

UC Merced

UC Merced Previously Published Works

Title

Wnt Antagonists in Hematopoietic and Immune Cell Fate: Implications for Osteoporosis Therapies.

Permalink

<https://escholarship.org/uc/item/6cz627tt>

Journal

Current osteoporosis reports, 17(2)

ISSN

1544-1873

Authors

Chicana, Betsabel
Donham, Cristine
Millan, Alberto J
et al.

Publication Date

2019-04-01

DOI

10.1007/s11914-019-00503-3

Peer reviewed



Wnt Antagonists in Hematopoietic and Immune Cell Fate: Implications for Osteoporosis Therapies

Betsabel Chicana¹ · Cristine Donham¹ · Alberto J. Millan¹ · Jennifer O. Manilay^{1,2}

Published online: 5 March 2019

© Springer Science+Business Media, LLC, part of Springer Nature 2019

Abstract

Purpose of Review We reviewed the current literature on the roles of the Wnt antagonists sclerostin (Sost) and sclerostin-containing domain protein 1 (Sostdc1) on bone homeostasis, the relationship of the hypoxia-inducible factor (Hif) and von Hippel-Lindau (Vhl) pathways on Sost expression, and how changes in bone induced by depletion of *Sost*, *Sostdc1*, and *Vhl* affect hematopoietic cells.

Recent Findings B cell development is adversely affected in *Sost*-knockout mice and is more severely affected in *Vhl*-knockout mice. Inflammation in the *Sost*^{-/-} bone microenvironment could alter hematopoietic stem cell behavior. *Sostdc1*^{-/-} mice display defects in natural killer cell development and cytotoxicity.

Summary Depletion of *Sost* and *Sostdc1* have effects on immune cell function that warrant investigation in patients receiving Wnt antagonist-depleting therapies for treatment of bone diseases. Additional clinical applications for manipulation of Wnt antagonists include cancer immunotherapies, stem cell transplantation, and directed differentiation to immune lineages.

Keywords Wnt · Wnt antagonists · Hematopoiesis · Immunology · Cell differentiation · Osteoimmunology

Introduction

Osteoblast, osteocyte, and osteoclast cells maintain bone homeostasis by building, supporting, and breaking down bone tissue, respectively. The Wnt signaling pathway plays a major role in the regulation of bone homeostasis and is a strategic target for the treatment of bone diseases such as osteoporosis [1–3]. Wnt signaling also plays a role in hematopoiesis and immune cell development [4, 5], but the mechanisms of crosstalk between the skeletal and hematopoietic systems is incompletely understood. Here, we briefly review the Wnt

signaling pathway and discuss how deficiencies or depletion of the Wnt antagonists sclerostin (*Sost*) and sclerostin domain-containing protein-1 (*Sostdc1*) affect bone homeostasis. In turn, we review current findings that suggest changes in bone homeostasis induced by altering expression of these Wnt antagonists (directly by targeted knockout strategies, or indirectly via manipulation of hypoxia pathway genes) influence hematopoietic stem cell fate and immune cell development and function (Table 1).

Canonical and Non-canonical Wnt Pathways

The canonical Wnt/ β -catenin pathways and non-canonical Wnt signaling pathways play important roles in bone tissue development, hematopoiesis, and immune cell development [4, 5, 16–18]. β -catenin is a key mediator of canonical Wnt signaling, where it is first sequestered in the cytoplasm by its interactions with Axin, GSK3, and APC proteins, and β -catenin activity is regulated by ubiquitin-initiated degradation [19]. Upon activation of Wnt signaling, β -catenin is translocated into the nucleus, and it cooperates with TCF1 and LEF1 co-activator proteins to control the transcription of Wnt target genes (Fig. 1a, left). Although binding of canonical Wnt ligands (such as Wnt3a, Wnt1, Wnt8) to Wnt receptors

Betsabel Chicana, Cristine Donham, and Alberto J. Millan contributed equally to the writing of this article.

This article is part of the Topical Collection on *Skeletal Biology and Regulation*

✉ Jennifer O. Manilay
jmanilay@ucmerced.edu

¹ Quantitative and Systems Biology Graduate Program, University of California, Merced, CA, USA

² Department of Molecular and Cell Biology, School of Natural Sciences, University of California, 5200 North Lake Road, Merced, CA 95343, USA

Table 1 Summary of effects of selected Wnt signaling antagonists on the skeletal and hematopoietic systems

Antagonist	Binding partners	Regulator of bone formation	Loss or gain of function model	Effects on bone	Immune or osteolineage cells expressing antagonist in mice	Effects on immune cells	References
Sostdc1	Lrp4 Lrp5/6 BMPs	Negative	Knockout mouse	Increased cortical bone mineral density, increased marrow area, enhanced bone fracture healing	Bulk CD4 ⁺ and CD8 ⁺ T cells, MSCs and periosteal cells, CD4 ⁺ PD-1 ⁺ T cells in spleen, T _H and T _{FR} cells in Peyer's Patch and peripheral lymph nodes	NK cells: maturation block and reduced cytotoxicity B cells: Increased memory B cell frequencies	[6, 7, 8••, 9••, 10•, 11]
Sost	Lrp5/6	Negative	Knockout mouse	Increased bone mass and total cortical area, decreased BM cavity.	Osteoblasts, osteocytes, chondrocytes, MSCs	B cells: differentiation and functional defects. Th17 cells: Promotes differentiation in vitro T _{regs} : decreased in vitro.	[12–15]

initiates the signal transduction pathways, the extent and intensity of this activation is influenced by multiple factors, including Wnt receptors, co-receptors, Wnt chaperones [20], and soluble Wnt antagonists [5, 21, 22]. Non-canonical Wnt signaling pathway is activated through ligands such as Wnt5a and Wnt11, and results in the phosphorylation of JNK, which subsequently translocates to the nucleus for gene transcription (Fig. 1a, right). Wnt signaling in bone may also be regulated by microenvironmental conditions, such as hypoxia, via Hif1 α [23–26] (Fig. 1b and c).

The Role of Wnt in Hematopoiesis and Immune Function

The regulation of adult hematopoiesis is a complex process, with contributions from many signaling pathways, including Wnt, Notch, FGF, and Hedgehog [27]. These pathways are involved in the differentiation, self-renewal, and quiescence of hematopoietic stem cells (HSCs). Early studies demonstrated that overexpression of a constitutively active form of β -catenin increased the proliferation and repopulation capacity of transplanted HSCs in mice [28, 29]. Subsequent work reported that overexpression of stable β -catenin led to exhaustion of long-term HSCs and a disruption in multilineage differentiation [30]. Mechanisms by which Wnt signaling regulates hematopoiesis in a dose-dependent fashion has more recently been revealed [31]. Low levels of Wnt signaling cause HSCs to display increased reconstitution ability, but higher levels of Wnt signaling cause failure of donor HSCs to reconstitute irradiated recipients. This lack of reconstitution potential in HSCs is due to increased differentiation and loss of stemness [31]. The distinct role of the non-canonical Wnt pathway in hematopoiesis and lymphopoiesis has also been described [32]. However, how Wnt antagonists differentially regulate canonical and non-canonical Wnt pathways, and if Wnt antagonists secreted by cells of the skeletal lineage can influence Wnt signaling activation in adjacent hematopoietic and immune cells, have not been fully addressed.

