). Using a
5 modified panel proposed by Chan et al. (12), we identified a small subset of CD51⁺CD200⁺
6 cells amongst non-hematopoietic cells within the synovial compartment and postulated that
7 this population might share progenitor properties of mSSC. This subset was decreased in
8 antigen-induced arthritis (AIA), reflecting an impaired osteogenic and chondrogenic
9 regeneration in arthritis (131). At the single cell level, Chan et al. determined the stage-
10 specific expression of BMP, Wnt, and TGF- β pathways, revealing their differential responses
11 to growth and differentiation stimuli. Co-expression of BMP-2 and its receptor (BMPRIa) in
12 28% of mSSCs pointed to their autocrine or paracrine signaling in sustaining the progenitor
13 pool or inducing its expansion (12). Committed mSSC progeny expressed antagonists of the
14 BMP2 pathway, such as Gremlin 2 and Noggin, suggesting their ability to control the
15 expansion of mSSCs via a negative feedback loop (12).

16 We aimed to further characterize the CD51⁺CD200⁺ population, reduced in arthritis, by next-
17 generation sequencing of the transcriptome of the CD45⁻CD31⁻TER119⁻
18 CD51⁺CD200⁺CD105⁻ cell subset from AIA and non-immunized (NI) mice. In addition, we
19 sequenced CD45⁻CD31⁻TER119⁻CD51⁺CD200⁻CD105⁺ cells from AIA mice to determine
20 whether they were an expanded committed progeny of CD51⁺CD200⁺ cells or represented a
21 population of CD105⁺ FLS enriched in arthritis. The comparison of gene expression profiles
22 of CD200⁺CD105⁻ cells between AIA and NI mice revealed no significant differences,
23 pointing to their preserved functional features in arthritis. However, in AIA, the
24 CD200⁺CD105⁻ population differed significantly from the CD200⁻CD105⁺ population, by
25 overexpression of BMP, Wnt, and TGF- β pathways (Table S1). In particular, CD200⁺CD105⁻
26 cells exhibit a significantly enhanced expression of *BMP-2*, *-4* and *-6*, *Gdf5*, as well as
27 receptors *BMPRI*, *BMPRII*, and *AcvR2b*. Several components of Wnt and TGF- β pathways
28 were also overexpressed in this population, as well as *Osx* and *Runx2* (Table S1). In addition
29 to *Grem1*, ascribed to SSCs in bone marrow (18), periosteum (19), and long bone metaphyses
30 (78), CD51⁺CD200⁺ cells also showed an enriched expression of *LepR*, another marker for
31 SSC identification (21), whereas expression of *Nes* was unchanged (Table S1). Zhou et al.
32 also showed that SSCs do not overlap for *LepR* and *Nes* expression. Our results, therefore,
33 suggest that the arthritis-reduced CD200⁺CD105⁻ population has osteochondroprogenitor
34 properties, whereas the CD200⁻CD105⁺ population might represent proliferating FLSs. The

1 transcriptome analysis also revealed a higher expression of Fas in CD200⁺CD105⁻ cells
2 compared to CD200⁻CD105⁺ cells, indicating that osteochondroprogenitors are effectively
3 removed by apoptosis under inflammatory conditions, while hypertrophic FLS survive and
4 proliferate, inducing damage to surrounding tissue. Interestingly, we previously observed a
5 preservation of the CD51⁺CD200⁺ population in AIA mice deficient for a functional Fas
6 receptor, which were protected from arthritis-induced subchondral bone loss (131).
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10 11 12 **3.2. Effects of joint inflammation on BMP signaling**

13 The inflammatory environment within the synovial compartment, accompanied by hypoxia
14 and reduced pH, is associated with insufficient osteoblast function, often attributed to direct
15 effects of proinflammatory cytokines, such as TNF- α , IL-1 and IL-6, which inhibit their
16 differentiation and maturation (132-135) (Fig1). Clinical studies applying TNF- α or IL-6
17 receptor blockers documented the persistence of bone erosions despite reduced inflammatory
18 activity in treated patients (136-138). Histological sections of arthritic samples, either from
19 humans or from murine models, have shown the presence of osteoblast lineage cells close to
20 the eroded bone once inflammation resolves (111, 114, 139). However, their regenerative
21 capacity to repair bone erosions is often defective (113, 140). Cartilage's capacity to heal is
22 intrinsically limited (141), and imposes a therapeutic challenge not only in classic
23 inflammatory arthritis but also in other pathological conditions involving cartilage damage.
24 Many studies have demonstrated a close relationship between the synovial inflammation and
25 pathways regulating bone formation, including BMP and Wnt. Increased production of Wnt
26 antagonists such as DKK-1 and SFRP1/2 by inflamed synovial tissue in arthritis impairs
27 osteoblast differentiation and bone formation (139, 142, 143). In contrast to RA, the synovial
28 fluid in SpA contains increased levels of the inflammatory cytokine IL-32g, which enhances
29 osteoblast differentiation via DKK-1 suppression (144). Indeed, lower levels of DKK-1 are
30 found in AS and PsA, promoting abnormal enthesial bone formation (142, 145, 146).
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48 Impairment in BMP signaling has been described at multiple levels in both experimental
49 arthritis and arthritic patients (147-155), but with contrasting results. By analyzing the global
50 gene expression profile in the tarsal joints harvested from mice with collagen-induced
51 arthritis (CIA), Denninger et al. reported complex time-dependent changes in BMP and Wnt
52 pathways, with an early stimulation of genes associated with bone induction (149). Daans et
53 al. also reported increasing activation of the BMP pathway during CIA progression (148),
54 detected immunohistochemically as phosphorylated Smad1/5. The expression pattern
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1 translated from the synovial lining in the early phase, towards deeper layers of invading
2 pannus in the destructive phase. Mild inflammation was associated with appearance of BMP-
3 7 in the synovial lining and superficial articular chondrocytes, whereas arthritis progression
4 was marked by expression in the subintimal layer (148). Furthermore, arthritis induced
5 opposing changes in the expression of *Gdf5*, with an early decrease in the arthritic synovium
6 and a later increase in the articular cartilage (148). Since arthritis destroys the cortical bone
7 barrier and exposes BM to synovial tissue, resulting changes in the bone marrow
8 compartment affect cortical bone remodeling. Gortz et al. found an increased osteoblast
9 number at the endosteal surface, close to cortical lesions, in the human TNF transgenic
10 (hTNFtg) mice. Indeed, accumulated B lymphocytes expressed BMP-6 and -7, contributing
11 to bone induction (150). Amongst negative regulators of the BMP pathway, upregulation of
12 BMP-3 was detected in osteoblasts, in the vicinity of bone erosions of developed AIA,
13 indicating that maturing osteoblasts may suppress differentiation of newly committed
14 progenitors (153). Maeda et al. determined the synovial expression of miRNAs at erosion
15 sites using the K/BxN serum transfer model (152). They identified several downregulated
16 miRNAs targeting inhibitors of Wnt and BMP pathways, including DKK3, GSK3b, sFRP-
17 1/2, Smad7, and Tob1/2, suggesting that decreased miRNAs in the inflamed synovium may
18 contribute to induction of BMP and Wnt antagonists, and limit bone formation at erosion
19 sites.
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21 Alterations in BMP expression have been reported in different tissues from arthritic patients.
22 Our group observed that peripheral blood cells had a reduced expression of *BMP-4*, *BMP-6*,
23 and *RUNX2* in patients with RA. A negative correlation with disease activity was found for
24 *BMP-4* in RA, while a positive correlation was found for *BMP-4* in PsA (151). Within the
25 affected joints, BMP-4 and -5 were reduced in the rheumatoid synovium, and, similar to
26 experimental studies, localized in deeper synovial layers in contrast to their superficial
27 expression in normal synovial lining (147). However, Verschueren et al. reported increased
28 activation of BMP signaling, detected by phosphorylated Smad1/5, in α SMA⁺ perivascular
29 cells, CD90⁺ synovial fibroblasts, and CD68⁺ synovial macrophages of RA synovium (154).
30 Proinflammatory cytokines produced by the inflamed synovium and subchondral bone
31 marrow contribute to the modulation of BMP signals. The addition of TNF- α and IL-1 β
32 arrested osteoblast differentiation and maturation *in vitro* (132, 156-165). Yamazaki et al.
33 used the MC3T3-E1 cell line to show that the NF- κ B subunit p65 is able to associate with the
34 Smad1/4 complex and suggested that TNF- α -induced NF- κ B-activation inhibits BMP
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1 signaling by interfering with the DNA binding of Smads (165). However, Sullivan et al.
2 could not prove the involvement of NF- κ B in TNF- α - and IL-1 β -mediated suppression of
3 bone formation from primary bone marrow MSCs (164). Huang et al. suggested that TNF-
4 α /IL-1 β and BMP-2 have opposing roles that converge on Runx2 to regulate osteoblastic
5 differentiation through p38 and ERK1/2 signaling (160). Similarly, IL-6 trans-signaling
6 inhibited differentiation of MC3T3-E1 cells and primary murine calvarial osteoblasts through
7 MEK/Erk and PI3K/Akt2 pathways. An anti-osteoblastogenic effect was detected as reduced
8 alkaline phosphatase (ALP) activity and mineralization as well as down-regulation of
9 osteoblast-specific genes (*Runx2*, *Osx* and *Bglap*) (135). TNF- α stimulates expression of
10 Smurf1 (Smad ubiquitin regulatory factor 1) in the C2C12 myoblast cell line and primary
11 cultured mouse calvarial cells, which can bind to BMP type I receptors and Smad1/5 complex
12 via I-Smads to induce their ubiquitination and degradation (133). Therefore, *in vivo* blocking
13 of TNF- α may be a promising approach for compromised bone healing (166, 167).
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24 However, several studies indicated that cytokines released at localized sites of inflammation,
25 including TNF- α , TGF- β , IFN- γ , and IL-17, may be the driving force for differentiation of
26 mesenchymal progenitors into the osteoblast phenotype (97). Osta et al. investigated the
27 effects of TNF- α and IL-17 on the osteogenic differentiation of isolated human bone
28 marrow-derived mesenchymal cells (168). They revealed that TNF- α and IL-17 increased
29 ALP activity in differentiating osteoblasts, but only TNF- α stimulated BMP-2 expression at
30 early time-points (6 and 12 h). In osteogenic cultures of adipose-derived mesenchymal cells
31 from RA and OA patients, TNF- α potentiated calcium deposition, up-regulated *RUNX2* and
32 *BMP-2*, but down-regulated *COL1a1* and *OPN* expression (169). In cultures of the MC3T3-
33 E1 murine cell line and primary murine calvarial osteoblasts, IL-6 stimulated osteoblast
34 differentiation through the interplay between STAT3 and the Smad/BMP pathway (135).
35 Similar effects were observed in the human osteoblast MG-63 cell line, where IL-6 trans-
36 signaling caused a marked elevation of ALP activity and a decrease in cell proliferation
37 through the JAK/STAT pathway (170). Although it seems that inflammation may have a
38 positive effect on the BMP pathway, the rate of bone formation in RA is insufficient to
39 counteract the greatly induced rate of bone resorption. However, in AS, where chronic
40 inflammation is accompanied with pathologic bone formation, TNF- α induced BMP-2
41 production in human chondrogenic cells (171, 172). Elevated IL-17 was associated with an
42 increased BMP-2 expression and heterotopic cartilage/bone formation in hyperplastic
43 enthesal tissues of mouse AS (173). Murine and human spinal ligament cells undergo
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1 osteogenic differentiation under BMP-2 treatment (174-176). Therefore, it seems that the
2 control of inflammation may reduce BMP pathway activation and abnormal osteogenic
3 differentiation within entheses.
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5 During inflammation, BMPs do not participate only in altered homeostasis of the skeletal
6 cells, but affect other cells, such as FLSs and macrophages (177). Lories et al. reported
7 increased BMP-2 and -6 expression in different cell types of RA hyperplastic synovia
8 including CD68⁺ macrophages and CD90⁺ FLS (178). TNF- α and IL-1 β upregulated both
9 BMPs in FLSs, which, in turn, had opposite autocrine effects, with BMP-2 acting as a
10 proapoptotic and BMP-6 as a protective factor (178). Varas et al. confirmed the expression of
11 BMP ligands (BMP-2, BMP-6, and BMP-7), receptors (mainly BMPRIA, ACTRIA and
12 BMPRII), and signal transducers (Smad1/5 and co-Smad4) in FLSs (179). These cells also
13 expressed BMP antagonists, which were highly upregulated after activation with TNF- α and
14 IL-17, suggesting that inhibition of the autocrine BMP pathway exacerbates the FLS pro-
15 inflammatory phenotype in RA. Wei et al. showed that BMP-2 down-regulated the
16 expression of inflammatory (M1) mediators, including IL-1 β , IL-6 and iNOS, and induced
17 the expression of reparative (M2) factors in macrophages, indicating a positive
18 immunoregulatory role of BMP-2 (180). Thus, it may be possible to utilize BMP-2
19 immunomodulatory properties to manipulate the osteoimmune environment for favorable
20 bone regeneration.
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37 **4. Conclusions**

