Genes & Diseases (2023) 10, 1291–1317

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REVIEW ARTICLE

Wnt signaling: Essential roles in osteoblast differentiation, bone metabolism and therapeutic implications for bone and skeletal disorders

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Received 6 June 2022; received in revised form 15 July 2022; accepted 16 July 2022
Available online 6 August 2022

KEYWORDS
Bone development;
Bone homeostasis;
Osteoblast differentiation;
Skeletal disorders;
Wnt signaling

Abstract Wnt signaling executes an indispensable performance in osteoblast differentiation, bone development, homeostasis, and remodeling. Wnt signals trigger the intracellular Wnt signaling cascade to initiate regulating the implication of β-catenin in the bone environment. Going through the novel discoveries done via high-throughput sequencing technologies on genetic mouse models, we highlighted the significant contribution of Wnt ligands, co-receptors, inhibitors, their related skeletal phenotypes in mouse models and the similar bone disorders clinically observed in human beings. Moreover, the crosstalk between Wnt signaling pathway and BMP, TGF-β, FGF, Hippo, Hedgehog, Notch and PDGF signaling pathways is thoroughly demonstrated to be the underlying gene regulatory network that orchestrates osteoblast differentiation and bone development. We also introspected the significance of Wnt signaling transduction in the reorganization of cellular metabolism by stimulating glycolysis, glutamine catabolism, and fatty acid oxidation in osteoblast-lineage cells that display an important regulatory arbor in the cellular bioenergetics of the bone. Throughout this evaluation, most to date therapeutical approaches towards osteoporosis and other bone maladies found in human beings, are formulated with an aspiration to holistically revamp the present clinical applications involving various monoclonal antibodies therapies that lack specificity, efficacy, and safety into more requisite advanced therapeutics that satisfy these three requirements for

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Peer review under responsibility of Chongqing Medical University.

https://doi.org/10.1016/j.gendis.2022.07.011

further clinical considerations. Conclusively, our review provides comprehensive scientific findings related to the fundamental significance of Wnt signaling cascades in skeletal system and the underlying gene regulatory network with other signaling pathways enlightening researchers with the possibility to further integrate the identified target molecules into therapeutic strategies for skeletal disorders treatment in the clinic.

Introduction

Bone is an exceptionally vigorous organ that goes through frequent resorption by osteoclasts and a brand-new development by osteoblasts during the remodeling process. The four types of cells that make up bone, the stiff tissue that constitutes the skeleton in vertebrates, are osteoblasts, bone lining cells, osteocytes, and osteoclasts.[1] It has significant roles in the body, including motility, resistance, and soft tissue protection, as well as Ca^{2+} and PO_4 storage and bone marrow nurturing.[2–4] Evidence also shows that osteocytes can sense mechanical stimuli and orchestrate the bone remodeling process by modifying their environment.[5–7] The old bone is replenished through a composite process of bone remodeling that includes three phases[1]: the resorption of bone tissue by osteoclasts,[2] the growth progression to new bone creation, and[3] the completed bone production by osteoblasts.[8,9] This process occurs as a result of the coordinated actions of osteoblasts, osteoclasts, osteocytes, and bone lining cells, within the bone cavities, which form the fundamental multicellular unit (BMU).[9,10] Besides its self-mending abilities, there are some deficiencies that bone is unable to provide regeneration for, therefore various signaling pathways actively respond to these deficiencies and one of them is the Wnt signaling pathway.[11]

The Wnt pathway is a developmentally conserved route that contains a series of signaling pathways that regulate a variety of biological activities ranging from embryonic growth to tissue regeneration.[12–15] In both human beings and mice mammals, there are 5 genes in *C. elegans* and 7 genes in *Drosophila* that encode for the large family of Wnt secreted protein growth factors and regulate the behavior, adhesion, and polarity of the cells. Wnt is an abbreviation for *wingless*, the *Drosophila* segment polarity gene, and *integrated (int-1)*, the vertebrate analog. This signaling pathway controls a plethora of functions with the help of Wnt ligands, R-spondin proteins, norrin and other receptors found on the cell surface that trigger downstream gene expression and determine the specificity of Wnt pathway.[12,16] In addition, the Wnt signaling pathway may be separated into the canonical mechanism that depends on β-catenin's activity and the non-canonical mechanism that doesn't depend on β-catenin's activity. These two major divisions are subdivided into the Wnt/planar cell polarity (PCP) and the Wnt/Ca^{2+} pathways and are required for osteocyte cell migration and patterning throughout embryonic maturation, for tissue homeostasis and regeneration and most notably during bone remodeling and homeostasis in bone.[17,18] Consequently, dysregulations of Wnt signaling have been associated with a variety of human skeletal diseases. Further studies are required to understand the underlying issues that lead to a cavity in targeted therapy of Wnt-linked diseases.[12,19]

Canonical and non-canonical Wnt signaling pathways

Wnt/β-catenin signaling: canonical Wnt pathway

Canonical Wnt signaling pathway promotes the production of β-catenin protein in the cytoplasm and is a requisite process for fetal maturity and metabolic equilibrium.[12] When the canonical Wnt ligand is missing in this specific pathway, it is a destructive complex of proteins, that includes serine–threonine kinases glycogen synthase kinase 3β (GSK-3β) and casein kinase 1α (CK1α), the protein Axin, the cytoplasmic phosphoproteins Dishevelled (DVL) and Adenomatous Polyposis Coli (APC), that calls for β-catenin's phosphorylation and eventually its destruction via the degrading actions of ubiquitin-proteasome system (UPS).[20,21] On the other side, when the canonical Wnt ligand is not missing, its cell surface receptor frizzled (FZD) and co-receptor low-density lipoprotein receptor-related protein 5/6 (LRP5/6) attach themselves to it causing DVL and Axin proteins to assemble near the plasma membrane, thus deconstructing the destructive complex of proteins. This process results into the accumulation of β-catenin in the cytoplasm and eventually its entrance into the nucleus thus triggering gene expression upon its molecular connection with T-cell factor/lymphoid enhancer factor (TCF/LEF)[16,22–25] (Fig. 1).

Wnt/PCP and Wnt/Ca^{2+} signaling: non-canonical Wnt pathways

The non-canonical Wnt/PCP pathway operates separately from β-catenin's activity. It combines cell mobility with tissue polarity via GTPase RhoA and/or c-Jun N-terminal kinase catalysis (JNK).[26] PCP pathway initiates functioning only when ligands like WNT5A, WNT5B, and/or WNT11, bind to their receptors Frizzled 3 or Frizzled 6 and their co-receptors ROR1/2 (Receptor tyrosine kinase-like orphan receptor 1 and 2), or PTK7 (Protein tyrosine kinase 7).[27] Other proteins such as VANG (11/12), Prickle (1/2), Cadherin EGF LAG (CELSR 1/2/3), DVL (1/2/3), and Ankyrin repeat domain 6 (ANKRD6) are major participants in this signaling