The Role of Wnt Antagonists in Skeletal and Immune Systems

Sclerostin

The neutralization of sclerostin results in increased bone mass in mice and humans through facilitation of Wnt signaling [33]. Sclerostin (Sost) is a protein secreted primarily by osteocytes that negatively regulates bone formation [34] through inhibition of canonical Wnt signaling in osteoblasts [35–37]. Sclerostin binds to the Wnt co-receptors Lrp4, Lrp5, and Lrp6 and inhibits downstream Wnt signaling [38, 39]. A Wnt target gene of interest is osteoprotegerin (OPG), an

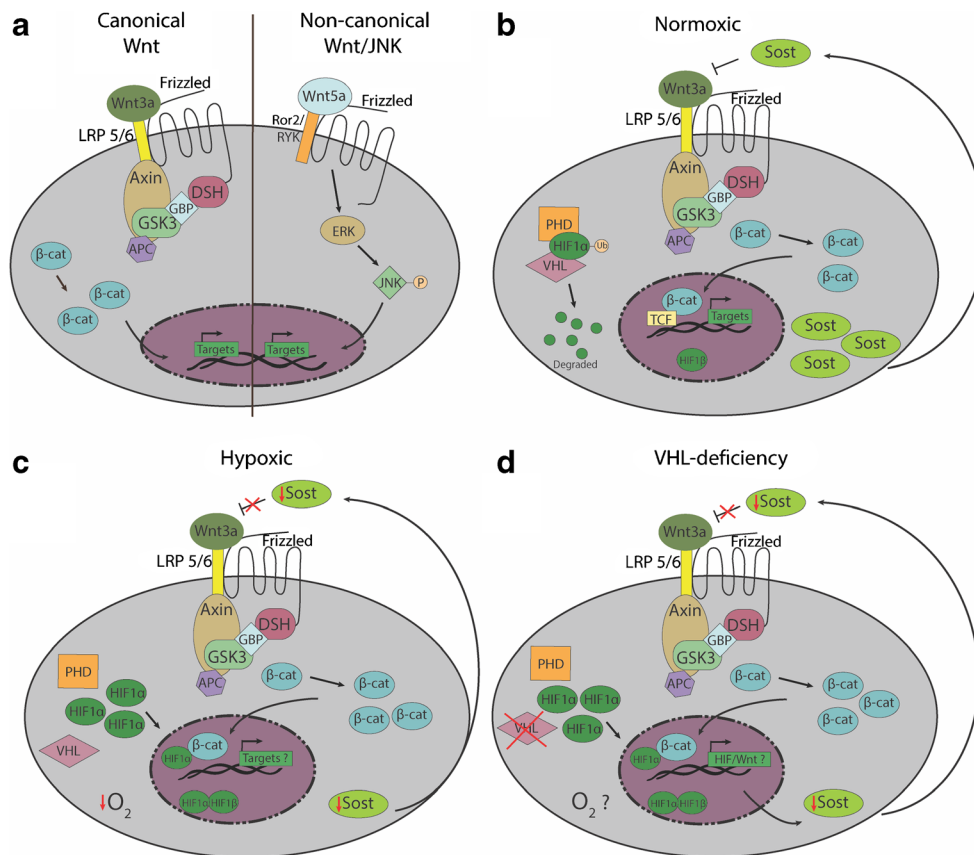


Fig. 1 Overview of Wnt signaling pathways and the regulation by Wnt antagonists and the bone microenvironment. **a** Schematic of canonical (left) and non-canonical (right) Wnt pathways. In canonical Wnt signaling, binding of ligands like Wnt3a to Frizzled family receptors results in the release of β-catenin from the Axin/GSK3/APC/DSH/GBP complex, allowing β-catenin to translocate from the cytoplasm into the nucleus for Wnt target gene transcription. In the non-canonical Wnt/JNK pathway, β-catenin is not utilized. Non-canonical Wnt ligands such as Wnt5a also bind to Frizzled receptors, but utilize the Ror2/Ryk co-receptors to activate downstream phosphorylation of JNK by ERK. Phosphorylated JNK then translocates into the nucleus to activate Wnt target gene transcription. **b** Under normoxic conditions, PHDs hydroxylate HIF1α, allowing von Hippel-Lindau (Vhl) protein to ubiquitinate Hif1α, which is subsequently degraded. In normal conditions, SOST binds to LRP 5/6 and antagonizes Wnt3a. **c** Under

hypoxic conditions, PHDs do not hydroxylate HIF1α, and VHL protein is inactivated. Therefore, HIF1α is not ubiquitinated or degraded, and is translocated into the nucleus to bind HIF1β to activate transcription of hypoxia-induced genes. Genetos et al. demonstrated that under hypoxic conditions, SOST is decreased in osteoblasts, which could lead to an increase in canonical Wnt signaling. There is evidence that Hif1α and β-catenin can act as co-activators, but how hypoxia affects Wnt signaling is not well understood. **d** In *Dmp-Cre* driven *Vhl*-knockout mice, which have been used as a model to study the effects of Hif1α stabilization in osteocytes in vivo, *Sost* expression is diminished. In turn, this decrease may result in increased canonical Wnt signaling and promote the building of bone mass. However, whether the osteocytes or the other cells within the bone and marrow microenvironments of *Vhl*-conditional knockout are truly hypoxic awaits further investigation

inhibitor of bone resorption. In *Sost*^{-/-} mice, where Wnt signaling is enhanced in bone, OPG is increased, and bone resorption decreases [40]. *Sost* also plays a role in the inhibition of the non-canonical Wnt/JNK pathway [41]. *Sost* is expressed in hypertrophic chondrocytes and is highly expressed in damaged cartilage, suggesting its role in bone and cartilage remodeling. Through *Sost* expression, restoration of anabolic genes normally inhibited by Wnt5a is achieved, allowing full metabolic activity of chondrocytes in cartilage lesion mending after mechanical stress.

Cain et al. were the first to demonstrate a role of *Sost* in the regulation of immune cell development, using global *Sost*^{-/-} mice (MGI:3797839). The number of B lymphocytes in the *Sost*^{-/-} bone marrow was reduced, due to impaired B

lymphocyte survival [12]. Importantly, this effect was mediated in an indirect, cell-extrinsic fashion, as B lymphocytes do not express *Sost*, and wild-type bone marrow cells transplanted into *Sost*^{-/-} mice displayed impaired B cell development. Further analysis demonstrated that the bone marrow stromal cells in *Sost*^{-/-} mice expressed low levels of CXCL12, a critical B cell growth factor, which might contribute to the defect in B cell development. *Sost* is also expressed by osteoblasts and mesenchymal stem cells and identified the contributions of *Sost* in specific osteolineage cells using conditional *Sost*-knockout mice (*Sost*^{COIN/COIN}, MGI:5544793) [42••]. Surprisingly, the deletion of *Sost* in osteocytes (using *Dmp-Cre*) did not affect B lymphocyte development, but the deletion of *Sost* in MSCs (using *Prx1-Cre*) resulted in a decrease

in the B cell precursors and immature B cell subsets. However, this decrease was not as pronounced as the global *Sost*^{-/-} mice, indicating another cell that expresses *Sost* is involved in B cell development. Taken together, these findings imply that patients receiving sclerostin-depleting therapies for osteoporosis might suffer from B cell defects. Functional analysis of B cells in *Sost*^{-/-} mice supports that B cell responses to antigens may be altered [13], but this has yet to be confirmed in humans.