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39 The role of inflammation is strongly context dependent, regarding local microenvironment,
40 cell types and mechanical forces, resulting in opposite net effects on bone tissue in different
41 diseases. In fracture healing, the inflammatory response induced immediately after injury is
42 crucial for recruitment of osteochondroprogenitor cells and creation of a microenvironment
43 that favors their expansion and differentiation into mature chondrocytes or osteoblasts. At the
44 site of injury, inflammation induces BMP signaling, and BMPs originate from various cell
45 types, ranging from osteochondroprogenitors themselves to inflammatory cells. BMP
46 signaling is beneficial in proper regeneration after fracture injury and has even been proposed
47 in therapeutical contexts. In inflammatory arthritides, inflammation promotes bone loss in the
48 joints, in periarticular areas, and systemically in RA, but drives bone formation at enthesial
49 and periosteal sites in diseases such as AS. Although most studies focus on the destructive
50 component of rheumatic diseases through the induction of osteoclasts, it has become
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1 increasingly evident that osteoblasts are also targeted by inflammation. We show that murine
2 synovial CD51⁺CD200⁺CD105⁻ cells most likely represent osteochondroprogenitors, which
3 are decreased in number in murine arthritis, suggesting that the progenitor pool is impaired.
4 Wnt and BMP signaling pathways have emerged as critical in the regulation of osteoblast
5 function and the net outcome for bone in rheumatic diseases, and these pathways have been
6 implicated in both impaired repair of articular erosions in RA, and in the pathological bone
7 formation in axial SpA. Proinflammatory molecules in the inflamed joint are known to
8 modulate BMP signaling, and altered BMP signaling is not only crucial for osteoblasts and
9 their progenitors, but also affects other cells, such as the FLS and macrophages. Although it
10 seems that inflammation may have a net stimulatory effect on BMP signaling pathway, the
11 rate of bone formation in arthritis is insufficient to counteract the greatly induced rate of bone
12 resorption. Therefore, more specific approaches to assess activation of the BMP pathway in
13 regard to the skeletal system at their various developmental stages might be useful in
14 addressing the question of impaired bone and cartilage regeneration during inflammatory
15 arthritides.
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References