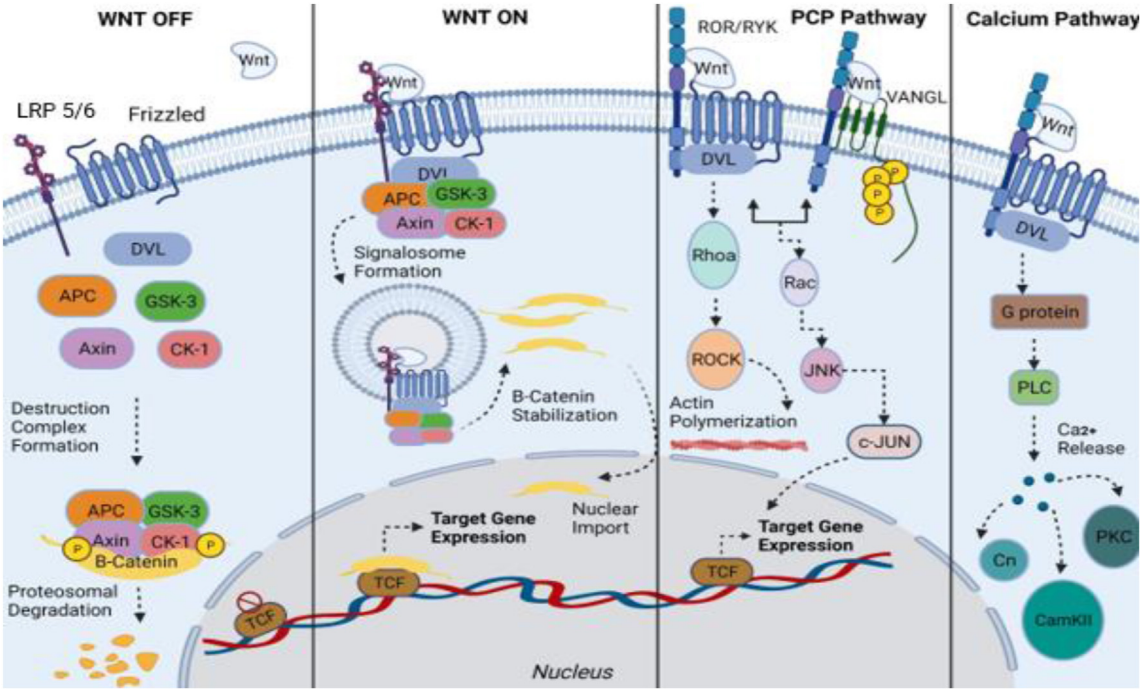

Figure 1 Canonical and non-canonical Wnt signaling pathways. *Canonical Signaling- Wnt-Off:* At the plasma membrane, both the Frizzled receptor (FZD) and LRP5/6 receptor stand inoperative, when in lack of Wnt ligand. In the cytoplasm, the destruction complex (consisted of GSK-3β, APC, CK-1 and Axin) bind and phosphorylate β-catenin, followed by its proteasomal degradation. In the nucleus, Wnt target gene expression remains off. *Canonical signaling- Wnt-On:* When Wnt ligand binds to the Frizzled receptor, an active receptor complex is formed by sequestering LRP5/6 receptor. This brings the inactivation destruction complex and signalosome formation mediated by Dishevelled (DVL). Thus β-catenin, after translocating into the nucleus, binds to T cell factor gene region and forms a transcriptional protein-activator complex in order for Wnt target genes to become expressable. *Non-canonical Wnt/PCP pathway:* Wnt ligand binds to the Frizzled receptor thus recruiting Dishevelled. They form a complex, with co-receptors such as ROR and RYK included, in order for RhoA/Rock pathway to be activated and actin to become polymerized. This particular pathway can instigate JNK pathway's operation via Frizzled-Dishevelled-ROR/RYK system or VANGL planar cell polarity protein-ROR/RYK system. *Non-canonical Wnt/Ca^{2+} pathway:* Frizzled receptor sequesters ROR&RYK receptors when Wnt ligand binds and trigger the membrane proteins Dishevelled and Guanine nucleotide-binding proteins to operate and consequently activate Plc enzyme to release Ca^{2+} from within the cell. High amounts of Ca^{2+} trigger other pathways to initiate their activity assisted by activating receptor molecules like CaN (calcium-dependent serine—threonine phosphatase), CaM-kinase II (Ca^{2+}/calmodulin-dependent protein kinase II) and PKC (protein kinase C).

catalyzation[28] (Fig. 1). Since this pathway is dependent on the Dishevelled protein's activity, it is exactly these group of proteins DAAM1 and DAAM2 (Dishevelled-associated activator of morphogenesis proteins) as well as MAP kinases (Mitogen activated protein) that trigger the Rho-associated protein kinase to initiate the RhoA-ROCK pathway or the RAC-JNK signaling pathway, sequentially. Contrastingly, in the situation of non-canonical Wnt pathway's independence from the activities of the Dishevelled protein, it is RTKs (Receptor tyrosine kinases), G proteins and PLC (Phospholipase C) that trigger the serine/threonine protein phosphatase calcineurin and cause the NFAT (Nuclear factor of activated T cells), via calcium release, to assemble inside the nucleus.[26,29,30] Other pathways, such as NLK (Nemo-like kinase) signaling can initiate functioning in the presence of high level of calcium released in the cytoplasm, via CaMKII (Calcium/calmodulin dependent kinase II)'s activity.

Mutations in Wnt components generate deficient skeletal phenotypes in mouse models and humans

Wnt ligands mutation in osteoblasts

WNT1. *WNT1* plays a crucial function during osteoblastogenesis. Given that WNT1 is found in mesenchymal stem cells (MSCs), osteoblasts, and osteocytes, it activates the canonical pathway and suppresses MSCs differentiation into adipocytes causing the production of osteoblasts to increase.[31,32] Studies have shown that WNT1 plays a principal role in the early stages of fetal growth. For instance, purebred *Wnt 1*-KO mice emerged with infant mortality whilst hybrid *Wnt1*-KO mice underwent lenient low bone density without a weakened bone development and ossification.[31] At a later stage, WNT1 activates mTORC1 pathway

promoting osteoblasts' differentiation and mineralization as studies have shown that the inhibition of mTORC1 in the *Wnt1* gain-of-function mouse model was associated with reduced mineralization and bone formation.[33] Due to WNT1's strong linkage with bone growth and formation, loss-of-function mutations associated with *WNT1* are responsible for diseases like premature osteoporosis and osteogenesis imperfecta in humans.[34–36]

WNT3A. WNT3A ligand has a major contribution to differentiating mesenchymal stem cells into osteoblastic cells via the canonical Wnt pathway as well as the non-canonical Wnt/Protein kinase C pathway.[37,38] It may also prevent the process of programmed cell death in both mature and immature osteoblastic cells[39–41] as well as mesenchymal stem cells' further progression to transform into chondrocytic cells.[42] Reported experiments have resulted in defective fetal development to non-surviving *Wnt3a*-KO mouse models.[43] Moreover, a patient diagnosed with skeletal dysplasia was found with a wild-type alteration on *WNT3A* gene.[44] Other findings have shown that the treatment of ESCs (embryonic stem cells) in mouse with recombinant *Wnt3a*, which positively affects the activity of β-catenin, downregulated the osteogenic expression.[45]

WNT4. WNT4 contributes to osteoblastic differentiation via activating the non-canonical Wnt pathway and hinders both the inflammation of bone as well as bone remodeling.[46,47] *Wnt 4*-KO mice have displayed a delay in chondrocyte maturation and an early postnatal death while *Wnt4*-cKO female mice showed decreased femoral BMD.[48,49] WNT4 can hinder RANK to attach to the TRAF6 in osteoclastic cells (TNF receptor-associated factor 6) suppressing the RANKL-induced osteoclast differentiation.[47] Experimenting with mouse models has shown that WNT4 ligand functions as a protective element against bone resorption as well as against bone destruction. Via *in vitro* reports, it was demonstrated that the main alterations of *Wnt4* gene as well as the knockdown of a protein coding gene named Zinc Finger and BTB/POZ Domain Containing 40 (ZBTB40) occurring in osteoblastic cells, resulted in a suspended osteoblastic development and ossification.[49–51]

WNT5A. WNT5A has a distinct role in stimulating the differentiation of both osteoblastic and osteoclastic cells by enhancing or hindering the canonical Wnt pathway in bone.[52–54,55] This ligand is responsible for independent-GSK-phosphorylation-β-catenin-deterioration.[58] It can also hinder the genes for β-catenin's adjustment to transcript via the non-canonical Wnt signaling pathway and T-cell factor (TCF) intervention.[54] Moreover, the *WNT5A* expression in osteoblasts is promoted by sphingosine-1-phosphate (S1p).[59] Experiments have shown *Wnt5a* knockout mouse models to display a diversity of developmental anomalies, a delay in chondrocyte hypertrophy and skeletal ossification, and perinatal death.[57,60] Meanwhile, the *Wnt5a* conditional knockout mouse models have displayed under-expressed RANK levels in osteoclast progenitors that led to breakdown of old bone, and a damaged osteoblast development.[57] In addition to that, the excessive signaling activity of WNT5A ligand and neurotrophic tyrosine kinase, receptor-related 2 (ROR2) has shown to cause bone depletion in rheumatoid arthritis.[56,57] Opposite to *Wnt3a*, the treatment of ESCs in mouse models with a recombinant *Wnt5a* upregulated various kinases' activity such as PKC, CaMKII and JNK while antagonizing the activity of β-catenin thus increasing the osteogenic proliferation and displaying a potential application in tissue engineering.[45] Loss of function alterations occurring in *WNT5A* gene have clinically shown to be a cause for Acral dysostosis with facial and genital abnormalities (Robinow syndrome).[53,61]

WNT5B. WNT5B is associated with MSC differentiation and chondrocyte proliferation during bone development.[60,62] Furthermore, *WNT5B* gene has been found to be overexpressed in female osteoarthritis (OA) patients.[63,64] *Wnt5b*-KO mice displayed a delayed bone ossification due to the overexpressed *Wnt5b* in chondrocytes.[60]

WNT7A. WNT7A is necessary in the development of cranium, face, and limbs during the formation process of the embryo.[65,66] Upregulation of WNT7A reduced RUNX2 expression thus reducing the osteogenic cell proliferation, while the downregulation of WNT7A in MSCs increased RUNX2 expression thus promoting osteogenic cell proliferation.[67] *Wnt7a*-KO mice models display impaired limb development.[68] Alterations like loss of functions for the *WNT7A* gene was shown to be a great cause for the Fuhrmann malady, the Al-Awadi-Raas-Rothschild disease (AARRS) and lastly the Santos affliction in human.[65,69,70] These diseases have the ectodermal dysplasia physical composition accompanied by acute limb deficiencies.[65,69]