Current studies are investigating whether exposure of hematopoietic progenitors to *Sost*-deficient microenvironments influences their differentiation. Transplantation of wild-type hematopoietic stem and progenitor cells (HSPC) into *Sost*^{-/-} recipient mice suggest that the *Sost*^{-/-} bone marrow niche tends to favor hematopoietic differentiation to the myeloid lineages. Further, extramedullary hematopoiesis in the spleen is clearly evident in aged *Sost* knockout mice (Manilay et al., unpublished data). These changes in hematopoiesis may be explained through *Wnt5a* overexpression. Staal and colleagues discovered that *Wnt5a* overexpression resulted in a marked increase in myeloid cells in the bone marrow and spleen, whereas *Wnt3a* overexpression resulted in an increase in lymphopoiesis [32]. In addition to the already known decrease in B cell lymphopoiesis, it is possible that lack of *Sost* in the bone influences myelopoiesis in mice. Increases in myeloid cells, and decreases in lymphoid cells, are indicative of an inflammatory aging hematopoietic development, and may lead to weakened immune responses. Although mice and patients with mutations in the *Sost* gene exhibit similar bone marrow cavity occlusion, as observed in *Sost*^{-/-} mice, it is unclear from the literature whether hematopoiesis has been monitored in these patients [43–45]. Our findings in *Sost*^{-/-} mice indicate that patients receiving anti-sclerostin antibodies may have altered developmental hematopoiesis in the bone marrow and spleen. This could have serious implications for patients with already diminished immune systems like the elderly, who are the primary recipient population for osteoporosis therapeutics.

von Hippel-Lindau and Hypoxia-Induced Factors

Hypoxia is a state in which the supply of oxygen is insufficient for normal cell function. Hypoxia interferes with oxygen transport and the synthesis of ATP, causing cell damage and cell death when ATP production fails to meet energy demands. One key cellular response to counter the hypoxic state is the triggering of hypoxia-inducible factor (Hif)-mediated gene expression. The Hif signaling pathway has a central role in oxygen homeostasis and it responds accordingly to oxygen tension. In normoxic conditions, prolyl hydroxylase-domain proteins (PHDs) hydroxylate hypoxia-inducible factor alpha (Hif1 α), allowing von Hippel-Lindau (Vhl) protein to ubiquitinate Hif1 α and Hif2 α for degradation [46, 47] (Fig. 1b). Under hypoxic conditions, PHD function is inhibited and Vhl protein is inactivated, resulting in HIF1 α

accumulation and activation of hypoxia-response genes in the nucleus (Fig. 1c). There are over 100 known direct HIF target genes with roles in diverse biological pathways such as angiogenesis, cell proliferation, redox homeostasis, and apoptosis [48–50].

Hypoxia regulates the processes of angiogenesis and osteogenesis during fracture healing and bone formation [51, 52], promotes osteoblast differentiation into osteocytes [53], and can stimulate osteoclast formation [54]. HIF stabilization may be a therapeutic option for treating bone fractures [55, 56] and osteoporosis [57–59], but the underlying molecular mechanism remains poorly understood. Vhl plays an important role regulating HIF expression, and disruption of VHL in bone cells leads to improper bone homeostasis. For example, *Vhl* depletion in osteochondral progenitor cells and osteocalcin-positive osteoblasts leads to an increase in bone mass through an increase in osteoblast number [60, 61]. Depletion of *Vhl* in osteoblasts also had accelerated bone repair and had implications for skeletal regeneration [62]. Furthermore, disrupting *Vhl* in osteoblasts induces expression of β -catenin, revealing the mechanism by which Vhl/Hif pathway affects bone formation through the Wnt pathway [63]. Recent work in *Vhl*-conditional knockout (*Vhl*^{fl/fl}; *Dmp1-Cre*) mice shows that *Sost* expression is decreased in the absence of *Vhl* [63, 64••] (Fig. 1d), but whether this change in *Sost* expression is due to actual reduction in oxygen tension awaits further investigation.

It is interesting that the global *Sost*^{-/-} and osteocytic-specific *Vhl*-knockout mice share some similarities in their bone and B cell phenotypes (although the effects are more severe in the *Vhl*-knockout). The mechanism by which Vhl/Hif pathways and Wnt pathways interact in normoxic and hypoxic bone microenvironments is not fully elucidated, but there are several lines of evidence that support they are dependent on each other. HIF1 α can directly bind to the promoter sequence of *Sost* [65]. Enhanced HIF1 α expression through PHD2 depletion in osteocytes decreased sclerostin expression and enhanced Wnt/ β -catenin signaling [66•]. In vitro, hypoxia decreases *Sost* expression in cultured osteoblasts and osteocytes through BMP [67], but contradictory results indicated that hypoxia can also induce *Sost* expression in osteoblasts [65]. These discrepancies could perhaps be explained by the different cell lines used in these in vitro studies. In vivo, deletion of *Vhl* in osteoblasts results in activation of HIF1 α , and a decrease in *Sost*-positive osteocytes [63]. *Vhl* deletion in osteocytes in vivo also results in a decrease in *Sost* [64••].

Osteoblasts also serve a supportive function in the maintenance of hematopoiesis and B lymphocytes [5, 68]. The bone marrow microenvironment is hypoxic, which is crucial for normal hematopoiesis [69]. Heterogeneities of local pO₂ exist within the bone marrow [70]. However, the implications of these variations in oxygen tension in hematopoietic stem cells and hematopoiesis remain uncharacterized. How hypoxia,

Vhl, and Wnt signaling crosstalk regulates bone homeostasis and B cell development is an area of active research. The role of HIF and its regulation of the immune system has been extensively reviewed, but the mechanism of Vhl in specific immune cell lineages has not fully been addressed [71]. Localized hypoxia and HIF stabilization are normal features of germinal centers. Development of robust antibody responses from conventional B lymphocytes (a.k.a. B-2 cells) is influenced by the relatively low oxygen levels in the germinal centers of the spleen and lymph nodes [72]. Cell-intrinsic deletion of Vhl in B cells in mice (*Vhl^{fl/fl}; ERT2-Cre*) stabilizes Hif1 α levels and affects B cell function by impairing cell proliferation, antibody class-switching, generation of high affinity antibodies, and antibody responses [72]. Analyses by Loots et al. suggest that specific deletion of *Vhl* in osteocytic cells results in cell-extrinsic changes that do not support development and survival of B-2 cells. For example, in the bones of *Vhl^{fl/fl}; Dmp1-Cre* mice, the number of hematopoietic cells is severely reduced, and B-2 B cell development is stunted. These mice also display splenomegaly, partly due to a movement of hematopoietic progenitors from the bone marrow to the spleen. Despite this increase in splenic hematopoiesis, the numbers of mature conventional B-2 cells is still reduced in the spleen [64•]. These data suggest that *Vhl* in osteocytic cells regulates B-2 cell development, but further studies are necessary to investigate the mechanisms underlying these observations.