1. Owen M, Friedenstein AJ. Stromal stem cells: marrow-derived osteogenic precursors. *Ciba Found Symp.* 1988;136:42-60.
2. Caplan AI. Mesenchymal stem cells. *J Orthop Res.* 1991;9(5):641-50.
3. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy.* 2006;8(4):315-7.
4. Sipp D, Robey PG, Turner L. Clear up this stem-cell mess. *Nature.* 2018;561(7724):455-7.
5. Bianco P, Robey PG. Skeletal stem cells. *Development.* 2015;142(6):1023-7.
6. Sacchetti B, Funari A, Remoli C, Giannicola G, Kogler G, Liedtke S, et al. No Identical "Mesenchymal Stem Cells" at Different Times and Sites: Human Committed Progenitors of Distinct Origin and Differentiation Potential Are Incorporated as Adventitial Cells in Microvessels. *Stem Cell Reports.* 2016;6(6):897-913.
7. Bianco P, Robey P. Skeletal Stem Cells. In: Lanza R, Atala A, eds. *Handbook of Adult and Fetal Stem Cells.* 2nd ed. Amsterdam: Elsevier. 2013;2:415-424.
8. Ambrosi TH, Longaker MT, Chan CKF. A Revised Perspective of Skeletal Stem Cell Biology. *Front Cell Dev Biol.* 2019;7:189.
9. Liu JQ, Li QW, Tan Z. New Insights on Properties and Spatial Distributions of Skeletal Stem Cells. *Stem Cells Int.* 2019;2019:9026729.
10. Morikawa S, Mabuchi Y, Kubota Y, Nagai Y, Niibe K, Hiratsu E, et al. Prospective identification, isolation, and systemic transplantation of multipotent mesenchymal stem cells in murine bone marrow. *J Exp Med.* 2009;206(11):2483-96.
11. Ambrosi TH, Scialdone A, Graja A, Gohlke S, Jank AM, Bocian C, et al. Adipocyte Accumulation in the Bone Marrow during Obesity and Aging Impairs Stem Cell-Based Hematopoietic and Bone Regeneration. *Cell Stem Cell.* 2017;20(6):771-84 e6.
12. Chan CK, Seo EY, Chen JY, Lo D, McArdele A, Sinha R, et al. Identification and specification of the mouse skeletal stem cell. *Cell.* 2015;160(1-2):285-98.
13. Gulati GS, Murphy MP, Marcic O, Lopez M, Brewer RE, Koepke LS, et al. Isolation and functional assessment of mouse skeletal stem cell lineage. *Nat Protoc.* 2018;13(6):1294-309.
14. Mendez-Ferrer S, Michurina TV, Ferraro F, Mazloom AR, Macarthur BD, Lira SA, et al. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. *Nature.* 2010;466(7308):829-34.
15. Grcevic D, Pejda S, Matthews BG, Repic D, Wang L, Li H, et al. In vivo fate mapping identifies mesenchymal progenitor cells. *Stem Cells.* 2012;30(2):187-96.
16. Park D, Spencer JA, Koh BI, Kobayashi T, Fujisaki J, Clemens TL, et al. Endogenous bone marrow MSCs are dynamic, fate-restricted participants in bone maintenance and regeneration. *Cell Stem Cell.* 2012;10(3):259-72.
17. Rux DR, Song JY, Swinehart IT, Pineault KM, Schlientz AJ, Trulik KG, et al. Regionally Restricted Hox Function in Adult Bone Marrow Multipotent Mesenchymal Stem/Stromal Cells. *Dev Cell.* 2016;39(6):653-66.
18. Duchamp de Lageneste O, Julien A, Abou-Khalil R, Frangi G, Carvalho C, Cagnard N, et al. Periosteum contains skeletal stem cells with high bone regenerative potential controlled by Periostin. *Nat Commun.* 2018;9(1):773.

- 1 19. Ortinau LC, Wang H, Lei K, Deveza L, Jeong Y, Hara Y, et al. Identification of
2 Functionally Distinct Mx1+alphaSMA+ Periosteal Skeletal Stem Cells. *Cell Stem Cell*.
3 2019;25(6):784-96 e5.
- 4 20. Mizuhashi K, Ono W, Matsushita Y, Sakagami N, Takahashi A, Saunders TL, et al.
5 Resting zone of the growth plate houses a unique class of skeletal stem cells. *Nature*.
6 2018;563(7730):254-8.
- 7 21. Zhou BO, Yue R, Murphy MM, Peyer JG, Morrison SJ. Leptin-receptor-expressing
8 mesenchymal stromal cells represent the main source of bone formed by adult bone marrow.
9 *Cell Stem Cell*. 2014;15(2):154-68.
- 10 22. Tikhonova AN, Dolgalev I, Hu H, Sivaraj KK, Hoxha E, Cuesta-Dominguez A, et al.
11 The bone marrow microenvironment at single-cell resolution. *Nature*. 2019;569(7755):222-8.
- 12 23. Baryawno N, Przybylski D, Kowalczyk MS, Kfoury Y, Severe N, Gustafsson K, et al.
13 A Cellular Taxonomy of the Bone Marrow Stroma in Homeostasis and Leukemia. *Cell*.
14 2019;177(7):1915-32 e16.
- 15 24. Sacchetti B, Funari A, Michienzi S, Di Cesare S, Piersanti S, Saggio I, et al. Self-
16 renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic
17 microenvironment. *Cell*. 2007;131(2):324-36.
- 18 25. Tormin A, Li O, Brune JC, Walsh S, Schutz B, Ehinger M, et al. CD146 expression
19 on primary nonhematopoietic bone marrow stem cells is correlated with in situ localization.
20 *Blood*. 2011;117(19):5067-77.
- 21 26. Li H, Ghazanfari R, Zacharaki D, Ditzel N, Isern J, Ekblom M, et al. Low/negative
22 expression of PDGFR-alpha identifies the candidate primary mesenchymal stromal cells in
23 adult human bone marrow. *Stem Cell Reports*. 2014;3(6):965-74.
- 24 27. Pinho S, Lacombe J, Hanoun M, Mizoguchi T, Bruns I, Kunisaki Y, et al.
25 PDGFRalpha and CD51 mark human nestin+ sphere-forming mesenchymal stem cells
26 capable of hematopoietic progenitor cell expansion. *J Exp Med*. 2013;210(7):1351-67.
- 27 28. Chan CKF, Gulati GS, Sinha R, Tompkins JV, Lopez M, Carter AC, et al.
28 Identification of the Human Skeletal Stem Cell. *Cell*. 2018;175(1):43-56 e21.
- 29 29. Lum L, Beachy PA. The Hedgehog response network: sensors, switches, and routers.
30 *Science*. 2004;304(5678):1755-9.
- 31 30. Gordon MD, Nusse R. Wnt signaling: multiple pathways, multiple receptors, and
32 multiple transcription factors. *J Biol Chem*. 2006;281(32):22429-33.
- 33 31. Baron R, Kneissel M. WNT signaling in bone homeostasis and disease: from human
34 mutations to treatments. *Nat Med*. 2013;19(2):179-92.
- 35 32. Wu M, Chen G, Li YP. TGF-beta and BMP signaling in osteoblast, skeletal
36 development, and bone formation, homeostasis and disease. *Bone Res*. 2016;4:16009.
- 37 33. Luu HH, Song WX, Luo X, Manning D, Luo J, Deng ZL, et al. Distinct roles of bone
38 morphogenetic proteins in osteogenic differentiation of mesenchymal stem cells. *J Orthop*
39 *Res*. 2007;25(5):665-77.
- 40 34. Grgurevic L, Macek B, Mercep M, Jelic M, Smoljanovic T, Erjavec I, et al. Bone
41 morphogenetic protein (BMP)1-3 enhances bone repair. *Biochem Biophys Res Commun*.
42 2011;408(1):25-31.
- 43 35. Daluiski A, Engstrand T, Bahamonde ME, Gamer LW, Agius E, Stevenson SL, et al.
44 Bone morphogenetic protein-3 is a negative regulator of bone density. *Nat Genet*.
45 2001;27(1):84-8.
- 46 36. Nickel J, Mueller TD. Specification of BMP Signaling. *Cells*. 2019;8(12).
- 47 37. Avsian-Kretchmer O, Hsueh AJ. Comparative genomic analysis of the eight-
48 membered ring cystine knot-containing bone morphogenetic protein antagonists. *Mol*
49 *Endocrinol*. 2004;18(1):1-12.