WNT7B. WNT7B ligand functions as an enhancing factor for osteoblast differentiation in mice, prenatally and postnatally[41] through the non-canonical activation of mTORC1 and PKC δ signaling.[37,41] *Wnt7b* knockout mouse models were shown perinatally dead.[71] In occasions where particular parts of *Wnt7b* gene in osteogenic cells were missing, a delayed chrondocytic development and a deficient osteoblastic differentiation mediated by the osterix transcription factor were displayed.[37,41] Overexpression of *Wnt7b* gene, in osteoblasts and chondrocytes of mice, resulted in accelerated bone formation and denser bones.[33,41] In humans, increased *WNT7B* expression has been linked to osteoarthritis (OA) patients and rheumatoid arthritis (RA) patients.[72,73]

WNT9A. WNT9A arranges appropriately chondrocyte development prior to ossification and maintains the joint stability.[48,74] During osteoblastic differentiation, *Wnt9a* expression decreases.[40] *Wnt9a* knockout mouse models have displayed bone malformations like, incomplete arthrodesis, low levels of bone ossification, indications of skeletal dysplasia, metaplastic cartilage and lastly, neonatal death.[48]

WNT10A. WNT10A favors ostosis by hindering the formation of adipocytes during osteoblastogenic process, via the Wnt-canonical signaling pathway.[56,75] *Wnt 10a* knockout mouse models have displayed reduced bone ossification, bone marrow adipose tissue development and tooth development.[76–78] Due to this ligand's significant role in dentinoblast differentiation, *WNT10A* gene alterations have displayed abnormal development of ectodermally-derived organs (also known as ectodermal dysplasia) in humans, causing absence of teeth (also known as anodontia) as well as the schöpf-schulz-passarge syndrome (also known as the odontoonycho-dermal dysplasia, OODD).[79–82]

WNT10B. WNT10B ligand functions as a regulator in the process of osteoblastic cells formation via the Wnt

canonical pathway. It hinders PPARγ and C/EBPα (also known as the family of transcription factors during adipogenesis) from expressing accurately and enhances RUNX2 to express properly.[83] WNT10B maintains and renews osteoblastic mesenchymal stem cells in the bone marrow[84] due to its highly expressed level in numerous progenitor cellular compartments.[84] In *Wnt 10b* knockout mouse models, the mineralized bone volume (BV/TV), bone density level (BMD) and the number of trabeculae (Tb.N) were all shown to be substantially reduced, when contrasted with homozygous mice.[32] *WNT10B* has been implied in various human disorders.[85] In humans, homozygous missense mutations in *WNT10B* cause split limb malformation with autosomal recessive inheritance, dental anomalies including here oligodontia and osteosarcoma.[53,86–88]

WNT16. WNT16 is involved in the maintenance of postnatal bone homeostasis by regulating both canonical and non-canonical Wnt targets in osteoblasts.[89] *Wnt 16* is found to be more highly expressed in cortical bone than in trabecular bone[90] as both *Wnt16* knockout and the conditional knockout mouse models displayed reduced bone density level, low density in cortex, weakened bones and increased impermeability in cortex, while the trabeculated bone tissue's volume remained unaltered.[90,91] Alternatively, mouse models with a high expression of *Wnt16* genes, in both their osteoblastic and osteocytic cells of trabeculae and cortex compartments, displayed an increased bone density level as well as a more resistant bone.[92–94] *WNT16* gene, has long been linked to with various bone physical compositions such as bone density level (BMD), the resistance of bone, bone linear framework, the density of cortex and lastly bone rupture possibility.[95–98] Researchers assume for *WNT16* ligand to contribute to hindering the process of bone formation in two ways, by increasing the osteoprotegerin (also known as the osteoclastogenesis inhibitory factor, OCIF) or activating the osteoclastic hematopoietic progenitors via ostoblastic mediation.[90,99]

Wnt-co-receptors in osteoblasts

LRP. LRP stands for low-density-lipoprotein-receptor-related-protein and belongs to the low density lipoprotein receptor family, recognized in the cell surface,[100] with endocytic and signaling functions.[101] These LRP integral polypeptides are composed of an outer-cell compartment which binds itself to proteins that are present in the extracellular matrix, and an inner-cell compartment that functions as an interceder in signaling mechanism transductions.[102] In experimental phases, both *Lrp5*-KOs and cKOs mice in skeletal cells display a low bone density level postnatally, caused by a decreased skeletal development processing.[103–108] Point-mutations occurring in *Lrp6* gene are linked to anodontia, the disorder related to congenital absence of more than six teeth (also known as ogliodontia) and lastly high bone mass phenotype.[109–111] The *Lrp6* knockout mice, during the development of embryo, display lost non-proximal limb appendage as well as shortened transverse bones.[105] Moreover, cKO mouse models have also confirmed the involvement of *Lrp6* in postnatal bone metabolism.[106,108,112] Various researchers tend to acknowledge the *Lrp5*-mediated regulation of Wnt-signaling pathway in mature osteoblast cells found in the cortex as well as the *Lrp5*-mediated regulation of Wnt-signaling pathway in premature osteoblast cells found in trabeculae, to enhance the bone mass density's well-development.[105,106,108,113] *Lrp4* is necessary to enhance the inhibitory function of sclerostin inhibitor in the canonical Wnt pathway[114–117] amongst other inhibitors such as DKK1 and SOSTdc1.[116,118] LRP4 ligand is also known to promote DKK1 and SOSTdc1's inhibiting activity in the Wnt-signaling pathway.[116,118] In addition, mutations in *Lrp4* cause Cenani–Lenz syndrome (CLS), sclerosteosis-2 and bilateral syndactyly.[114,119–122] *Lrp4*-KO mutant mouse models absent neuromuscular functions and the inability to breathe[115] whilst, *Lrp4*-cKO mouse models do not.[115,117] *Lrp4* is also shown to apply its functions in hindering the canonical pathway and eventually affect the osteoblastic development process, during *in-vitro* and *in-vivo* animal experimental phases.

In humans, alterations related to *LRP5* gene appear linked to two types of skeletal physical compositions such as, osteogenesis perfecta (OPPG) and increased bone mineral density (HBM). OPPG is highly linked to loss of function mutations in *LRP5* gene that cause the Wnt-canonical pathway to become inoperative, while gain of function mutations are linked to the reduction of propinquity for other inhibiting elements found in the cell's surface such as SOSTdc1 and DKK1, an event that eventually generated the increased bone mineral density phenotype to display.[123–128]

ROR. ROR1/2 are type I transmembrane protein tyrosine kinases that belong to the ROR family of kinases. *Ror1/2* genes are highly expressed in most main skeletal compartments during the formation of embryo but significantly reduced in mature tissue compartments.[129] *Ror2* gene, especially, is distinctly expressed in fully-developed osteoclastic cells[130] and has been outlined to modulate bone length, via interacting with two other Wnt ligands such as WNT5A and WNT9A.[129,131] The *Ror2* knockout mouse prototypes have shown traits of nanism characterized by shortened or misshaped bones, anomalous face typology, congenital defective ventricle, and lastly blue skin disorder (also known as cyanosis).[57,132–134] ROR2-WNT5A moderate signaling controls the osteoclastic development and ossification therewith preserving proper amounts of BMD while being initiated by the constitutively active form of RhoA.[57,130,134] Inactive loss of function *ROR2* gene modification is clinically shown to be linked to the less common type of robinow syndrome (RRS). On the other hand, gain of function alterations are associated with BDB (also known as brachydactyly type B), a condition characterized hypoplasia or aplasia, also in human beings.[130,135–138]

KRM. KRM1/2 are single-spanning trans-membrane receptors that display excessive binding interaction for Wnt inhibitors like Dickkorpf 1 and 2 (DKK1 and DKK2).[139,140] However, Kremen 1 is shown to have a more extensive expression habit while Kremen 2 is mainly highly expressed in bone cells [109]. Therefore, when the inhibitor DKK1 is not present, KRM 1/2 attaches to receptor LRP6 thus activating the Wnt signaling pathway.[141,142] *Krm1* knockout mouse models and premature *Krm2* knockout mouse models appear alive with no malformations and a normalized BMD in general,[143] while mature *Krm2* knockout mouse models

show an increased BMD.[141] In humans, *KRM1* alterations have been associated with ectrodactyly and the congenital absence of more than six teeth (also known as oligodontia).[144] Putting these data altogether, KRM 1 and KRM 2 appear superfluously present in amount during skeletal development with especially, KRM 2, playing a significant role during bone remodeling process.