Sostdc1

Sclerostin domain-containing protein-1 (Sostdc1), also known as Wise, Ectodin, Usag-1, and Sost-like, has been widely studied in the framework of tooth development, kidney disease, hair follicle formation, bone fracture, and cancers [73–76]. Sostdc1 and Sost share 55% protein sequence homology and both antagonize Wnt signaling by binding Lrp5 and Lrp6, whereas Sostdc1 additionally antagonizes BMP signaling and preferentially binds to Lrp4 [6, 7]. *Sostdc1^{-/-}* (*Sostdc1^{tm1.1(KOMP)Vlcr}*, MGI:5695910) mice have increased cortical bone area (TA) and bone mineral density (BMD), suggesting that Sostdc1, like Sost, are both negative regulators of bone formation [8•]. However, *Sostdc1^{-/-}* mice have enlarged bone marrow areas, which is contrary to *Sost*-deficient mice, which have diminished bone marrow area [8•, 12]. Consistent with these findings, we observed an increase in bone marrow and spleen cellularity in *Sostdc1^{-/-}* mice compared to wild-type control mice [9•]. *Sostdc1^{-/-}* mice display enhanced callus bone formation and remodeling after 28-day post-fracture, with an increase of mesenchymal stem cells (MSCs) and mature osteoblasts at the site of fracture. In addition, *Sostdc1* expression was highly expressed in the periosteal region of mice femora and MSCs during bone fracture, suggesting that *Sostdc1* may prompt MSCs out of quiescence and promote bone healing [8•].

New work is revealing how deletion of *Sostdc1* in the bone microenvironment may affect immune cell development. Contrary to observations in *Sost*- and *VHL*-knockout mice, B cell development in *Sostdc1^{-/-}* mice is normal. However, Millan et al. have revealed a novel role for *Sostdc1* in natural killer (NK) cell maturation and function [9•]. NK cells are lymphocytes from the innate immune system that eliminate virally infected and cancerous cells. As a function of age, *Sostdc1^{-/-}* mice have progressively diminished numbers of mature NK cells in bone marrow and spleens, indicating a partial block in NK cell maturation. NK cells recognize target cells by Ly49 receptors that are involved in cellular self- and non-self-recognition [77, 78]. NK cells from *Sostdc1^{-/-}* mice express an altered Ly49 repertoire and are hyporesponsive against MHC-I-deficient cell targets in vitro and in vivo. Consistent with Sostdc1's known role in antagonizing Wnt signaling, Wnt/ β -catenin signaling is increased in NK cells from *Sostdc1^{-/-}* mice. These data suggest that *Sostdc1* is required to modulate Wnt/ β -catenin signaling in NK cells for maturation and function. *Sostdc1* is not expressed in NK cells themselves, and studies by Millan et al. suggest that *Sostdc1* expressed by both non-hematopoietic (MSCs) and hematopoietic cells (CD4⁺ and CD8⁺ T lymphocytes) regulate NK cell maturation, Ly49 repertoire expression, and NK cell cytotoxicity.

It is now evident that specialized T lymphocytes express *Sostdc1*, but the interaction between T cells and NK cells in maturation and function is understudied. For example, T follicular helper (T_{FH}) and T follicular regulatory (T_{FR}) cells are found in the peripheral lymph node (pLN) and Peyer's Patches (PP). These cells are a small subset of CD4⁺ T cells that express high levels of *Sostdc1* [10•]. Additionally, T_{FR} cells in the PP secrete NK cell regulatory cytokines, IL-2 and IL-21, which play an important role in NK cell maturation and function. Analysis of the PP in *Sostdc1^{-/-}* mice further demonstrated a decrease in memory B cell frequency and fecal IgA secretion, but no differences were observed in T_{FH} and T_{FR} cell frequencies. Additionally, CD4⁺PD-1⁺ T cells with memory phenotype (CD44^{High}CD62L^{Low}) expresses high levels of *Sostdc1* in the spleen of aged mice [11]. In vitro anti-CD3 and anti-CD28 stimulation of CD4⁺ PD-1⁺ T cells result in an increase of *Sostdc1*, *Srr1*, and osteopontin [11]. It is yet to be determined if CD4⁺ PD-1⁺ T cells, T_{FH} and T_{FR} cells, directly or indirectly regulate NK cell maturation and function via Sostdc1.

Future Directions

Can Wnt Antagonists Be Used for HSC Expansion and to Direct Hematopoietic Differentiation In Vitro?

An important goal in hematopoietic studies is expansion of patient HSCs ex vivo for different hematological treatments such as bone marrow transplantation. It has been proposed

that exploitation of highly evolutionarily conserved pathways such as Wnt signaling could be used in this instance [79, 80]. By increasing canonical Wnt signaling through inhibition of Wnt antagonists like SOST, it might be possible to manipulate patient hematopoietic stem cells *ex vivo* towards expansion without differentiation. Wnt signaling in conjunction with organoid stem cell culture systems has made recent advances in maintaining and expanding other stem cell types in culture [81]. An organoid system is defined as a culture system with self-renewing cells that are self-organizing and differentiate into multiple cell types that recapitulate normal development. Organoid-initiating cells often express the Wnt target gene *Lgr5* and these cells can be maintained in culture for long periods of time when provided with the favorable conditions and cytokines [81]. However, *Lgr5* is expressed at very low levels in adult hematopoietic stem and progenitor cells. It is expressed, however, at slightly higher levels in HSCs derived from the embryonic aorta-gonad-mesonephros (AGM) region and fetal liver. These embryonically derived hematopoietic stem cells only are able to reconstitute myeloid and lymphoid lineages for a short period of time of 2 months [82]. Titration of soluble canonical and non-canonical Wnt ligands and Wnt antagonists might be useful to optimize the conditions for the expansion of long-lived self-renewing HSCs or directed differentiation to specific hematopoietic lineages *in vitro* [32, 83, 84], for subsequent *in vivo* transplantation.

Recent studies have also shown that secretion of Dkk1, another Wnt antagonist protein, from bone marrow-derived osteoprogenitors, promoted hematopoietic regeneration directly through inhibition of HSC quiescence as well as indirectly through EGF secretion by BM endothelial cells [85•]. Dkk1 inhibition increases *Sost* expression, which suggests a compensatory role for *Sost* in the absence of other Wnt antagonist [86]. Similarly, the activation of Wnt signaling through inhibition of *Sost* increases Dkk1 expression and limits bone formation in a negative feedback loop; therefore, perhaps combinations of Wnt antagonists could be used directly to promote hematopoietic regeneration in patients [87].

Could Wnt Antagonists Be Used to Improve Cancer Immunotherapies?