- 1 38. Karp SJ, Schipani E, St-Jacques B, Hunzelman J, Kronenberg H, McMahon AP. Indian hedgehog coordinates endochondral bone growth and morphogenesis via parathyroid
2 hormone related-protein-dependent and -independent pathways. *Development*.
3 2000;127(3):543-8.
4
5 39. Kronenberg HM. Developmental regulation of the growth plate. *Nature*.
6 2003;423(6937):332-6.
7
8 40. Martin TJ. Parathyroid Hormone-Related Protein, Its Regulation of Cartilage and
9 Bone Development, and Role in Treating Bone Diseases. *Physiol Rev*. 2016;96(3):831-71.
10
11 41. St-Jacques B, Hammerschmidt M, McMahon AP. Indian hedgehog signaling
12 regulates proliferation and differentiation of chondrocytes and is essential for bone formation.
13 *Genes Dev*. 1999;13(16):2072-86.
14
15 42. Kobayashi T, Chung UI, Schipani E, Starbuck M, Karsenty G, Katagiri T, et al.
16 PTHrP and Indian hedgehog control differentiation of growth plate chondrocytes at multiple
17 steps. *Development*. 2002;129(12):2977-86.
18
19 43. Itoh N, Ornitz DM. Functional evolutionary history of the mouse Fgf gene family.
20 *Dev Dyn*. 2008;237(1):18-27.
21
22 44. Long F. Building strong bones: molecular regulation of the osteoblast lineage. *Nat*
23 *Rev Mol Cell Biol*. 2011;13(1):27-38.
24
25 45. Teven CM, Farina EM, Rivas J, Reid RR. Fibroblast growth factor (FGF) signaling in
26 development and skeletal diseases. *Genes Dis*. 2014;1(2):199-213.
27
28 46. Chikazu D, Katagiri M, Ogasawara T, Ogata N, Shimoaka T, Takato T, et al.
29 Regulation of osteoclast differentiation by fibroblast growth factor 2: stimulation of receptor
30 activator of nuclear factor kappaB ligand/osteoclast differentiation factor expression in
31 osteoblasts and inhibition of macrophage colony-stimulating factor function in osteoclast
32 precursors. *J Bone Miner Res*. 2001;16(11):2074-81.
33
34 47. Dailey L, Ambrosetti D, Mansukhani A, Basilico C. Mechanisms underlying
35 differential responses to FGF signaling. *Cytokine Growth Factor Rev*. 2005;16(2):233-47.
36
37 48. Xiao L, Fei Y, Hurley MM. FGF2 crosstalk with Wnt signaling in mediating the
38 anabolic action of PTH on bone formation. *Bone Rep*. 2018;9:136-44.
39
40 49. Dishowitz MI, Terkhorn SP, Bostic SA, Hankenson KD. Notch signaling components
41 are upregulated during both endochondral and intramembranous bone regeneration. *J Orthop*
42 *Res*. 2012;30(2):296-303.
43
44 50. Matthews BG, Grcevic D, Wang L, Hagiwara Y, Roguljic H, Joshi P, et al. Analysis
45 of alphaSMA-labeled progenitor cell commitment identifies notch signaling as an important
46 pathway in fracture healing. *J Bone Miner Res*. 2014;29(5):1283-94.
47
48 51. Novak S, Roeder E, Sinder BP, Adams DJ, Siebel CW, Grcevic D, et al. Modulation
49 of Notch1 signaling regulates bone fracture healing. *J Orthop Res*. 2020. in print
50 doi.org/10.1002/jor.24650
51
52 52. Wang C, Inzana JA, Mirando AJ, Ren Y, Liu Z, Shen J, et al. NOTCH signaling in
53 skeletal progenitors is critical for fracture repair. *J Clin Invest*. 2016;126(4):1471-81.
54
55 53. Zanotti S, Canalis E. Notch Signaling and the Skeleton. *Endocr Rev*. 2016;37(3):223-
56 53.
57
58 54. Tao J, Chen S, Lee B. Alteration of Notch signaling in skeletal development and
59 disease. *Ann N Y Acad Sci*. 2010;1192:257-68.
60
61 55. Canalis E, Parker K, Feng JQ, Zanotti S. Osteoblast lineage-specific effects of notch
62 activation in the skeleton. *Endocrinology*. 2013;154(2):623-34.
63
64 56. Canalis E, Bridgewater D, Schilling L, Zanotti S. Canonical Notch activation in
65 osteocytes causes osteopetrosis. *Am J Physiol Endocrinol Metab*. 2016;310(2):E171-82.
57
58 57. Karsenty G, Kronenberg HM, Settembre C. Genetic control of bone formation. *Annu*
59 *Rev Cell Dev Biol*. 2009;25:629-48.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
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46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
58. Murakami S, Kan M, McKeehan WL, de Crombrughe B. Up-regulation of the chondrogenic Sox9 gene by fibroblast growth factors is mediated by the mitogen-activated protein kinase pathway. *Proc Natl Acad Sci U S A.* 2000;97(3):1113-8.
 59. Dy P, Wang W, Bhattaram P, Wang Q, Wang L, Ballock RT, et al. Sox9 directs hypertrophic maturation and blocks osteoblast differentiation of growth plate chondrocytes. *Dev Cell.* 2012;22(3):597-609.
 60. Nakashima K, Zhou X, Kunkel G, Zhang Z, Deng JM, Behringer RR, et al. The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. *Cell.* 2002;108(1):17-29.
 61. Zhou X, Zhang Z, Feng JQ, Dusevich VM, Sinha K, Zhang H, et al. Multiple functions of Osterix are required for bone growth and homeostasis in postnatal mice. *Proc Natl Acad Sci U S A.* 2010;107(29):12919-24.
 62. Colnot C. Skeletal cell fate decisions within periosteum and bone marrow during bone regeneration. *J Bone Miner Res.* 2009;24(2):274-82.
 63. Bais M, McLean J, Sebastiani P, Young M, Wigner N, Smith T, et al. Transcriptional analysis of fracture healing and the induction of embryonic stem cell-related genes. *PLoS One.* 2009;4(5):e5393.
 64. Abou-Khalil R, Yang F, Lieu S, Julien A, Perry J, Pereira C, et al. Role of muscle stem cells during skeletal regeneration. *Stem Cells.* 2015;33(5):1501-11.
 65. Terhi JH, Teuvo AH. Differentiation of Osteoblasts and Osteocytes from Mesenchymal Stem Cells. *Current Stem Cell Research & Therapy.* 2008;3(2):131-45.
 66. Friedenstein AJ, Piatetzky S, II, Petrakova KV. Osteogenesis in transplants of bone marrow cells. *J Embryol Exp Morphol.* 1966;16(3):381-90.
 67. Tavassoli M, Crosby WH. Transplantation of marrow to extramedullary sites. *Science.* 1968;161(3836):54-6.
 68. Chan CK, Lindau P, Jiang W, Chen JY, Zhang LF, Chen CC, et al. Clonal precursor of bone, cartilage, and hematopoietic niche stromal cells. *Proc Natl Acad Sci U S A.* 2013;110(31):12643-8.
 69. Marecic O, Tevlin R, McArdle A, Seo EY, Wearda T, Duldulao C, et al. Identification and characterization of an injury-induced skeletal progenitor. *Proc Natl Acad Sci U S A.* 2015;112(32):9920-5.
 70. Grcevic D, Sironi M, Valentino S, Deban L, Cvija H, Inforzato A, et al. The Long Pentraxin 3 Plays a Role in Bone Turnover and Repair. *Front Immunol.* 2018;9:417.
 71. Cserjesi P, Lilly B, Bryson L, Wang Y, Sassoon DA, Olson EN. MHOX: a mesodermally restricted homeodomain protein that binds an essential site in the muscle creatine kinase enhancer. *Development.* 1992;115(4):1087-101.
 72. Kawanami A, Matsushita T, Chan YY, Murakami S. Mice expressing GFP and CreER in osteochondro progenitor cells in the periosteum. *Biochem Biophys Res Commun.* 2009;386(3):477-82.
 73. Wang Y, Chen L, Kang M, Ling L, Tian F, Won-Kim SH, et al. The Fracture Callus Is Formed by Progenitors of Different Skeletal Origins in a Site-Specific Manner. *JBMR Plus.* 2019;3(9):e10193.
 74. Kalajzic Z, Li H, Wang LP, Jiang X, Lamothe K, Adams DJ, et al. Use of an alpha-smooth muscle actin GFP reporter to identify an osteoprogenitor population. *Bone.* 2008;43(3):501-10.
 75. Sinder BP, Novak S, Wee NKY, Basile M, Maye P, Matthews BG, et al. Engraftment of skeletal progenitor cells by bone directed transplantation improves osteogenesis imperfecta murine bone phenotype. *Stem Cells.* 2020;38(4):530–541. doi:10.1002/stem.3133.