LGR. G-protein-coupled receptors rich in leucine (LGRs) subfamily consists of three specific types 4, 5 and 6.[145] Specifically, LGR4 is broadly expressed in various body compartments, like bone cells, adipocytic cells and myocytic cells since premature stages of embryo's formation and development until the maturity stages.[146–148] LGR4 has also demonstrated to inhibit the development of osteoclasts mediated by a type II membrane known as RANKL.[149] *Lgr4* fully knockout mouse models are shown at risk of dying in their early stages of life exhibiting an abnormal light and small body as well as shortened bones.[147–150] *Lgr4* knockout mouse models appear to have a reduced bone mineral density caused by a diminished process of ossification and bone mineralization thus obstructing the regular process of osteoblastogenesis in the early stages of embryogenesis and reducing the uncalcified bone matrix (also known as the osteoid) development.[147,150] *Lgr5* is expressed in osteoblastic progenitors during bone development and formation. *Lgr5* knockout mouse models display traits of tongue-tie disease (also known as ankyloglossia) a congenital oral anomaly that reduces the mobility of the tongue tip accompanied by acute respiratory distress syndrome (ARDS) and lastly death in their newly days of being born.[151] LGR6 enhances Wnt pathway downstream of the BMP signaling pathway.[152] Although *Lgr6* knockout mouse models have displayed a normal pattern of development, LGR6 is compulsory for multi-tissue restoration (also known as the digit tip regeneration) of bone in mouse stereotypes.[153]

Wnt inhibitors in osteoblasts

DKK. Dickkopf (DKK) is comprised of four genes that code for the Dickkopf-related proteins.[1–4] Members DKK1, DKK2 and DKK4 have a similar structure and act through the canonical Wnt pathway, while DKK3 has no effect through the canonical Wnt pathway[154] as DKK1, DKK2 and DKK4 can interact with LRP5/6 while DKK3 does not.[155–157] DKK1 binds to LRP5/6 co-receptors and inhibits the canonical Wnt pathway and is mostly found in osteoprogenitors, osteoblasts, osteocytes, and adipocytes.[158,159] DKK1 is known to hinder osteoblastic and chondrocytic differentiation, promote adipocytic development and prompt osteoclastic development thereby enhancing the osteoprotegerin-RANKL binding.[158–162] *Dkk 1* knockout mouse models manifest a deficiency in bone development, like head and limb abnormalities and ending up dying in a short time, while the hybrid *Dkk1* knockout mice and those characterized by a specialized lineage of ostoblastic cells appeared to have a high bone mineral density as well as bone composition.[159,160,163–165] Deregulated expression levels of *Dkk1* are linked to different skeletal afflictions, characterized by the death of osteocytes and bone marrow (also known as femoral head avascular necrosis/osteonecrosis (ONFH)),[166] bone deformity (also known as osteitis deformans/Paget disease) as well as rheumatoid arthritis, osteoarthritis, and multiple myeloma.[162,167,168] DKK2 attaches strongly to Kremen 1/2 and LRP5/6 to prevent Wnt to attach to the respective cell-surface molecules. However, when Kremen 2 is not present, LRP 6 gets activated without any DVL-mediation activity. Consequently, the *Dkk 2* knockout mice display a reduced level of osteoblastogenesis and a high level of osteclastogenesis mediated by the osteoprotegerin-RANKL activity, causing low bone mass (osteopenia).[140,169]

SOST. SOST is a glycoprotein rich in cysteine that acts as an inhibitor of the Wnt pathway, by blocking LRP5/6 co-receptors' activity.[170,171] *SOST* transcripts are found in high levels in osteocytes as well as in bone marrow and cartilage.[172] High BMD and bone strength were seen in *Sost*-KO mice, as well as an increase in the number of osteoblasts that recapitulate the phenotypic of sclerosteosis or van buchem disease.[173,174] In addition, the mice with specific overexpression in osteocytes have shown increased levels of RANKL.[175,176] In humans, alterations of *SOST* gene are linked to diseases such as sclerosteosis, van buchem disease and craniodiaphyseal dysplasia, an extremely rare sclerosing bone dysplasia, that is caused by missense mutations in *SOST* gene and decreases its extracellular secretion.[177] SOST inhibits osteoblast function and stimulates osteocyte function by detaching bone formation and resorption. In addition, explorations done *in vitro* and *in vivo* have shown SOST to increase and promote adipogenesis by inhibiting the Wnt pathway.[178–180] As a result, the findings show that SOST inhibits bone growth while also stimulating bone resorption.

SFRPS. SFRPS are secreted glycoproteins that share similarity with FZD receptors. They can bind to Wnt ligands and FZD receptors and eventually block both canonical and non-canonical Wnt pathways.[181] SFRP1 and 2, amongst others, attach themselves to WNT5A whilst SFRP3 only prevents WNT1 and 8.[182] In bone, during ossification process the *Sfrp1* gene is overexpressed thus deactivating the canonical Wnt pathway and boosting both the osteoblast and osteocyte cell death.[183,184] *Sfrp1*-KO mice have resulted with an increased bone trabecular area, a decrease in osteoblast apoptosis and enhancement of osteogenesis and mineralization.[183,184] On the other side, when *Sfrp2* gene was overly expressed, the mice resulted with an augmented production of mesenchymal stem cells and an abatement in apoptosis, thus inhibiting the accurate procession of osteogenesis and chondrogenesis. SFRP3 is also linked to mature osteoblastogenesis and programmed cell death (also known as apoptosis) promotion in mesenchymal stem cells.[182,185] Both osteoblasts and osteoclasts hold an over-expressed level of *SFRP4* in embryogenesis and postpartum periods, displaying the significance of this glycoprotein in the growth of bone and the loss of bone linked to stress oxidation and age.[186,187] Gene alterations in *SFRP4* are known to cause bone disorders such as metaphyseal chondrodysplasia (also known as the Pyle's disease), characterized flaring of the ends of long bones amongst other abnormalities.[188] Upraised *SFRP4* levels are linked to osteomalacia, characterized by a soft and more flexible bone.[189]

WIF-1. Wnt inhibitory factor-1 is an excreted Wnt inhibitor that acts as a negative feedback regulator of the Wnt canonical pathway. *WIF-1* gene expression is increased during osteoblast differentiation as well as in the treatment of MSCs with Bmp 2.[185,190,191] Alterations of *WIF-1* gene are

closely related to its methylation and this fact has been used to propose, Gossypol, for treatment against osteoporosis as it promotes bone proliferation through methylation and inhibition of the *WIF-1* promoter.[192] However, *WIF-1* methylation is associated with bone tumors although it is epigenetically silenced in human osteosarcoma cell lines.[190]

Ultimately, a documentation of the roles of most Wnt signaling pathway constituents during osteoblastogenesis, osteclastogenesis and osteocytogenesis are outlined in Table 1. Gene variations of Wnt elements are linked to their respective physical compositions in mouse stereotypes. Their related human disorder is also presented in detail. These encompassing interpretations provided from both past and current research highlight the importance of Wnt signaling in assisting the differentiation of MSCs precursors into osteoblastogenesis as well as in impeding the bone development process.

Table 1 Genetic alterations of Wnt signaling molecular components in mouse phenotype and their related human disease.

Gene	Method	Cre line (Tissue)	Phenotype(s)	Reference	Related human skeletal disorder
Wnt 1	KO	Germline	Homozygous resulted in perinatal death. Heterozygous resulted with mild osteopenia.	[31]	LOF mutations can cause early-onset of osteoporosis (OMIM: 166710) or osteogenesis imperfecta (OI) (OMIM: 166200)
Wnt 3	cKO	*RARβ-Cre* (Forelimb Ectoderm) or *MSX2-Cre* (Hindlimb Ectoderm)	Resulted in the loss of forelimbs (*RARβ-Cre*) or hindlimbs (*Msx2-Cre*).	[193]	N/A
Wnt3a	KO	Germline	Heterozygous resulted in low amounts of BMD, BV/TV, Tb N, and a high amount of Tb Sp.	[43,44]	May be linked to Skeletal Dysplasia (OMIM: 618870)
Wnt 4	KO	Germline	Homozygotes resulted in delayed chondrocyte maturation and early postnatal death.	[48,49]	N/A
Wnt 4	cKO	Germline	Female mice resulted in a decreased femoral BMD.	[48,49]	N/A
Wnt5a	KO	Germline	Homozygotes resulted in delay in chondrocyte hypertrophy and skeletal ossification as well as perinatal death. Heterozygotes resulted in low BMD, BV/TV, Tb N, and a high Tb Sp.	[57,61]	Robinow Syndrome (OMIM: 180700)
Wnt5a	cKO	Germline	Osteoclast and bone formation as well as bone resorption.	[57]	N/A
Wnt5b	OE	Chondro-osteo progenitors	Resulted in a delayed bone ossification due to the overexpressed Wnt5b in chondrocytes.	[60,63]	Overexpressed Osteoarthritis in female patients (OMIM: 165720).
Wnt7a	KO	Germline	Frequent impaired limb development.	[65,69,70]	Al-Awadi/Raas-Rothschild/ Schinzel phocomelia syndrome (OMIM:276820); Santos syndrome (OMIM: 613005).
Wnt7b	cKO	Dermo1-Cre (Mesoderm)	Homozygotes resulted alive with a low ossification and anomalous chondrocytes and osteoblasts.	[37]	N/A

(*continued on next page*)

Table 1 (*continued*)