Natural killer cells provide an immediate response for the control of tumors and virally infected cells, which makes them an attractive source for immunotherapies. Current NK cell immunotherapies include engineering NK cells with chimeric antigen receptors (CAR), which had milder side effects in patients as compared to the CAR-T cell-based therapies [88]. However, CAR-NK immunotherapies are limited by small NK cell numbers, survival, and proliferation once administered to the patient [88]. Increasing evidence now suggests Wnt/ β -catenin signaling may promote cancer progression by negatively regulating immune cell cytotoxicity [89–91]. Our

studies have demonstrated that *Sostdc1*^{-/-} mice have altered bone marrow and splenic microenvironments and defective NK cell cytotoxicity against MHC-I-deficient targets. In the absence of *Sostdc1*, Wnt/ β -catenin signaling in NK cells is increased [9••]. Cytotoxic T cells (CD3⁺CD8⁺) are also negatively regulated by Wnt/ β -catenin signaling [89]. Taken together, these data support that activation of Wnt/ β -catenin signaling in NK and T cells reduces their potential cytotoxicity towards tumor cell targets. Thus, it is possible that enhancing the expression of *Sostdc1* and decreasing Wnt/ β -catenin signaling in the bone marrow and spleen of cancer patients may promote the immune microenvironment to support robust functional T and NK cell responses against cancerous cells. Furthermore, modulating the levels of *Sostdc1* may be used jointly with the already accessible CAR-NK cell-based therapy to increase the success of this emerging technology.

Can Hypoxia That Is Induced in the Bone Be Used with Wnt Antagonists to Regulate Immune Cell Differentiation?

The crosstalk between hypoxia, bone, immune cells, and metabolic regulation could present a novel therapeutic approach to immune deficiencies. Hypoxia also has implications on glucose homeostasis and little is known about the metabolic pathways used by osteoblast lineage cells. Glucose metabolism and glycolysis is important during osteoblast differentiation and for HIF-driven bone formation [92, 93]. Aerobic glycolysis is important for osteoblast differentiation and bone formation regulated by Wnt and HIF pathways [92, 94]. A recent study showed that disrupting *Vhl* in osteoblasts leads to an increase in glucose tolerance and hypoglycemia [95••], altering the whole-body glucose homeostatic process. These findings indicate that metabolic regulation in osteoblasts can have effects beyond the bone environment, and might be controlled by hypoxia via the *Vhl/Hif* pathway. In turn, changes in osteoblast cellular metabolism could impact immune cell fate, function, and glycolytic gene expression in hypoxic environment through the *Hif* pathway [96]. *Vhl* depletion in osteocytes has a detrimental effect on B lymphocyte development [64••], but the mechanism underlying requires additional investigation. Whether the whole-body glucose dysregulation in mice with *Vhl*-deficient osteoblasts has an impact on immune cells and response, and whether it can be prevented by overexpression of Wnt antagonists (like *Sost*), remains to be explored.

Conclusion

Basic biomedical and clinical research has demonstrated a clear role for the Wnt antagonists in the control of bone homeostasis, a therapeutic strategy that is already being applied

to osteoporosis. Although these treatments are promising, there are subtle defects in the immune cell development in *Sost*-deficient and *Sostdc1*-deficient mice that may worsen over time and affect immune responses to pathogens and transformed cells. The changes in the bone microenvironment induced by depletion of Wnt antagonists and changes in oxygen tension may have effects in hematopoietic stem cells that could reduce their capacity to replenish the blood system or create new hematopoietic niches outside of the bone. Future studies to investigate mechanisms controlling the crosstalk between the skeletal and immune systems should help to identify solutions for possible side effects of Wnt antagonist-depleting therapies for osteoporosis. It also provide additional avenues to influence hematopoietic differentiation in vitro and in vivo.

Acknowledgments The authors thank Dr. Damian Genetos and Dr. Gabriela Loots for their comments on the manuscript.

Author Contributions Drafting and revising manuscript: BC, CD, AM and JOM; approving final version of manuscript: JOM.

Funding Information This work was supported by University of California (UC), Merced faculty research funding, University of California Cancer Research Coordinating Committee Grant CRC-15-380532, National Institutes of Health Award 1R15HL121786-01A1, Halcyon-Dixon Trust award to JOM, and UC Graduate Student Fellowships to AM, CD, and BC.

Compliance with Ethical Standards

All reported studies/experiments with human or animal subjects performed by the authors have been previously published and complied with all applicable ethical standards and institutional approvals.

Conflict of Interest Betsabel Chicana, Cristine, Alberto Millan, and Jennifer Manilay declare no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

Papers of particular interest, published recently, have been highlighted as:

- Of importance
 - Of major importance
1. Canalis E. Wnt signalling in osteoporosis: mechanisms and novel therapeutic approaches. *Nat Rev Endocrinol*. 2013;9(10):575–83.
 2. Canalis E. Management of endocrine disease: novel anabolic treatments for osteoporosis. *Eur J Endocrinol*. 2018;178(2):R33–44.
 3. Langdahl BL, Libanati C, Crittenden DB, Bolognese MA, Brown JP, Daizadeh NS, et al. Romosozumab (sclerostin monoclonal antibody) versus teriparatide in postmenopausal women with osteoporosis transitioning from oral bisphosphonate therapy: a randomised, open-label, phase 3 trial. *Lancet*. 2017;390(10102):1585–94.
 4. Richter J, Traver D, Willert K. The role of Wnt signaling in hematopoietic stem cell development. *Crit Rev Biochem Mol Biol*. 2017;52(4):414–24.
 5. Cain CJ, Manilay JO. Hematopoietic stem cell fate decisions are regulated by Wnt antagonists: comparisons and current controversies. *Exp Hematol*. 2013;41(1):3–16.
 6. Laurikkala J, Kassai Y, Pakkasjarvi L, Thesleff I, Itoh N. Identification of a secreted BMP antagonist, ectodin, integrating BMP, FGF, and SHH signals from the tooth enamel knot. *Dev Biol*. 2003;264(1):91–105.
 7. Ahn Y, Sanderson BW, Klein OD, Krumlauf R. Inhibition of Wnt signaling by *wise* (*Sostdc1*) and negative feedback from *Shh* controls tooth number and patterning. *Development*. 2010;137(19):3221–31.
 8. Collette NM, Yee CS, Hum NR, Murugesk DK, Christiansen BA, Xie L, et al. *Sostdc1* deficiency accelerates fracture healing by promoting the expansion of periosteal mesenchymal stem cells. *Bone*. 2016;88:20–30 **This study used genetic knockout mice to outline the role of *Sostdc1* as a negative regulator of bone fracture repair and identified *Sostdc1* expression in periosteal cells and MSCs and their role in bone fracture repair. Furthermore, *Sostdc1*^{-/-} mice were found to have enlarged cortical bones with higher bone marrow density.**
 9. Millan A, Elizaldi S, Lee E, Aceves J, Murugesk D, L GG, et al. *Sostdc1* regulates natural killer cell maturation and cytotoxicity. *J. of Immunology*, 2019, in press **This study demonstrates that *Sostdc1* plays a crucial role in NK cell maturation and cytotoxicity, and reveals *Sostdc1* from both hematopoietic and non-hematopoietic sources differentially regulate on NK cell development.**
 10. Georgiev H, Ravens I, Papadogianni G, Halle S, Malissen B, Loots GG, et al. Shared and unique features distinguishing follicular T helper and regulatory cells of peripheral lymph node and Peyer's Patches. *Front Immunol*. 2018;9:714 **This study found *Sostdc1* expression in CD4⁺ T follicular helper (T_{FH}) and T follicular regulatory (T_{FR}) cells in the peripheral lymph node and Peyer's Patches, and revealed that lack of *Sostdc1* in mice increases frequencies of memory B cells increases fecal IgA secretion.**
 11. Shimatani K, Nakashima Y, Hattori M, Hamazaki Y, Minato N. PD-1+ memory phenotype CD4+ T cells expressing C/EBPalpha underlie T cell immunodepression in senescence and leukemia. *Proc Natl Acad Sci U S A*. 2009;106(37):15807–12.
 12. Cain CJ, Rueda R, McLelland B, Collette NM, Loots GG, Manilay JO. Absence of sclerostin adversely affects B-cell survival. *J Bone Miner Res*. 2012;27(7):1451–61.
 13. Chow A, Mason J, Coney L, Bajwa J, Zaslavsky A, Pellman Y, et al. Sclerostin deficiency alters peripheral B lymphocyte responses in mice. *BioRxiv* 2018. <https://doi.org/10.1101/357772>.
 14. Winkler DG, Sutherland MK, Geoghegan JC, Yu C, Hayes T, Skonier JE, et al. Osteocyte control of bone formation via sclerostin, a novel BMP antagonist. *EMBO J*. 2003;22(23):6267–76.
 15. You L, Chen L, Pan L, Peng Y, Chen J. SOST gene inhibits osteogenesis from adipose-derived mesenchymal stem cells by inducing Th17 cell differentiation. *Cell Physiol Biochem*. 2018;48(3):1030–40.
 16. Divieti Pajevic P, Krause DS. Osteocyte regulation of bone and blood. *Bone*. 2019;119:13–18.
 17. Kobayashi Y, Thirukonda GJ, Nakamura Y, Koide M, Yamashita T, Uehara S, et al. Wnt16 regulates osteoclast differentiation in