- 1 76. Kramann R, Schneider RK, DiRocco DP, Machado F, Fleig S, Bondzie PA, et al.
2 Perivascular Gli1+ progenitors are key contributors to injury-induced organ fibrosis. *Cell*
3 *Stem Cell*. 2015;16(1):51-66.
- 4 77. Shi Y, He G, Lee WC, McKenzie JA, Silva MJ, Long F. Gli1 identifies osteogenic
5 progenitors for bone formation and fracture repair. *Nat Commun*. 2017;8(1):2043.
- 6 78. Worthley DL, Churchill M, Compton JT, Taylor Y, Rao M, Si Y, et al. Gremlin 1
7 identifies a skeletal stem cell with bone, cartilage, and reticular stromal potential. *Cell*.
8 2015;160(1-2):269-84.
- 9 79. Matsushita Y, Nagata M, Kozloff KM, Welch JD, Mizuhashi K, Tokavanich N, et al.
10 A Wnt-mediated transformation of the bone marrow stromal cell identity orchestrates skeletal
11 regeneration. *Nat Commun*. 2020;11(1):332.
- 12 80. Mizoguchi T, Pinho S, Ahmed J, Kunisaki Y, Hanoun M, Mendelson A, et al. Osterix
13 marks distinct waves of primitive and definitive stromal progenitors during bone marrow
14 development. *Dev Cell*. 2014;29(3):340-9.
- 15 81. Tournaire G, Stegen S, Giacomini G, Stockmans I, Moermans K, Carmeliet G, et al.
16 Nestin-GFP transgene labels skeletal progenitors in the periosteum. *Bone*. 2020;133:115259.
- 17 82. Murao H, Yamamoto K, Matsuda S, Akiyama H. Periosteal cells are a major source
18 of soft callus in bone fracture. *J Bone Miner Metab*. 2013;31(4):390-8.
- 19 83. Root SH, Wee NK, Novak S, Rosen CJ, Baron R, Matthews BG, et al. Perivascular
20 osteoprogenitors are associated with trans-cortical channels of long bones. *Stem Cells*. in
21 print doi:10.1002/stem.3159.
- 22 84. Tsuji K, Cox K, Gamer L, Graf D, Economides A, Rosen V. Conditional deletion of
23 BMP7 from the limb skeleton does not affect bone formation or fracture repair. *J Orthop Res*.
24 2010;28(3):384-9.
- 25 85. Tsuji K, Bandyopadhyay A, Harfe BD, Cox K, Kakar S, Gerstenfeld L, et al. BMP2
26 activity, although dispensable for bone formation, is required for the initiation of fracture
27 healing. *Nat Genet*. 2006;38(12):1424-9.
- 28 86. Mi M, Jin H, Wang B, Yukata K, Sheu TJ, Ke QH, et al. Chondrocyte BMP2
29 signaling plays an essential role in bone fracture healing. *Gene*. 2013;512(2):211-8.
- 30 87. Chhabra A, Zijerdi D, Zhang J, Kline A, Balian G, Hurwitz S. BMP-14 deficiency
31 inhibits long bone fracture healing: a biochemical, histologic, and radiographic assessment. *J*
32 *Orthop Trauma*. 2005;19(9):629-34.
- 33 88. Massagué J. TGF- β signal transduction. *Annual Review of Biochemistry*.
34 1998;67(1):753-91.
- 35 89. Domic-Cule I, Peric M, Kucko L, Grgurevic L, Pecina M, Vukicevic S. Bone
36 morphogenetic proteins in fracture repair. *Int Orthop*. 2018;42(11):2619-26.
- 37 90. El Bialy I, Jiskoot W, Reza Nejadnik M. Formulation, Delivery and Stability of Bone
38 Morphogenetic Proteins for Effective Bone Regeneration. *Pharm Res*. 2017;34(6):1152-70.
- 39 91. Bastian O, Pillay J, Alblas J, Leenen L, Koenderman L, Blokhuis T. Systemic
40 inflammation and fracture healing. *J Leukoc Biol*. 2011;89(5):669-73.
- 41 92. Yang X, Ricciardi BF, Hernandez-Soria A, Shi Y, Pleshko Camacho N, Bostrom MP.
42 Callus mineralization and maturation are delayed during fracture healing in interleukin-6
43 knockout mice. *Bone*. 2007;41(6):928-36.
- 44 93. Gerstenfeld LC, Cho TJ, Kon T, Aizawa T, Tsay A, Fitch J, et al. Impaired fracture
45 healing in the absence of TNF-alpha signaling: the role of TNF-alpha in endochondral
46 cartilage resorption. *J Bone Miner Res*. 2003;18(9):1584-92.
- 47 94. Prystaz K, Kaiser K, Kovtun A, Haffner-Luntzer M, Fischer V, Rapp AE, et al.
48 Distinct Effects of IL-6 Classic and Trans-Signaling in Bone Fracture Healing. *Am J Pathol*.
49 2018;188(2):474-90.
- 50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

- 1 95. Rapp AE, Bindl R, Recknagel S, Erbacher A, Muller I, Schrezenmeier H, et al.
2 Fracture Healing Is Delayed in Immunodeficient NOD/scidIL2Rgammacnull Mice. *PLoS*
3 *One*. 2016;11(2):e0147465.
- 4 96. Toben D, Schroeder I, El Khassawna T, Mehta M, Hoffmann JE, Frisch JT, et al.
5 Fracture healing is accelerated in the absence of the adaptive immune system. *J Bone Miner*
6 *Res*. 2011;26(1):113-24.
- 7 97. Rifas L. T-cell cytokine induction of BMP-2 regulates human mesenchymal stromal
8 cell differentiation and mineralization. *J Cell Biochem*. 2006;98(4):706-14.
- 9 98. Claes L, Recknagel S, Ignatius A. Fracture healing under healthy and inflammatory
10 conditions. *Nat Rev Rheumatol*. 2012;8(3):133-43.
- 11 99. Hoff P, Gaber T, Strehl C, Jakstadt M, Hoff H, Schmidt-Bleek K, et al. A Pronounced
12 Inflammatory Activity Characterizes the Early Fracture Healing Phase in Immunologically
13 Restricted Patients. *Int J Mol Sci*. 2017;18(3).
- 14 100. Dube PR, Birnbaumer L, Vazquez G. Evidence for constitutive bone morphogenetic
15 protein-2 secretion by M1 macrophages: Constitutive auto/paracrine osteogenic signaling by
16 BMP-2 in M1 macrophages. *Biochem Biophys Res Commun*. 2017;491(1):154-8.
- 17 101. Zhang Y, Bose T, Unger RE, Jansen JA, Kirkpatrick CJ, van den Beucken J.
18 Macrophage type modulates osteogenic differentiation of adipose tissue MSCs. *Cell Tissue*
19 *Res*. 2017;369(2):273-86.
- 20 102. Rahman MS, Akhtar N, Jamil HM, Banik RS, Asaduzzaman SM. TGF-beta/BMP
21 signaling and other molecular events: regulation of osteoblastogenesis and bone formation.
22 *Bone Res*. 2015;3:15005.
- 23 103. Niikura T, Hak DJ, Reddi AH. Global gene profiling reveals a downregulation of
24 BMP gene expression in experimental atrophic nonunions compared to standard healing
25 fractures. *J Orthop Res*. 2006;24(7):1463-71.
- 26 104. Cho TJ, Gerstenfeld LC, Einhorn TA. Differential temporal expression of members of
27 the transforming growth factor beta superfamily during murine fracture healing. *J Bone*
28 *Miner Res*. 2002;17(3):513-20.
- 29 105. Benn A, Hiepen C, Osterland M, Schutte C, Zwijsen A, Knaus P. Role of bone
30 morphogenetic proteins in sprouting angiogenesis: differential BMP receptor-dependent
31 signaling pathways balance stalk vs. tip cell competence. *FASEB J*. 2017;31(11):4720-33.
- 32 106. Kimura Y, Miyazaki N, Hayashi N, Otsuru S, Tamai K, Kaneda Y, et al. Controlled
33 release of bone morphogenetic protein-2 enhances recruitment of osteogenic progenitor cells
34 for de novo generation of bone tissue. *Tissue Eng Part A*. 2010;16(4):1263-70.
- 35 107. Kloen P, Paola M, Borens O, Richmond J, Perino G, Helfet D, et al. BMP signaling
36 components are expressed in human fracture callus. *Bone*. 2003;33:362-71.
- 37 108. Yu YY, Lieu S, Lu C, Miclau T, Marcucio RS, Colnot C. Immunolocalization of
38 BMPs, BMP antagonists, receptors, and effectors during fracture repair. *Bone*.
39 2010;46(3):841-51.
- 40 109. Kloen P, Lauzier D, Hamdy RC. Co-expression of BMPs and BMP-inhibitors in
41 human fractures and non-unions. *Bone*. 2012;51(1):59-68.
- 42 110. Wang X, Matthews BG, Yu J, Novak S, Grcevic D, Sanjay A, et al. PDGF Modulates
43 BMP2-Induced Osteogenesis in Periosteal Progenitor Cells. *JBMR Plus*. 2019;3(5):e10127.
- 44 111. Coury F, Peyruchaud O, Machuca-Gayet I. Osteoimmunology of Bone Loss in
45 Inflammatory Rheumatic Diseases. *Front Immunol*. 2019;10:679.
- 46 112. Baum R, Gravallese EM. Bone as a Target Organ in Rheumatic Disease: Impact on
47 Osteoclasts and Osteoblasts. *Clin Rev Allergy Immunol*. 2016;51(1):1-15.
- 48 113. Schett G, Gravallese E. Bone erosion in rheumatoid arthritis: mechanisms, diagnosis
49 and treatment. *Nat Rev Rheumatol*. 2012;8(11):656-64.
- 50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