Gene	Method	Cre line (Tissue)	Phenotype(s)	Reference	Related human skeletal disorder
Wnt7b	KO	Germline	Resulted in delay in chondrocyte maturation, accelerated bone formation and perinatal death.	[41,71,73]	Linked to osteoarthritis (OMIM: 165720); Rheumatoid arthritis patients (OMIM: 180300).
Wnt9a	KO	Germline	Resulted in skeletal abnormalities, reduced mineralization and chondroid metaplasia and probable prenatal death.	[48]	N/A
Wnt 10a	KO	Germline	Resulted in decrease in bone mineralization, adipose tissue, bone marrow and dental formation.	[77,80]	Linked to odontoonychodermal dysplasia OODD (OMIM: 257980)
Wnt 10b	KO	Germline	Resulted in decrease in bone volume fraction, BMD, and tb.N.	[32,86,88]	Homozygous missense mutations are linked to split limb malformation, oligodontia (OMIM: 106600) as well as osteosarcoma (OMIM: 165660).
Wnt 16	KO & cKO	Germline	Resulted in a decreased BMD level, cortex density, skeletal strength and an increased porous cortex.	[93,96]	N/A
Wls	cKO	*OC-Cre* (Osteoblasts)	Resulted in low BMD, trabeculae, calvaria and BV/TV.	[194]	N/A
Fzd2	KO-LacZ	Germline	Homozygotes resulted less viable with smaller sized skeletal elements.	[195]	N/A
Fzd9	KO	Germline	Resulted in low vertebral Tb N, osteoblastic surface, osteoid apposition, and mechanical strength in long bones.	[196]	LoF mutations may be linked to Williams-Beuren syndrome (OMIM: 194050)
Lrp4	KO	Germline	Homozygotes resulted in perinatal death.	[197]	Cenani-Lenz Syndactyly Syndrome (OMIM: 212780)
Lrp5	KO-Genetrap	Germline	Decreased BMC, BMD, as well as cortical and trabecular bone mass.	[103]	Osteoporosis-Pseudoglioma Syndrome (OMIM: 259770)
CGOF		*DMP1-Cre* (Osteocytes), *PRX1-Cre* (Limb mesenchyme)	Resulted in high bone mass with *DMP1-Cre* and high bone mass with *PRX1-Cre*.	[107]	Osteosclerosis, autosomal dominant (OMIM: 144750)
GOF		Germline	Resulted in high in cortical bone mass, BV/TV, Tb N and very thick trabeculae with a low Tb Sp.	[107,198]	Osteopetrosis, autosomal dominant 1 (OMIM: 607634)
CGOF		*DMP1-Cre* (Osteocytes), *PRX1-Cre* (Limb mesenchyme), *COL1A1-Cre* (Mature osteoblasts)	Resulted in high bone mass with *DMP1-Cre*, high bone mass with Prx1-Cre and no enhancement of bone mass with *COL1A1-Cre*.	[107,199]	High bone mass (OMIM: 601884)

Table 1 (*continued*)

Gene	Method	Cre line (Tissue)	Phenotype(s)	Reference	Related human skeletal disorder
Lrp6	KO-LacZ	Germline	Homozygotes resulted in close to perinatal death, shortening of the axial skeleton and limb defects. Heterozygotes resulted in a low BV/TV.	[113,200]	Associated with low bone mass (OMIM: 610947) in CAD.
Dkk 1	KO	Germline	Homozygotes resulted in perinatal death, severe craniofacial malformation, and ectopic digits. Heterozygotes resulted in high BV/TV, mineral apposition rate, osteoblast surface, and mechanical strength.	[159,163]	Associated with rheumatoid arthritis (OMIM: 180300).
Dkk 2	KO	Germline	Homozygotes were viable. Resulted in osteopenia with defective mineralization level and high osteoclast numbers.	[201]	N/A
Sost	KO-LacZ	Germline	Resulted in high bone mass.	[173,198,202]	Sclerosteosis 1 (OMIM: 269500)
	cKO-Hypomorphic	*COL1A1-Cre* (Mature osteoblasts)	Resulted in increased BV/TV and osteoblast surface.	[203]	Van Buchem disease (OMIM: 239100) and Craniodiaphyseal dysplasia (OMIM: 112860)
	OE	Osteoblasts and osteocytes	Resulted in osteopenia with disarranged bone construction, thin cortex, low Tb.N and osteochondrodysplasia.	[204]	N/A
Sfrp1	KO-LacZ	Germline	Resulted in reduced age-related trabecular bone loss mostly in female mice.	[183]	N/A
Sfrp2	KO	Germline	Resulted in truncated metacarpus and phalanges with a retarded ossification and a low rate of chondrocytic cells.	[205]	N/A
Sfrp3	cKO	*E2A-Cre* (Germline)	Resulted in high BMD level, and cortex density.	[206]	N/A
Sfrp4	OE	Various	Resulted in decreased rate of bone acquisition, BV/TV, and Tb size.	[207]	N/A
Dvl2	KO	Germline	Resulted in viable mice with twisted tails, anomalous vertebras, and fusion in ribs.	[208]	N/A
Axin 1	KO (Fu-TG1)	Germline	Homozygotes died at days 9.5. Heterozygotes had tail bisections and fused ribs.	[209]	Caudal duplication anomaly (OMIM: 607864)
Axin 2	KO-LacZ	Germline	Resulted in skull bone fusion from high mineralization rate and bone ossification, and low chondrocyte growth zone.	[210—212]	Oligodontia-colorectal cancer syndrome (OMIM: 608615)

(*continued on next page*)

Table 1 (*continued*)

Gene	Method	Cre line (Tissue)	Phenotype(s)	Reference	Related human skeletal disorder
Apc	cKO	*COL2A1-Cre* (Chondro-osteo progenitors)	Homozygotes resulted in PNM, truncated limbs, abnormal craniofacial construction, highly abnormal vertebras, and immature chondrocyte and osteoblast cells.	[213]	N/A
Gsk3β	Germline		Resulted in nanism with truncated limbs and vertebras, and a COL2A1 under-expression.	[214]	N/A
Gsk3β	Germline		Homozygotes resulted in neonatal mortality. Resulted in low skull ossification and craniofacial features. Heterozygotes resulted in high density of cortex and trabeculae bones.	[215–217]	N/A
β-catenin	*COL2A1-Cre* (Chondro-osteo progenitors)		Homozygotes resulted in neonatal mortality with bone deficiencies with truncated limbs, loss of transverse tarsal joint leading to a fused bone and a dome-like skull.	[218]	N/A
Tcfl (Tcf 7)	Germline		Homozygotes displayed viability. Resulted in low bone mass.	[219]	N/A

Notes: BMD, Bone mineral density; CAD, Coronary artery disease; CGOF, Conditional gain of function; COL1A1/COL2A1, Collagen, type I/type II, alpha 1; DMP1, Dentin matrix acidic phosphoprotein 1; E2a/Tcf 3, Transcription factor 3/E2A immunoglobulin enhancer-binding factors E12/*E47*; GOF, Gain of function; LOF, Loss of function; MSX2, Homeobox protein MSX-2; NA, Not applicable; OC, Osteocalcin; OE, Overexpression; OMIM, Online Mendelian Inheritance in Man; PNM, Perinatal mortality; PRX1, Peroxiredoxin 1; RARβ, Retinoic acid receptor beta; Tb.N, Trabecular number; Tb.Sp, Trabecular separation; TMD, Tissue mineral density.

Crosstalk between Wnt pathways and other signaling pathways in osteoblasts differentiation and bone metabolism

Multiple former and recent publications have investigated the integration of Wnt signaling pathway with other signaling pathways such as BMP, TGF-β, FGF, Notch, Hedgehog, and PDGF in osteoblast differentiation. This cross–functional interaction serves as a gene regulatory network that coordinates bone orientation, development, and flexibility, which are an attribute to the skeletal well-development (Fig. 2 and Table 2).

Wnt and Bmp signaling in osteoblasts

Wnt and BMP pathway interact in mesenchymal cells during chondrocyte or osteoblast differentiation. For instance, BMP2 can promote differentiation of chondrogenic cells while β-catenin has no effect or even inhibits the mechanism.[220,221] Moreover, BMP2 blocks ALP (Alkaline phosphatase) induction while, the Wnt/β-catenin pathway plays a direct role in upregulating ALP induction in mesenchymal cells. β-Catenin interacts with SOX9, that mediates the transcription of collagen, and causes ubiquitination-mediated degradation in chondrocytes.[222–224] However, BMP2 inhibits β-catenin–SOX9 interaction by activating p38 MAPK factor suggesting that BMP signaling indirectly promotes chondrogenesis by blocking Wnt/β-catenin signaling. Wnt signaling can repress BMP-induced activation by over-expressing *Dkk1* gene that automatically enhance BMP4-triggered apoptosis in vertebrate limb development thus indicating that Wnt signaling inhibits apoptosis induced by BMP signaling.[225] Other results confirm the cooperative interaction between SMAD1/4 and β-catenin in gene expression during mesenchymal cell differentiation.[226] Also, the loss of bone morphogenetic protein receptor type 1a (*Bmpr1a*) gene in both osteoblastic and osteocytic cells has shown to block the proper expression of various metabolic-promoting Wnt genes in the cortex and bring about an overly-expressed *Wnt7b* gene that displayed an effective operation in completely

Table 2 Cross-functional signaling between Wnt and other signaling pathways in osteoblasts.