- conjunction with Wnt5a. *Biochem Biophys Res Commun.* 2015;463(4):1278–83.
18. Chae W-J, Bothwell A. Canonical and non-canonical Wnt signaling in immune cells. *Trends Immunol.* 2018. <https://doi.org/10.1016/j.it.2018.08.006>.
 19. Chitalia VC, Foy RL, Bachschmid MM, Zeng L, Panchenko MV, Zhou MI, et al. Jade-1 inhibits Wnt signalling by ubiquitylating beta-catenin and mediates Wnt pathway inhibition by pVHL. *Nat Cell Biol.* 2008;10(10):1208–16.
 20. Zhong Z, Zylstra-Diegel CR, Schumacher CA, Baker JJ, Carpenter AC, Rao S, et al. Wntless functions in mature osteoblasts to regulate bone mass. *Proc Natl Acad Sci U S A.* 2012;109(33):E2197–204.
 21. Zhong Z, Ethen NJ, Williams BO. WNT signaling in bone development and homeostasis. *Wiley Interdiscip Rev Dev Biol.* 2014;3(6):489–500.
 22. Leucht P, Lee S, Yim N. Wnt signaling and bone regeneration: can't have one without the other. *Biomaterials.* 2018.
 23. Mazumdar J, O'Brien WT, Johnson RS, LaManna JC, Chavez JC, Klein PS, et al. O2 regulates stem cells through Wnt/beta-catenin signalling. *Nat Cell Biol.* 2010;12(10):1007–13.
 24. Newton IP, Kenneth NS, Appleton PL, Nathke I, Rocha S. Adenomatous polyposis coli and hypoxia-inducible factor-1 {alpha} have an antagonistic connection. *Mol Biol Cell.* 2010;21(21):3630–8.
 25. Kaidi A, Williams AC, Paraskeva C. Interaction between beta-catenin and HIF-1 promotes cellular adaptation to hypoxia. *Nat Cell Biol.* 2007;9(2):210–7.
 26. Iqbal S, Zhang S, Driss A, Liu ZR, Kim HR, Wang Y, et al. PDGF upregulates Mcl-1 through activation of beta-catenin and HIF-1alpha-dependent signaling in human prostate cancer cells. *PLoS One.* 2012;7(1):e30764.
 27. Blank U, Karlsson G, Karlsson S. Signaling pathways governing stem-cell fate. *Blood.* 2008;111(2):492–503.
 28. Reya T, Duncan AW, Ailles L, Domen J, Scherer DC, Willert K, et al. A role for Wnt signalling in self-renewal of haematopoietic stem cells. *Nature.* 2003;423(6938):409–14.
 29. Scheller M, Huelsken J, Rosenbauer F, Taketo MM, Birchmeier W, Tenen DG, et al. Hematopoietic stem cell and multilineage defects generated by constitutive beta-catenin activation. *Nat Immunol.* 2006;7(10):1037–47.
 30. Kirstetter P, Anderson K, Porse BT, Jacobsen SE, Nerlov C. Activation of the canonical Wnt pathway leads to loss of hematopoietic stem cell repopulation and multilineage differentiation block. *Nat Immunol.* 2006;7(10):1048–56.
 31. Staal FJ, Chhatta A, Mikkers H. Caught in a Wnt storm: complexities of Wnt signaling in hematopoiesis. *Exp Hematol.* 2016;44(6):451–7.
 32. Famili F, Naber BA, Vloemans S, de Haas EF, Tiemessen MM, Staal FJ. Discrete roles of canonical and non-canonical Wnt signaling in hematopoiesis and lymphopoiesis. *Cell Death Dis.* 2015;6:e1981.
 33. McClung MR. Sclerostin antibodies in osteoporosis: latest evidence and therapeutic potential. *Ther Adv Musculoskelet Dis.* 2017;9(10):263–70.
 34. Li X, Ominsky MS, Niu QT, Sun N, Daugherty B, D'Agostin D, et al. Targeted deletion of the sclerostin gene in mice results in increased bone formation and bone strength. *J Bone Miner Res.* 2008;23(6):860–9.
 35. van Bezooijen RL, Svensson JP, Eefting D, Visser A, van der Horst G, Karperien M, et al. Wnt but not BMP signaling is involved in the inhibitory action of sclerostin on BMP-stimulated bone formation. *J Bone Miner Res.* 2007;22(1):19–28.
 36. Poole KE, van Bezooijen RL, Loveridge N, Hamersma H, Papapoulos SE, Lowik CW, et al. Sclerostin is a delayed secreted product of osteocytes that inhibits bone formation. *FASEB J.* 2005;19(13):1842–4.
 37. Gori F, Lerner U, Ohlsson C, Baron R. A new WNT on the bone: WNT16, cortical bone thickness, porosity and fractures. *Bonekey Rep.* 2015;4:669.
 38. Leupin O, Piters E, Halleux C, Hu S, Kramer I, Morvan F, et al. Bone overgrowth-associated mutations in the LRP4 gene impair sclerostin facilitator function. *J Biol Chem.* 2011;286(22):19489–500.
 39. Semenov M, Tamai K, He X. SOST is a ligand for LRP5/LRP6 and a Wnt signaling inhibitor. *J Biol Chem.* 2005;280(29):26770–5.
 40. Tu X, Delgado-Calle J, Condon KW, Maycas M, Zhang H, Carlesso N, et al. Osteocytes mediate the anabolic actions of canonical Wnt/beta-catenin signaling in bone. *Proc Natl Acad Sci U S A.* 2015;112(5):E478–86.
 41. Bouaziz W, Funck-Brentano T, Lin H, Marty C, Ea HK, Hay E, et al. Loss of sclerostin promotes osteoarthritis in mice via beta-catenin-dependent and -independent Wnt pathways. *Arthritis Res Ther.* 2015;17:24.
 42. Yee CS, Manilay JO, Chang JC, Hum NR, Muruges DK, Bajwa J, et al. Conditional deletion of Sost in MSC-derived lineages identifies specific cell type contributions to bone mass and B cell development. *J Bone Miner Res.* 2018;33(10):1748–59 **This study utilized conditional Sost knockout mice to reveal novel roles of mesenchymal stem cells in control of bone homeostasis and B cell development and revealed that osteocyte specific Sost is not the main driver of these processes.**
 43. Balemans W, Patel N, Ebeling M, Van Hul E, Wuyts W, Laczka C, et al. Identification of a 52 kb deletion downstream of the SOST gene in patients with van Buchem disease. *J Med Genet.* 2002;39(2):91–7.
 44. van Lierop AH, Hamdy NA, van Egmond ME, Bakker E, Dikkers FG, Papapoulos SE. Van Buchem disease: clinical, biochemical, and densitometric features of patients and disease carriers. *J Bone Miner Res.* 2013;28(4):848–54.
 45. Nassar K, Rachidi W, Janani S, Mkinsi O. Van Buchem's disease. *Joint Bone Spine.* 2016;83(6):737–8.
 46. Kageyama Y, Koshiji M, To KK, Tian YM, Ratcliffe PJ, Huang LE. Leu-574 of human HIF-1alpha is a molecular determinant of prolyl hydroxylation. *FASEB J.* 2004;18(9):1028–30.
 47. Kaelin WG Jr, Ratcliffe PJ. Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. *Mol Cell.* 2008;30(4):393–402.
 48. Wang V, Davis DA, Haque M, Huang LE, Yarchoan R. Differential gene up-regulation by hypoxia-inducible factor-1alpha and hypoxia-inducible factor-2alpha in HEK293T cells. *Cancer Res.* 2005;65(8):3299–306.
 49. Liao SH, Zhao XY, Han YH, Zhang J, Wang LS, Xia L, et al. Proteomics-based identification of two novel direct targets of hypoxia-inducible factor-1 and their potential roles in migration/invasion of cancer cells. *Proteomics.* 2009;9(15):3901–12.
 50. Semenza GL. Hypoxia-inducible factors: mediators of cancer progression and targets for cancer therapy. *Trends Pharmacol Sci.* 2012;33(4):207–14.
 51. Riddle RC, Khatri R, Schipani E, Clemens TL. Role of hypoxia-inducible factor-1alpha in angiogenic-osteogenic coupling. *J Mol Med (Berl).* 2009;87(6):583–90.
 52. Lu C, Saless N, Wang X, Sinha A, Decker S, Kazakia G, et al. The role of oxygen during fracture healing. *Bone.* 2013;52(1):220–9.
 53. Hirao M, Hashimoto J, Yamasaki N, Ando W, Tsuboi H, Myoui A, et al. Oxygen tension is an important mediator of the transformation of osteoblasts to osteocytes. *J Bone Miner Metab.* 2007;25(5):266–76.
 54. Knowles HJ. Hypoxic regulation of osteoclast differentiation and bone resorption activity. *Hypoxia (Auckl).* 2015;3:73–82.