114. Gravallesse E, Goldring S. Cellular mechanism and the role of cytokines in bone erosions in rheumatoid arthritis. *Arthritis and rheumatism*. 2000;43:2143-51.
115. Walsh NC, Gravallesse EM. Bone remodeling in rheumatic disease: a question of balance. *Immunol Rev*. 2010;233(1):301-12.
116. FitzGerald O, Haroon M, Giles JT, Winchester R. Concepts of pathogenesis in psoriatic arthritis: genotype determines clinical phenotype. *Arthritis Res Ther*. 2015;17:115.
117. Lories RJ, Luyten FP, de Vlam K. Progress in spondylarthritis. Mechanisms of new bone formation in spondyloarthritis. *Arthritis Res Ther*. 2009;11(2):221.
118. Tsui FW, Tsui HW, Akram A, Haroon N, Inman RD. The genetic basis of ankylosing spondylitis: new insights into disease pathogenesis. *Appl Clin Genet*. 2014;7:105-15.
119. Jung YK, Kang YM, Han S. Osteoclasts in the Inflammatory Arthritis: Implications for Pathologic Osteolysis. *Immune Netw*. 2019;19(1):e2.
120. Sato K, Takayanagi H. Osteoclasts, rheumatoid arthritis, and osteoimmunology. *Curr Opin Rheumatol*. 2006;18(4):419-26.
121. Bianco P, Robey PG, Simmons PJ. Mesenchymal stem cells: revisiting history, concepts, and assays. *Cell Stem Cell*. 2008;2(4):313-9.
122. De Bari C, Dell'Accio F, Tylzanowski P, Luyten F. Multipotent mesenchymal stem cells from adult human synovial membrane. *Arthritis & Rheumatism*. 2001;44:1928-42.
123. Jones EA, English A, Henshaw K, Kinsey SE, Markham AF, Emery P, et al. Enumeration and phenotypic characterization of synovial fluid multipotential mesenchymal progenitor cells in inflammatory and degenerative arthritis. *Arthritis Rheum*. 2004;50(3):817-27.
124. Sakaguchi Y, Sekiya I, Yagishita K, Muneta T. Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source. *Arthritis Rheum*. 2005;52(8):2521-9.
125. Futami I, Ishijima M, Kaneko H, Tsuji K, Ichikawa-Tomikawa N, Sadatsuki R, et al. Isolation and characterization of multipotential mesenchymal cells from the mouse synovium. *PLoS One*. 2012;7(9):e45517.
126. Decker RS, Koyama E, Pacifici M. Genesis and morphogenesis of limb synovial joints and articular cartilage. *Matrix Biol*. 2014;39:5-10.
127. Roelofs AJ, Zupan J, Riemen AHK, Kania K, Ansboro S, White N, et al. Joint morphogenetic cells in the adult mammalian synovium. *Nat Commun*. 2017;8:15040.
128. Li F, Tang Y, Song B, Yu M, Li Q, Zhang C, et al. Nomenclature clarification: synovial fibroblasts and synovial mesenchymal stem cells. *Stem Cell Res Ther*. 2019;10(1):260.
129. Mazor M, Cesaro A, Ali M, Best TM, Lespessaille E, Toumi H. Progenitor Cells from Cartilage: Grade Specific Differences in Stem Cell Marker Expression. *Int J Mol Sci*. 2017;18(8).
130. Matsukura Y, Muneta T, Tsuji K, Miyatake K, Yamada J, Abula K, et al. Mouse synovial mesenchymal stem cells increase in yield with knee inflammation. *J Orthop Res*. 2015;33(2):246-53.
131. Mosler E, Kuzmac S, Ivcevic S, Grcevic D, Marusic A, Kovacic N. Fas deficiency attenuates bone loss during antigen induced arthritis in mice. *Arthritis Research & Therapy*. 2012;14(Suppl 1).
132. Gilbert L, He X, Farmer P, Rubin J, Drissi H, van Wijnen AJ, et al. Expression of the osteoblast differentiation factor RUNX2 (Cbfa1/AML3/Pebp2alpha A) is inhibited by tumor necrosis factor-alpha. *J Biol Chem*. 2002;277(4):2695-701.
133. Kaneki H, Guo R, Chen D, Yao Z, Schwarz EM, Zhang YE, et al. Tumor necrosis factor promotes Runx2 degradation through up-regulation of Smurf1 and Smurf2 in osteoblasts. *J Biol Chem*. 2006;281(7):4326-33.

134. Stashenko P, Dewhirst FE, Rooney ML, Desjardins LA, Heeley JD. Interleukin-1 beta is a potent inhibitor of bone formation in vitro. *J Bone Miner Res.* 1987;2(6):559-65.
135. Kaneshiro S, Ebina K, Shi K, Higuchi C, Hirao M, Okamoto M, et al. IL-6 negatively regulates osteoblast differentiation through the SHP2/MEK2 and SHP2/Akt2 pathways in vitro. *J Bone Miner Metab.* 2014;32(4):378-92.
136. Dohn UM, Ejbjerg B, Boonen A, Hetland ML, Hansen MS, Knudsen LS, et al. No overall progression and occasional repair of erosions despite persistent inflammation in adalimumab-treated rheumatoid arthritis patients: results from a longitudinal comparative MRI, ultrasonography, CT and radiography study. *Ann Rheum Dis.* 2011;70(2):252-8.
137. Finzel S, Rech J, Schmidt S, Engelke K, Englbrecht M, Schett G. Interleukin-6 receptor blockade induces limited repair of bone erosions in rheumatoid arthritis: a micro CT study. *Ann Rheum Dis.* 2013;72(3):396-400.
138. Moller Dohn U, Boonen A, Hetland ML, Hansen MS, Knudsen LS, Hansen A, et al. Erosive progression is minimal, but erosion healing rare, in patients with rheumatoid arthritis treated with adalimumab. A 1 year investigator-initiated follow-up study using high-resolution computed tomography as the primary outcome measure. *Ann Rheum Dis.* 2009;68(10):1585-90.
139. Matzelle MM, Gallant MA, Condon KW, Walsh NC, Manning CA, Stein GS, et al. Resolution of inflammation induces osteoblast function and regulates the Wnt signaling pathway. *Arthritis Rheum.* 2012;64(5):1540-50.
140. Schett G, Stolina M, Dwyer D, Zack D, Uderhardt S, Kronke G, et al. Tumor necrosis factor alpha and RANKL blockade cannot halt bony spur formation in experimental inflammatory arthritis. *Arthritis Rheum.* 2009;60(9):2644-54.
141. Buckwalter JA. Articular cartilage injuries. *Clin Orthop Relat Res.* 2002(402):21-37.
142. Diarra D, Stolina M, Polzer K, Zwerina J, Ominsky MS, Dwyer D, et al. Dickkopf-1 is a master regulator of joint remodeling. *Nat Med.* 2007;13(2):156-63.
143. Walsh NC, Reinwald S, Manning CA, Condon KW, Iwata K, Burr DB, et al. Osteoblast function is compromised at sites of focal bone erosion in inflammatory arthritis. *J Bone Miner Res.* 2009;24(9):1572-85.
144. Lee EJ, Lee EJ, Chung YH, Song DH, Hong S, Lee CK, et al. High level of interleukin-32 gamma in the joint of ankylosing spondylitis is associated with osteoblast differentiation. *Arthritis Res Ther.* 2015;17:350.
145. Daoussis D, Liossis S-N, Solomou E, Tsanaktsi A, Bounia K, Karampetsou M, et al. Evidence That Dkk-1 Is Dysfunctional in Ankylosing Spondylitis. *Arthritis and rheumatism.* 2010;62:150-8.
146. Fassio A, Idolazzi L, Viapiana O, Benini C, Vantaggiato E, Bertoldo F, et al. In psoriatic arthritis Dkk-1 and PTH are lower than in rheumatoid arthritis and healthy controls. *Clin Rheumatol.* 2017;36(10):2377-81.
147. Bramlage CP, Haupl T, Kaps C, Ungethum U, Krenn V, Pruss A, et al. Decrease in expression of bone morphogenetic proteins 4 and 5 in synovial tissue of patients with osteoarthritis and rheumatoid arthritis. *Arthritis Res Ther.* 2006;8(3):R58.
148. Daans M, Lories R, Luyten F. Dynamic activation of bone morphogenetic protein signaling in collagen-induced arthritis supports their role in joint homeostasis and disease. *Arthritis research & therapy.* 2008;10:R115.
149. Denninger KC, Litman T, Marstrand T, Moller K, Svensson L, Labuda T, et al. Kinetics of gene expression and bone remodelling in the clinical phase of collagen-induced arthritis. *Arthritis Res Ther.* 2015;17:43.
150. Gortz B, Hayer S, Redlich K, Zwerina J, Tohidast-Akrad M, Tuerk B, et al. Arthritis induces lymphocytic bone marrow inflammation and endosteal bone formation. *J Bone Miner Res.* 2004;19(6):990-8.