Molecules of respective pathway	Crosstalk signaling target molecules	Molecular mechanism's results	Reference
BMP and WNT	SMAD complex with TCF/LEF/β-catenin	Increased osteoblast differentiation	256,257
TGFβ1	WNTs increased, Axin 1/2 decreased	Increased chondrocyte differentiation, Decreased adipocyte differentiation	258,259
TGF-β1 stimulation	Increased BMP2	Stimulates ectopic bone formation	260
Decreased level of SMAD4	β-catenin activity increased	Increased bone formation	261,262
BMPR1A stimulation	SOST increased, increased DKK1 stimulates β-catenin to decrease	Decreased bone mass	263
BMP2	Decreased β-TRCP stimulates β-catenin to increase.	Increased osteoblast differentiation, Increased chondrocyte hypertrophy.	264,265
FGF-FGFR3	TGF-β	Mediates embryonic bone formation	266
FGFR stimulation	Increased BMP2 and TGF-β1	Increased osteogenic expression	267
FGR2 stimulation	Increased Bmp-induced osteogenesis	Increased osteoblast differentiation	268,269
BMP2 stimulation	Increased Fgf-induced osteogenesis	Increased osteoblast differentiation	270
Notch	Increased Bmp-induced ALP	Stimulated SMAD and Notch	271
Ihh stimulation	Increased Bmp-induced osteogenesis	Bone formation	272
Ihh and Bmp stimulation	Increased ALP; Increased Ihh	Long bone development	272
YAP	β-catenin	Increased β-catenin transcriptional activity and downstream genes	273,274
TAZ	β-catenin, DVL	Knockdown of TAZ significantly increased DVL phosphorylation and β-catenin nuclear levels	275
MST1/2	β-catenin	Deletion of MST1/2 led to activation of β-catenin	276
GLI stimulation	Increased BMP2	Normal osteoblast differentiation	277
PDGF	Increased MSCs	Highly mitogenic factor and chemotactic for MSCs, osteoblasts and perivascular cells	278

Notes: BMP, bone morphogenetic protein; FGF, fibroblast growth factor; GLI, glioma-associated oncogene; Ihh, Indian hedgehog; MSCs, meristematic stem cells; MST1/2, macrophage-stimulating protein; TGF-β, transforming growth factor-β; PDGF, platelet-derived growth factor; TAZ, transcription adaptor putative zinc finger; YAP, Yes-associated protein.

rescuing the deficiency in periosteum's growth.[227,228] These data illustrate the functional role of Wnt signaling in BMP2-regulated mesenchymal cell differentiation.

Wnt and TGF-β signaling in osteoblasts

β-Catenin signaling pathway is a process through which TGF-β regulates osteoblastogenesis in hMSCs (human mesenchymal stem cells). Through RNAi approaches, was shown that TGF-β1 uplifted the amounts of β-catenin and augmented β-catenin- T-cell factor/lymphoid enhancer factor transcription rates in human mesenchymal stem cells through both SMADand non-SMAD pathways. Moreover, it was shown that after knockdown of β-catenin with siRNA, transforming growth factor beta 1 (TGF-β1), activin receptor-like kinase 5 (ALK-5), protein kinase A (PKA) and Jun N-terminal Kinase (JNK), hinder osteoblastogenesis in hMSCs *in vitro*. In recent findings it was demonstrated that when mesenchymal progenitor cells are treated with TGF-β, a β-catenin-responsive firefly luciferase reporter plasmid (also known as TOPflash), is activated and eventually β-catenin is translocated into the nucleus without the activation of Wnt signaling pathway.[229] Also, cooperation between TGF-β and WNT3A led to high amounts of nucleic β-catenin. Transcriptionally, SMADs and β-catenin can form a complex that is imported into the nucleus to trigger osteoblast-dependent gene expressions however, further investigation is needed to better-understand this interaction.

Wnt, Fgf and Bmp signaling in osteoblasts

During limb development, a regulatory system displayed by FGF, Wnt and BMP signaling interaction is observed to manage the cell proliferation and development.[230] This regulatory system induces cell death in the anterior margin of the limb by either inhibiting Wnt or FGF signaling or by activating Bmp signaling through failing in regulating *Dkk*, *Fgf 8* or *Bmp 4* expression.[231] Through experiments, the repression of FGF signaling is considered crucial in inducing DKK gene expression, by hindering its expression. Similarly, DKK together with BMP4 hinder *Fgf8* gene expression, playing a dominative role in apoptosis.[231] Glycogen synthase kinase 3 (GSK-3β) also functions as an intermediator in assisting the Wnt-FGF signaling amalgamation.[232] Moreover, osteoblast differentiation during bone development can be stimulated by *Fgf 2* via Wnt pathway where *Fgf2* deletion reduced the expression of *WNT10B*, *LRP5* and *β-catenin* genes.[233] During exogenous *Fgf2* administration in marrow stromal cells of *Fgf2*-KO mice, β-catenin was accumulated in nucleus thus increasing the effect of mineralization.[233] In the absence of endogenous *Fgf2*, *SOST* mRNA and SOST protein expression