55. Komatsu DE, Hadjiargyrou M. Activation of the transcription factor HIF-1 and its target genes, VEGF, HO-1, iNOS, during fracture repair. *Bone*. 2004;34(4):680–8.
56. Danis A. Mechanism of bone lengthening by the Ilizarov technique. *Bull Mem Acad R Med Belg*. 2001;156(1–2):107–12.
57. Zhao Q, Shen X, Zhang W, Zhu G, Qi J, Deng L. Mice with increased angiogenesis and osteogenesis due to conditional activation of HIF pathway in osteoblasts are protected from ovariectomy induced bone loss. *Bone*. 2012;50(3):763–70.
58. Tando T, Sato Y, Miyamoto K, Morita M, Kobayashi T, Funayama A, et al. Hif1alpha is required for osteoclast activation and bone loss in male osteoporosis. *Biochem Biophys Res Commun*. 2016;470(2):391–6.
59. Miyauchi Y, Sato Y, Kobayashi T, Yoshida S, Mori T, Kanagawa H, et al. HIF1alpha is required for osteoclast activation by estrogen deficiency in postmenopausal osteoporosis. *Proc Natl Acad Sci U S A*. 2013;110(41):16568–73.
60. Weng T, Xie Y, Huang J, Luo F, Yi L, He Q, et al. Inactivation of Vhl in osteochondral progenitor cells causes high bone mass phenotype and protects against age-related bone loss in adult mice. *J Bone Miner Res*. 2014;29(4):820–9.
61. Wang Y, Wan C, Deng L, Liu X, Cao X, Gilbert SR, et al. The hypoxia-inducible factor alpha pathway couples angiogenesis to osteogenesis during skeletal development. *J Clin Invest*. 2007;117(6):1616–26.
62. Wan C, Gilbert SR, Wang Y, Cao X, Shen X, Ramaswamy G, et al. Activation of the hypoxia-inducible factor-1alpha pathway accelerates bone regeneration. *Proc Natl Acad Sci U S A*. 2008;105(2):686–91.
63. Zuo GL, Zhang LF, Qi J, Kang H, Jia P, Chen H, et al. Activation of HIFa pathway in mature osteoblasts disrupts the integrity of the osteocyte/canalicular network. *PLoS One*. 2015;10(3):e0121266.
64. Looops GG, Robling AG, Chang JC, Muruges DK, Bajwa J, Carlisle C, et al. Vhl deficiency in osteocytes produces high bone mass and hematopoietic defects. *Bone*. 2018;116:307–14 **The findings in this article highlight the VHL and HIF influences in skeletal development and repair. It has an additional focus on the implications in the immune system in particular of B cell development.**
65. Chen D, Li Y, Zhou Z, Wu C, Xing Y, Zou X, et al. HIF-1alpha inhibits Wnt signaling pathway by activating Sost expression in osteoblasts. *PLoS One*. 2013;8(6):e65940.
66. Stegen S, Stockmans I, Moermans K, Thienpont B, Maxwell PH, Carmeliet P, et al. Osteocytic oxygen sensing controls bone mass through epigenetic regulation of sclerostin. *Nat Commun*. 2018;9(1):2557 **This article revealed the role of PHD2 role in the bone homeostasis through epigenetic regulation of sclerostin expression through the Wnt signaling pathway, and a possible strategy to treat osteoporosis.**
67. Genetos DC, Toupadakis CA, Raheja LF, Wong A, Papanicolaou SE, Fyhrie DP, et al. Hypoxia decreases sclerostin expression and increases Wnt signaling in osteoblasts. *J Cell Biochem*. 2010;110(2):457–67.
68. Wu JY, Scadden DT, Kronenberg HM. Role of the osteoblast lineage in the bone marrow hematopoietic niches. *J Bone Miner Res*. 2009;24(5):759–64.
69. Parmar K, Mauch P, Vergilio JA, Sackstein R, Down JD. Distribution of hematopoietic stem cells in the bone marrow according to regional hypoxia. *Proc Natl Acad Sci U S A*. 2007;104(13):5431–6.
70. Spencer JA, Ferraro F, Roussakis E, Klein A, Wu J, Runnels JM, et al. Direct measurement of local oxygen concentration in the bone marrow of live animals. *Nature*. 2014;508(7495):269–73.
71. Bader HL, Hsu T. Systemic VHL gene functions and the VHL disease. *FEBS Lett*. 2012;586(11):1562–9.
72. Cho SH, Raybuck AL, Stengel K, Wei M, Beck TC, Volanakis E, et al. Germinal centre hypoxia and regulation of antibody qualities by a hypoxia response system. *Nature*. 2016;537(7619):234–8.
73. Togo Y, Takahashi K, Saito K, Kiso H, Tsukamoto H, Huang B, et al. Antagonistic functions of USAG-1 and RUNX2 during tooth development. *PLoS One*. 2016;11(8):e0161067.
74. Kiso H, Takahashi K, Saito K, Togo Y, Tsukamoto H, Huang B, et al. Interactions between BMP-7 and USAG-1 (uterine sensitization-associated gene-1) regulate supernumerary organ formations. *PLoS One*. 2014;9(5):e96938.
75. Collette NM, Yee CS, Muruges D, Sebastian A, Taher L, Gale NW, et al. Sost and its paralog Sostdc1 coordinate digit number in a Gli3-dependent manner. *Dev Biol*. 2013;383(1):90–105.
76. Tanaka M, Endo S, Okuda T, Economides AN, Valenzuela DM, Murphy AJ, et al. Expression of BMP-7 and USAG-1 (a BMP antagonist) in kidney development and injury. *Kidney Int*. 2008;73(2):181–91.
77. Manilay JO, Waneck GL, Sykes M. Altered expression of Ly-49 receptors on NK cells developing in mixed allogeneic bone marrow chimeras. *Int Immunol*. 1998;10(12):1943–55.
78. Tu MM, Mahmoud AB, Makrigiannis AP. Licensed and unlicensed NK cells: differential roles in cancer and viral control. *Front Immunol*. 2016;7:166.
79. Goessling W, North TE, Loewer S, Lord AM, Lee S, Stoick-Cooper CL, et al. Genetic interaction of PGE2 and Wnt signaling regulates developmental specification of stem cells and regeneration. *Cell*. 2009;136(6):1136–47.
80. Huang J, Nguyen-McCarty M, Hexner EO, Danet-Desnoyers G, Klein PS. Maintenance of hematopoietic stem cells through regulation of Wnt and mTOR pathways. *Nat Med*. 2012;18(12):1778–85.
81. de Lau W, Barker N, Low TY, Koo BK, Li VS, Teunissen H, et al. Lgr5 homologues associate with Wnt receptors and mediate R-spondin signalling. *Nature*. 2011;476(7360):293–7.
82. Liu D, He XC, Qian P, Barker N, Trainor PA, Clevers H, et al. Leucine-rich repeat-containing G-protein-coupled receptor 5 marks short-term hematopoietic stem and progenitor cells during mouse embryonic development. *J Biol Chem*. 2014;289(34):23809–16.
83. Sugimura R, He XC, Venkatraman A, Arai F, Box A, Semerad C, et al. Noncanonical Wnt signaling maintains hematopoietic stem cells in the niche. *Cell*. 2012;150(2):351–65.
84. Florian MC, Nattamai KJ, Dorr K, Marka G, Uberle B, Vas V, et al. A canonical to non-canonical Wnt signalling switch in haematopoietic stem-cell ageing. *Nature*. 2013;503(7476):392–6.
85. Himgburg HA, Doan PL, Quarmyne M, Yan X, Sasine J, Zhao L, et al. Dickkopf-1 promotes hematopoietic regeneration via direct and niche-mediated mechanisms. *Nat Med*. 2017;23(1):91–9 **This study showed that secretion of Dkk1 by BM osteoprogenitors regulates promotes hematopoietic regeneration directly through inhibition of HSC quiescence as well as indirectly through EGF secretion by BM endothelial cells.**
86. Witcher PC, Miner SE, Horan DJ, Bullock WA, Lim KE, Kang KS, et al. Sclerostin neutralization unleashes the osteoanabolic effects of Dkk1 inhibition. *JCI Insight*. 2018;3(11).
87. Florio M, Gunasekaran K, Stolina M, Li X, Liu L, Tipton B, et al. A bispecific antibody targeting sclerostin and DKK-1 promotes bone mass accrual and fracture repair. *Nat Commun*. 2016;7:11505.
88. Li Y, Hermanson DL, Moriarity BS, Kaufman DS. Human iPSC-derived natural killer cells engineered with chimeric antigen receptors enhance anti-tumor activity. *Cell Stem Cell*. 2018;23(2):181–92 e5.
89. Wang B, Tian T, Kalland KH, Ke X, Qu Y. Targeting Wnt/beta-catenin signaling for cancer immunotherapy. *Trends Pharmacol Sci*. 2018;39(7):648–58.