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151. Grcevic D, Jajic Z, Kovacic N, Lukic IK, Velagic V, Grubisic F, et al. Peripheral blood expression profiles of bone morphogenetic proteins, tumor necrosis factor-superfamily molecules, and transcription factor Runx2 could be used as markers of the form of arthritis, disease activity, and therapeutic responsiveness. *J Rheumatol.* 2010;37(2):246-56.
 152. Maeda Y, Farina NH, Matzelle MM, Fanning PJ, Lian JB, Gravallesse EM. Synovium-Derived MicroRNAs Regulate Bone Pathways in Rheumatoid Arthritis. *J Bone Miner Res.* 2017;32(3):461-72.
 153. Matzelle MM, Shaw AT, Baum R, Maeda Y, Li J, Karmakar S, et al. Inflammation in arthritis induces expression of BMP3, an inhibitor of bone formation. *Scand J Rheumatol.* 2016;45(5):379-83.
 154. Verschuere PC, Lories RJ, Daans M, Theate I, Durez P, Westhovens R, et al. Detection, identification and in vivo treatment responsiveness of bone morphogenetic protein (BMP)-activated cell populations in the synovium of patients with rheumatoid arthritis. *Ann Rheum Dis.* 2009;68(1):117-23.
 155. Zoricic S, Maric I, Bobinac D, Vukicevic S. Expression of bone morphogenetic proteins and cartilage-derived morphogenetic proteins during osteophyte formation in humans. *J Anat.* 2003;202(Pt 3):269-77.
 156. Abbas S, Zhang Y-H, Clohisy J, Abu-Amer Y. Tumor necrosis factor-alpha inhibits pre-osteoblast differentiation through its type-1 receptor. *Cytokine.* 2003;22:33-41.
 157. Bertolini DR, Nedwin GE, Bringman TS, Smith DD, Mundy GR. Stimulation of bone resorption and inhibition of bone formation in vitro by human tumour necrosis factors. *Nature.* 1986;319(6053):516-8.
 158. Centrella M, McCarthy TL, Canalis E. Tumor Necrosis Factor- α Inhibits Collagen Synthesis and Alkaline Phosphatase Activity Independently of Its Effect on Deoxyribonucleic Acid Synthesis in Osteoblast-Enriched Bone Cell Cultures*. *Endocrinology.* 1988;123(3):1442-8.
 159. Gilbert L, He X, Farmer P, Boden S, Kozlowski M, Rubin J, et al. Inhibition of osteoblast differentiation by tumor necrosis factor-alpha. *Endocrinology.* 2000;141(11):3956-64.
 160. Huang RL, Yuan Y, Tu J, Zou GM, Li Q. Opposing TNF-alpha/IL-1beta- and BMP-2-activated MAPK signaling pathways converge on Runx2 to regulate BMP-2-induced osteoblastic differentiation. *Cell Death Dis.* 2014;5:e1187.
 161. Kotake S, Nanke Y. Effect of TNFalpha on osteoblastogenesis from mesenchymal stem cells. *Biochim Biophys Acta.* 2014;1840(3):1209-13.
 162. Lacey DC, Simmons PJ, Graves SE, Hamilton JA. Proinflammatory cytokines inhibit osteogenic differentiation from stem cells: implications for bone repair during inflammation. *Osteoarthritis Cartilage.* 2009;17(6):735-42.
 163. Mukai T, Otsuka F, Otani H, Yamashita M, Takasugi K, Inagaki K, et al. TNF-alpha inhibits BMP-induced osteoblast differentiation through activating SAPK/JNK signaling. *Biochem Biophys Res Commun.* 2007;356(4):1004-10.
 164. Sullivan CB, Porter RM, Evans CH, Ritter T, Shaw G, Barry F, et al. TNFalpha and IL-1beta influence the differentiation and migration of murine MSCs independently of the NF-kappaB pathway. *Stem Cell Res Ther.* 2014;5(4):104.
 165. Yamazaki M, Fukushima H, Shin M, Katagiri T, Doi T, Takahashi T, et al. Tumor necrosis factor alpha represses bone morphogenetic protein (BMP) signaling by interfering with the DNA binding of Smads through the activation of NF-kappaB. *J Biol Chem.* 2009;284(51):35987-95.
 166. Chen TH, Weber FE, Malina-Altzinger J, Ghayor C. Epigenetic drugs as new therapy for tumor necrosis factor-alpha-compromised bone healing. *Bone.* 2019;127:49-58.

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167. Eguchi Y, Wakitani S, Imai Y, Naka Y, Hashimoto Y, Nakamura H, et al. Antitumor necrotic factor agent promotes BMP-2-induced ectopic bone formation. *J Bone Miner Metab.* 2010;28(2):157-64.
168. Osta B, Benedetti G, Miossec P. Classical and Paradoxical Effects of TNF-alpha on Bone Homeostasis. *Front Immunol.* 2014;5:48.
169. Skalska U, Prochorec-Sobieszek M, Kontny E. Osteoblastic potential of infrapatellar fat pad-derived mesenchymal stem cells from rheumatoid arthritis and osteoarthritis patients. *Int J Rheum Dis.* 2016;19(6):577-85.
170. Nishimura R, Moriyama K, Yasukawa K, Mundy GR, Yoneda T. Combination of interleukin-6 and soluble interleukin-6 receptors induces differentiation and activation of JAK-STAT and MAP kinase pathways in MG-63 human osteoblastic cells. *J Bone Miner Res.* 1998;13(5):777-85.
171. Fukui N, Ikeda Y, Ohnuki T, Hikita A, Tanaka S, Yamane S, et al. Pro-inflammatory cytokine tumor necrosis factor-alpha induces bone morphogenetic protein-2 in chondrocytes via mRNA stabilization and transcriptional up-regulation. *J Biol Chem.* 2006;281(37):27229-41.
172. Neve A, Maruotti N, Corrado A, Cantatore FP. Pathogenesis of ligaments ossification in spondyloarthritis: insights and doubts. *Ann Med.* 2017;49(3):196-205.
173. Abe Y, Ohtsuji M, Ohtsuji N, Lin Q, Tsurui H, Nakae S, et al. Ankylosing enthesitis associated with up-regulated IFN-gamma and IL-17 production in (BXSB x NZB) F(1) male mice: a new mouse model. *Mod Rheumatol.* 2009;19(3):316-22.
174. Hayashi K, Ishidou Y, Yonemori K, Nagamine T, Origuchi N, Maeda S, et al. Expression and localization of bone morphogenetic proteins (BMPs) and BMP receptors in ossification of the ligamentum flavum. *Bone.* 1997;21(1):23-30.
175. Hoshi K, Amizuka N, Sakou T, Kurokawa T, Ozawa H. Fibroblasts of spinal ligaments pathologically differentiate into chondrocytes induced by recombinant human bone morphogenetic protein-2: morphological examinations for ossification of spinal ligaments. *Bone.* 1997;21(2):155-62.
176. Tanaka H, Nagai E, Murata H, Tsubone T, Shirakura Y, Sugiyama T, et al. Involvement of bone morphogenetic protein-2 (BMP-2) in the pathological ossification process of the spinal ligament. *Rheumatology (Oxford).* 2001;40(10):1163-8.
177. Salazar VS, Gamer LW, Rosen V. BMP signalling in skeletal development, disease and repair. *Nat Rev Endocrinol.* 2016;12(4):203-21.
178. Lories RJ, Derese I, Ceuppens JL, Luyten FP. Bone morphogenetic proteins 2 and 6, expressed in arthritic synovium, are regulated by proinflammatory cytokines and differentially modulate fibroblast-like synoviocyte apoptosis. *Arthritis Rheum.* 2003;48(10):2807-18.
179. Varas A, Valencia J, Lavocat F, Martinez VG, Thiam NN, Hidalgo L, et al. Blockade of bone morphogenetic protein signaling potentiates the pro-inflammatory phenotype induced by interleukin-17 and tumor necrosis factor-alpha combination in rheumatoid synoviocytes. *Arthritis Res Ther.* 2015;17:192.
180. Wei F, Zhou Y, Wang J, Liu C, Xiao Y. The Immunomodulatory Role of BMP-2 on Macrophages to Accelerate Osteogenesis. *Tissue Eng Part A.* 2018;24(7-8):584-94.