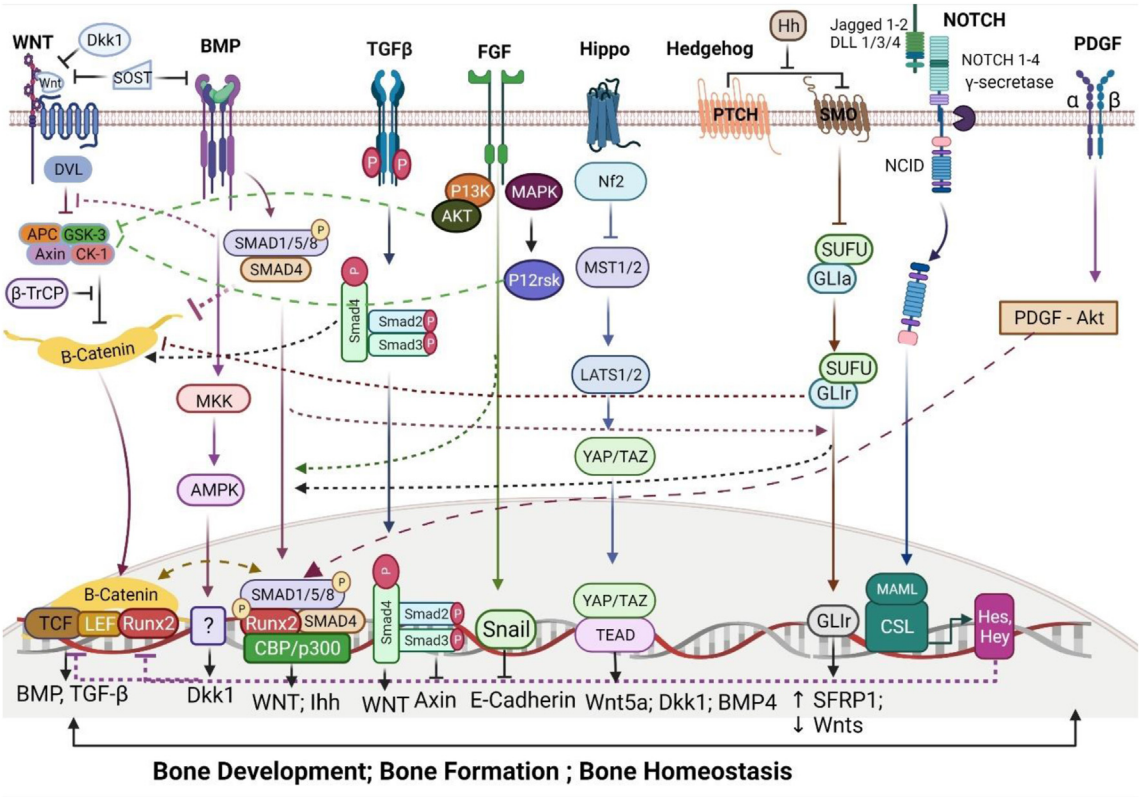

Figure 2 Cross-functional signaling between Wnt and other signaling pathways in osteoblasts. *Wnt and Bmp*: Wnt/β-catenin signaling is stimulated by Bmp signaling activity by creating β-catenin/TCF/LEF/RUNX2 complex thus increasing the Wnt expression, inhibiting DVL and decreasing BTRC expression, oppositely Wnt signaling is inhibited when Bmp activates Wnt antagonist Dkk 1 and Sost expression thus preventing β-catenin translocation in the nucleus. *Wnt and TGF-β*: TGF-β stimulates Wnt ligands gene expression to increase and Axin protein gene expression to decrease via Wnt signaling activation. *Wnt, BMP and FGF:* FGF expression is increased by Bmp signaling and vice versa, thus indirectly increasing and decreasing DKK levels, respectively. The Fgf-mediated activation of P13K-AKT pathway or MAP kinase pathway causes the GSK-3β to phosphorylate and further translocate β-catenin into the nuclei while the inhibition of SNAIL phosphorylation hinders the E-cadherin-mediated translocation of β-catenin into the nuclei. *Wnt and Hippo:* In the nucleus, YAP forms transcriptional complexes with TEADs (Transcriptional enhanced associate domains), inducing downstream gene expression. *Wnt and Hh:* Smoothened (SMO) catalyzes a series of reactions in order to release glioma-associated oncogene (GLI) from the deactivated compound and translocate it to the nucleus to operate target gene expression. During the post-translational modification (PTM) phase, GLI and Suppressor of fused homolog (SUFU) negatively regulate the Hh signaling and hinder β-catenin to translocate to the nuclei, while Smoothened positively promotes β-catenin production. *Wnt and Notch:* Notch proteins initiate the expression of *Hes* and *Hey* genes thus hindering the well-functioning of Wnt signaling and restricting β-catenin-mediated transcription. *Wnt and PDGF:* PDGF signaling pathway can stimulate the SMAD1/5/8/RUNX2 complex to induce gene production and guide the differentiation of bone.

were increased thus reducing osteoblast differentiation and bone development in *Fgf2*-KO mice.[234]

Wnt and Hippo signaling in osteoblasts

Wnt and Hippo signaling cooperate to regulate each other's activity for precise regulation of biological phenomenon. YAP/TAZ (Yes-associated protein 1/Transcription adaptor putative zinc finger) interact with β-catenin and DVL and regulate their levels and activities.[235] Apparently, there is a combinational interaction of YAP/TAZ co-activators in promoting skeletal formation. The absence of genes coding for the transcriptional YAP/TAZ proteins has shown to decrease bone size therefore causing a condition similar to osteogenesis imperfecta in *Osx-Cre* mouse line.[236] *Yap* and/or *Taz* gene deletion in *Osx-Cre* mouse line has also shown to produce a damaged thickness in periosteum by negatively affecting the process of differentiation in osteo-chondro-progenitor cells.[237] Other transgenic mouse lines with a *Yap/Taz* deletion, such as *Prx-Cre* and *Dmp-Cre*, have resulted in fetal death and a disbalance in the osteoblast/osteoclast cells ratio with a low number of osteoblastic cells and a high number of osteoclastic cells, respectively.[238] This phenomenon occurs due to the suppressive effect of YAP/TAZ co-activators in osteo-chondro-progenitor cells that cause the osteoblasts number to decrease and a promotive effect in adult osteoblastic cells that cause the osteoblasts number to increase and positively enhance the skeletal development and fracture repair.[238,239] Altogether, the given data identify YAP/TAZ as key transcriptional regulators in osteoblast, osteoclast, and osteocyte mediated bone remodeling.[236,240]

Wnt and hedgehog signaling in osteoblasts

Wnt and Hh signaling integration is pivotal for the development and differentiation of embryogenesis. During the limb regeneration process, Hh pathway inhibits the activation of Wnt signaling[241] and hinders the expression of β-catenin in order to cause cartilaginous disorders.[242] The Ihh signaling activation during bone repair is another important evidence that demonstrates the synergetic interaction of these two pathways.[243] While Wnt signaling pathway's activity is enhanced in mature osteoblast cells, the Ihh signaling pathway's activity is enhanced in early phases of osteoblast cells differentiation in order to promote bone repair and formation.[243] The Ihh signaling is also highly operative in skeletal cancerous cells and the deletion of *PTCH1* gene (protein patched homolog 1) causing the benign bone tumor of enchondroma and the malignant neoplasm known as osteosarcoma, demonstrates that. This phenomenon occurs while *PTCH1* deletion enhances the WNT5A and WNT6 ligands expression causing the β-catenin's activity to increase and originate bone abnormalities.[244]

Wnt and Notch signaling in osteoblasts

Wnt and Notch signaling pathways are two highly-cooperative pathways that well-manage cell fate proliferation especially the bone cell formation process.[245] In human cells, both inactive and active form of Notch signaling pathway when limited to the membrane fraction and nucleus can inhibit Wnt/β-catenin signaling by seizing β-catenin through endocytosis to prevent its interactivity with T cell factor/lymphoid enhancer factor family (TCF/LEF). The nuclear-limited Notch binds to β-catenin forming a composite that interrupts Wnt/β-catenin signaling and restricts β-catenin-induced transcription through the expression of downstream target genes HES (Hairy and enhancer of split-1) and HEY (Hairy/enhancer-of-split related with YRPW motif protein) repressors.[246] When the activation of HES and HEY repressors is blocked, there is no displayed modification in the function of Notch intracellular domain (NICD), in order to inhibit Wnt signaling.[246] In bone cells, the link between these two pathways is represented by JAG1, one of the five surface-interactive ligands of Notch pathway,[247] and osteocytes with JAG1 deletion containing a higher amount of other Notch ligands and receptors, demonstrates this cooperation. Also, Notch stimulates β-catenin's activity by hindering the GSK-3β activity in order to stabilize the β-catenin-activator SNAIL (Zinc finger protein SNAI1).[248,249] Commonly, Wnt and Notch have opposite functions and whenever one of them is obstructed, the other pathway reacts to the Wnt—Notch interaction.[250] Among other factors, HIF-1α (hypoxia-inducible factor-1α) is one that upregulates the NICD domain levels and promotes Notch pathway but, on the other side, it obstructs the activity of Wnt pathway therefore modifying the growth of osteoblast cells.[251]

Wnt and PDGF signaling in osteoblasts

Platelet-derived growth factor receptor alpha (PDGF-Rα) is necessary for a normal development of skull and face bones.[252] In general, PDGF-Rβ can potentially regulate the role of MSCs however and reduce mitogenesis and enhances osteogenesis.[253] PDGF-R is involved in osteoblatogenesis process mediated by WNT3A in MC3T3-E1 cells (subclone 4 pre-osteoblasts) with PDGF-R reducing osteoblast proliferation induced by the WNT3A ligand.[254] PDGF-R is transactivated by WNT3A ligand via SRC (non-receptor tyrosine kinase) anchoring to DVL2 and stimulating the activity of its tyrosine kinases.[255] Moreover, WNT3A binds to LRP5/6-FZD receptors and activates DVL, which stimulates β-catenin-independent pathways via the transactivation of PDGF-R in osteoprogenitors and osteoblastic cells and therefore result in bone formation increment.[254]

Osteoblastic utilization of glucose, glutamine and fatty acids via Wnt-stimulation

Wnt stimulates β-oxidation in osteoblasts

Wnt-mediated mechanisms of metabolism in osteoblastic cells were observed to maintain LRP5 and LRP6 effectors' intersecting activities.[279] *LRP5* variants developed modifications in body configuration not seen in the *LRP6* variants such as white adipose tissue depots increased in size and whole-body energy expenditure reduced. The genetic fingerprinting of osteoblastic cells containing defective *LRP5* genes displayed physical traits of varied triglyceride catabolic reaction caused by an under-expressed gene activity taking place in mitochondria in deviant osteoblasts during the saturated fats oxidation.[279] Frey et al[280] explored if the operation of this mitochondrial type of oxidation executed by *LRP5* gene was associated with β-catenin's stimulation. Treatments of primary osteoblasts *in vitro* demonstrated that only WNT3A, WNT10B and WNT16 ligands were shown to be linked to β-catenin activation, suggesting the canonical mechanism to be the cause of alteration induction. On the other side, mature osteoblastic cells that were missing the activation of β-catenin, encountered a hindering of osteoblastic cells' further development as well as a significant depletion in oxidative reactions and adenosine triphosphate's accumulation, in spite of glycolysis activity being enhanced. Anyhow, the manipulated β-catenin *in vivo*, resulted in loss of function for LRP5 as well as an increase in adipose tissue mass and serum fatty acids (Fig. 3).[280]

Kim et al[281] examined the skeletal and metabolic phenotypes of a mouse model to investigate the requirements and contributions of lipid homeostasis in bone development and vice versa and eventually, the study demonstrated the disruption of the expression of carnitine palmitoyl transferase 2 (CPT2), a highly required enzyme for mitochondrial fatty-acid β-oxidation in osteoblasts and osteocytes being caused by CPT2 deficiency. However, the seriousness of the matter was determined by the sex of the mutant. *Cpt2*-male-mouse-variants demonstrated an impermanent low mechanical competence in trabeculae or Tbvf (also known as the trabecular bone volume fraction). On the other side, *Cpt2*-female-mouse-variants showed a low mechanical competence in trabeculae in thighbones (distal femur) and the fifth lumbar spine vertebrae (L5) all the time. It is important to point out that *Cpt2*-female-mouse-variants cumulated osteoid with an enhanced adjustment period