90. Gattinoni L, Zhong XS, Palmer DC, Ji Y, Hinrichs CS, Yu Z, et al. Wnt signaling arrests effector T cell differentiation and generates CD8+ memory stem cells. *Nat Med*. 2009;15(7):808–13.
91. Gajewski TF, Corrales L, Williams J, Horton B, Sivan A, Spranger S. Cancer immunotherapy targets based on understanding the T cell-inflamed versus non-T cell-inflamed tumor microenvironment. *Adv Exp Med Biol*. 2017;1036:19–31.
92. Regan JN, Lim J, Shi Y, Joeng KS, Arbeit JM, Shohet RV, et al. Up-regulation of glycolytic metabolism is required for HIF1 α -driven bone formation. *Proc Natl Acad Sci U S A*. 2014;111(23):8673–8.
93. Li Z, Frey JL, Wong GW, Faugere MC, Wolfgang MJ, Kim JK, et al. Glucose transporter-4 facilitates insulin-stimulated glucose uptake in osteoblasts. *Endocrinology*. 2016;157(11):4094–103.
94. Esen E, Long F. Aerobic glycolysis in osteoblasts. *Curr Osteoporos Rep*. 2014;12(4):433–8.
95. Dirckx N, Tower RJ, Mercken EM, Vangoitsenhoven R, Moreau-Triby C, Breugelmans T, et al. Vhl deletion in osteoblasts boosts cellular glycolysis and improves global glucose metabolism. *J Clin Invest*. 2018;128(3):1087–105 **The findings in this article indicate important metabolic regulation in osteoblasts can extend beyond the bone environment. It showed that deleting VHL in osteoblast cells alters metabolic homeostasis through the VHL/HIF pathway. This highlights the skeleton's role in global nutrient homeostasis.**
96. Krzywinska E, Stockmann C. Hypoxia, metabolism and immune cell function. *Biomedicines*. 2018;6(2):56.