Figure legends

Figure 1. Murine/human osteochondroprogenitor phenotype and major mechanisms of the inflammation-induced BMP pathway modulation. Murine mesenchymal osteochondroprogenitor populations express various combinations of surface markers such as stem cell antigen 1 (Sca1), endoglin (CD105), differentiation antigen 1 (BP1, 6C3), membrane glycoprotein Thy-1 (CD90), membrane glycoprotein OX-2 (CD200), vascular cell adhesion protein 1 (VCAM-1, CD106), platelet-derived growth factor receptor alpha (PDGFR α , CD140a), integrin alpha-V (CD51, vitronectin receptor), and membrane glycoprotein MUC18 (MCAM, CD146). Human mesenchymal progenitors have been identified by a number of surface markers including transferrin receptor (CD73), CD105, podoplanin (PDPN), CD90, CD51, CD146, endolyn (CD164), low-affinity nerve growth factor receptor (CD271), CD140a, PDGFR β (CD140b), and stromal cell surface marker-1 (STRO-1). In addition, a variety of progenitor markers have been applied in murine lineage tracing models, including *Acta2*, *Hox11*, *Mx1*, *Nes*, *Gli1*, and *Prrx1*. Osteogenic pathways in progenitor cells are influenced by a variety of modulators produced by surrounding cells, which determine the net effect on their proliferation, differentiation, and lifespan. Under inflammatory conditions, the balance between anabolic factors, cytokines and osteogenic pathway agonists/antagonists may be affected in opposite ways – either enhancing bone formation or suppressing bone repair. The final outcome mostly relies on the fine interplay between major intracellular signaling pathways. Under the anabolic arm, bone morphogenetic proteins (BMPs) act in a paracrine and autocrine manner and together with growth factors (PDGF, VEGF), inflammatory cytokines (IL-6, IL-17, TNF- α), and Wnt-agonists induce transcription of RUNX2 and OSX, further production of BMPs, osteoblast differentiation, alkaline phosphatase activity and matrix mineralization, at the same time suppressing progenitor proliferation and apoptosis (left). Catabolic pathways include production of BMP- and Wnt-antagonists, apoptotic factors (CD95L), and an uncontrolled release of inflammatory cytokines (TNF- α , IL-1 β , IL-6) that suppress differentiation, transcription of RUNX2 and OSX, inhibit collagen synthesis and alkaline phosphatase activity, often leading to cell apoptosis or deregulated proliferation (right). For clarity, only the most commonly described pathways that are influenced by inflammatory signals are schematically presented. More data are required to further confirm their *in vivo* importance and possible use as therapeutic targets.

Table S1. Differences in gene expression of members of BMP family, Wnt and TGF- β pathway and other selected genes between CD51⁺CD200⁻CD105⁺ and CD51⁺CD200⁻CD105⁻ populations in murine antigen-induced arthritis (AIA). Arthritis was induced in 12 week old female C57BL6 mice by intra-articular injection of methylated bovine serum albumin (mBSA) in mice previously immunized with mBSA in complete Freund's adjuvant. On day 10 of arthritis, cells were released by collagenase digestion, and 200-500 live CD45⁻CD31⁻TER119⁻CD51⁺CD200⁻CD105⁺ and CD45⁻CD31⁻TER119⁻CD51⁺CD200⁺CD105⁻ cells were sorted by FACS Aria IIu, pre-amplified and converted to cDNA by Smartseq v4 Ultra® Low Input RNA Kit for Sequencing (TakaRa). Libraries were prepared using Nextera XT DNA Library Preparation Kit (Illumina). Total of five (n=5) libraries for each population separated from individual mouse, with satisfactory concentration and quality were sequenced, with 50 million 2x75bp reads using NextSeq 500 (Illumina) and High Output Kit v2.5 (150 Cycles) (Illumina). Read quality was assessed by FASTQC, reads were trimmed with cutadapt (1), and sequences aligned by HISAT2 (2). Transcripts assembly and quantification was performed by Stringtie (3) and count matrices were normalized with trimmed mean of M values normalization (TMM) in *edgeR* (4). *limma voom* was used to assess the differential gene expression (5). Genes with absolute log₂ of fold change (FC) higher than 1.5 and adjusted p value (BH correction) lower than 0.05 were considered significantly changed. Log₂FC, adjusted p value and average expression log₂ counts per million mapped reads (CPM) of members of BMP family, Wnt and TGF- β pathway and changes in expression of other selected genes encoding for CD200, CD105, CD90, 6C3, Osterix, Runx2, RANKL, Nestin and Fas are shown in the table. All animal protocols were approved by the Ethics Committee of the University of Zagreb, School of Medicine (380-59-10106-15-168/235) and the National ethics committee (EP 07-2/2015), and conducted in accordance with accepted standards of ethical care and use of laboratory animals.

Members of the BMP family					
Gene symbol	Gene description	log₂FC	adjusted p value	log₂CPM in CD51⁺CD200⁺CD105⁻	log₂CPM in CD51⁺CD200⁻CD105⁺
Bmp2	bone morphogenetic protein 2	8.27	1.92×10⁻⁵	4.69	-3.52
Bmp3	bone morphogenetic protein 3	8.25	0.00056	4.20	-4.30
Bmp4	bone morphogenetic protein 4	9.59	1.87×10⁻⁶	7.06	-2.96
Bmp6	bone morphogenetic protein 6	8.84	3.52×10⁻⁶	5.81	-3.12
Gdf3	growth differentiation factor 3	-9.37	1.72×10⁻⁷	-4.06	5.45

Gdf6	growth differentiation factor 6	6.76	0.003886	2.31	-4.30
Acvr2b	activin receptor IIB	5.52	0.028873	2.57	-2.93
Bmpr1a	bone morphogenetic protein receptor, type 1A	4.72	0.006831	6.44	1.77
Bmpr2	bone morphogenetic protein receptor, type II	3.55	0.015029	6.82	3.17
Smad1	SMAD family member 1	4.59	0.008171	5.59	1.09
Smad3	SMAD family member 3	4.31	0.037767	4.57	0.72
Grem1	gremlin 1, DAN family BMP antagonist	6.53	0.007645	2.98	-3.22
Members of the WNT pathway					
Fzd4	frizzled class receptor 4	4.69	0.00563	5.64	0.70
Fzd7	frizzled class receptor 7	5.43	0.018986	2.66	-2.50
Fzd8	frizzled class receptor 8	5.79	0.020296	2.08	-3.45
Wnt4	wingless-type MMTV integration site family, member 4	5.75	0.024414	2.82	-2.77
Members of the TGF-β pathway					
Tgfb2	transforming growth factor, beta 2	7.10	0.000358	6.19	-0.71
Tgfb1	transforming growth factor, beta receptor I	2.73	0.024076	6.37	3.79
Tgfb3	transforming growth factor, beta receptor III	4.47	0.000777	7.98	3.37
Other					
CD200	CD200 antigen	11.29	2.66×10^{-6}	8.74	-2.72
Eng	CD105, endoglin	-3.55	0.103711	-1.38	1.88
Thy1 (CD90.1)	thymus cell antigen 1, theta	-1.03	0.753003	0.17	1.08
Enpep (6C3)	glutamyl aminopeptidase	6.10	0.022652	2.67	-3.10
Sp7 (Osterix)	Sp7 transcription factor 7	7.41	0.001808	3.59	-3.49
Runx2	runt related transcription factor 2	7.68	0.005623	2.66	-4.30
Tnfsf11 (RANKL)	tumor necrosis factor (ligand) superfamily, member 11 (RANKL)	6.03	0.024547	1.51	-4.30
Nes	Nestin	1.49	0.636425	1.18	-0.27
Fas	Fas, CD95	6.41	0.002375	4.93	-1.68

References:

1. Martin M. Cutadapt Removes Adapter Sequences From High-Throughput Sequencing Reads. EMBnet journal. 2006;17(1).
2. Kim D, Langmead B, Salzberg SL. HISAT: a fast spliced aligner with low memory requirements. Nat Methods. 2015;12:357-60.
3. Pertea M, Pertea GM, Antonescu CM, Chang TC, Mendell JT, Salzberg SL. StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. Nat Biotechnol. 2015;33:290-5.

4. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*. 2010;26:139-40.
5. Law CW, Chen Y, Shi W, Smyth GK. voom: Precision weights unlock linear model analysis tools for RNA-seq read counts. *Genome Biol*. 2014;15:R29.

Figure(s)
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