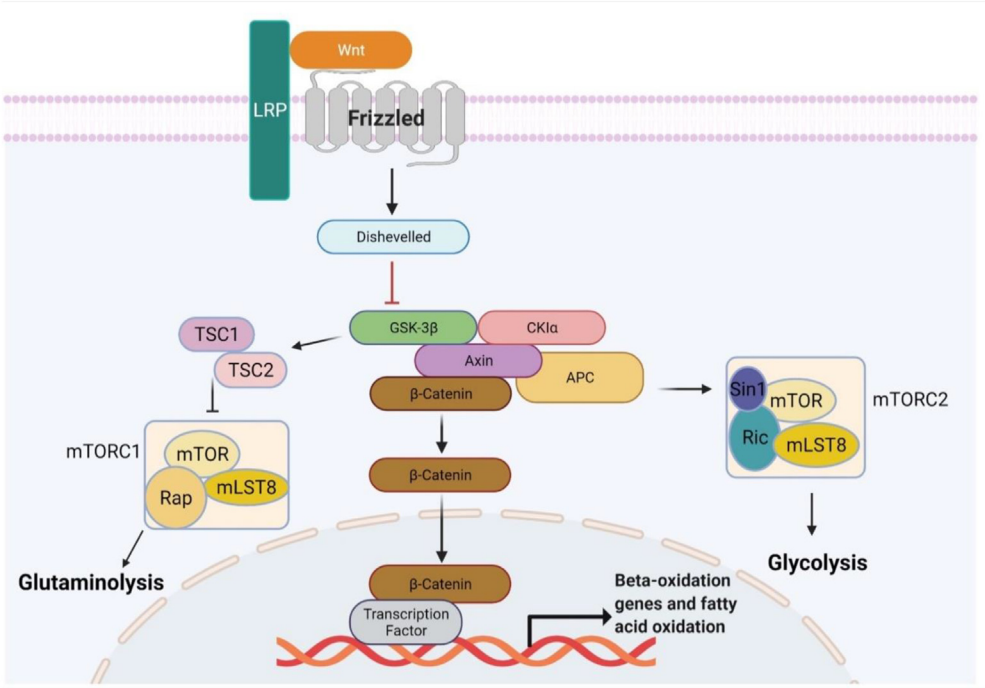

Figure 3 Glucose, glutamine and fatty acids utilization in osteoblasts via Wnt signaling regulation. Wnt ligand binds to FZD and LRP5/6 receptors which leads to degradation compound's deactivation, which is composed of Dishevelled, Axin, GSK-3β, APC's and CKIα (casein kinase 1). Through this mechanism, in mature osteoblastic cells, β-catenin's amount increases in the cytoplasm then translocates to the nuclei and assisted by the TF (also known as sequence-specific DNA-binding factor), the mitochondrial fatty-acid β-oxidation gene expression initiates. In osteogenic cells, mTORC1 signaling pathway is stimulated by Wnt pathway and causes the amount of glutamine amidohydrolase, the initiative enzyme in the glutaminolytic pathway, to increase. Wnt pathway also stimulates mTORC2 signaling pathway in order to adjust the proper number of proteins engaged in the glycolytic pathway. **Notes:** MLST8, mTOR associated protein, LST8 homolog; RAP, RAS/RAP GTP-binding protein; RIC, resistance to inhibitors of cholinesterase; SIN1, stress activated protein kinase interacting protein 1; TSC1/2, tuberous sclerosis proteins 1 and 2.

(lag period) during the mineralizing process. The sex dimorphic phenotype is linked to whether the estrogen hormone adjusts fuel selection according to both *in vivo* and *in vitro* studies. In male mutants' CPT2 deficient osteoblasts, cultured *in vitro*, the glucose uptake was increased, which was not shown in female mutants' CPT2 deficient osteoblast cultures treated with exogenic estrogen.[281] Similarly important is the fact that the abnormal amount of lipids (dyslipidemia) has shown significant alterations in bone metabolism equilibrium and caused trabeculae to decline in hyperlipidemic and high cholesterol diet mice.[282] Altogether, these studies illustrate the importance of fatty acid catabolism in normal osteoblast function and bone mass development which is highly influenced by both sex and food diet.

Wnt stimulates glycolysis in osteoblasts

Glucose is the vitality origin for most mammalian cell types and here it is required to normalize osteoblast function. Wnt signaling pathway is intrinsically linked to carbohydrate metabolism, therefore, Esen and colleagues[283] illustrated the increase of carbohydrate attainment via the stimulation of various gene expressions, such as glucose transporter 1 (*GLUT1*), *HK2* (known as hexokinase II), PDC kinase (known as pyruvate dehydrogenase complex kinase) and lactic acid dehydrogenase (LDH) via WNT3A and WNT10B ligands. This was shown to be more efficacious than the usage of insulin-cellosaurus cell line (ST2) as a stimulant. Interestingly enough, it was shown that the Wnt3a stimulated lactate production suggesting that Wnt signaling activated aerobic glycolysis instead of oxidative phosphorylation.[284]

Esen et al[283] also demonstrated via gene knockdown studies, that in both *lrp5*$^{-/-}$ mice and those in which *Lrp5* expression was eradicated by *Osterix-Cre*, displayed a reduction in glycolytic enzyme expression in bone which did not continue via alterations in the activity of either GSK3β or β-catenin activity because these effectors were altered as inhibitors and did not impact glucose utilization. Alternatively, the mammalian target of rapamycin complex 2 (mTORC2) was promoted by the activity of WNT3A ligand assisted by the RAC Family Small GTPase 1 (RAC1) and consequently enhancing the carbohydrate utilization via gene overexpression (Fig. 3).

Through RNA-Seq technique, it was demonstrated that the increased number of genes caused by Wnt3a-stimulated cellosaurus cells (ST2) was exceeded by the number of genes with a decreasing effect in gene expression.[285] Gene downregulation is linked to chondrocytic and adipocytic differentiation identified via CCAAT and CEBPA detectors. To conclude, Wnt signaling controls the genomic landscape and

fate-specification of osteoprogenitors by hindering carbohydrate to enter the Krebs or citric acid cycle whereby both citric acid and acetyl coenzyme A catalyzation are decreased.

Wnt stimulates glutaminolysis in osteoblasts

Glutamine is the most abundant free amino acid in circulation and is not only an important oxidative fuel, but also a precursor for the synthesis of non-essential amino acids, nucleotides, and the antioxidant. Experimental research has put forward the necessity for GLN (glutamine) addition in order to assist the process of osteoblast-mediated ossification.[286]

The stimulation of the ST2 cell is caused by Wnt factors during osteoblast differentiation and bone associated with glutamine metabolism.[287] This stimulation increased glutamine content. Glutamine catabolism, resulted from toxicological hindrance of glutaminase catalyzation, was considered necessary for stimulating osteoblastogenesis in an artificial environment and enhancing BMD via the Hemoglobin subunit mu (*Hbm*) gene well-expression found in the LRP5 cell surface receptor in the living body of an *Hbm*-variant. Simultaneously, the "fight or flight" reaction and the over-expression of particular genes encoding for protein utilization and their three-dimensional structure acquisition were initiated by glutaminolysis. Furthermore, research on this mechanism, stipulate that the mTORC1 (mechanistic target of rapamycin complex 1) pathway stimulates glutamine usage, therefore, glutamine attainment and consumption in response to Wnt signaling constitutes a molecular resistance mechanism that sustains metabolism, ER (endoplasmic reticulum) status[287] and oxidoreduction equilibrium.[288]

The association of Wnt signals with various human skeletal disorders and their forms of treatment

Taking into consideration to date therapeutical advancements related to Wnt signaling pathway, we elaborate the association of numerous muscular-skeletal maladies with Wnt ligands, recent and innovative prospective forms of treatment, pre-symptomatic observations acquired by both human and animal samples, potential novel therapies, and lastly the aspects at issue that should be taken into consideration when performing clinical interventions (Table 3).

Therapeutic targeting of Wnt signaling components in clinical trials

Wnt molecules are implicated to well-maintain bone mass, repair bone fractures, and provide remission for tumor treatments during separate phases.[30,338] *ETC1922159* and *WNT-974* (selective molecule inhibitors of O-acyltransferase porcupine) reach the first phase of clinical trial and palmitoleate Wnt ligands to moderate the β-catenin productivity and maintain the uncontrolled cell-growth,[339–341] Ipafricept (IPA) (transgenic integrating protein containing an extracellular human frizzled 8 receptor) engages with a Cr-domain (Cysteine-rich) of Wnt ligand and eventually hinders the activation of the Wnt signaling,[342–344] Tabituximab barzuxetan (monoclonal immunoglobulin G1 targeting frizzled 10 receptor), reaching the first phase of clinical trial, experiments have shown a provision of potent remission for synovial sarcoma in mice stereotype,[345,346] Vantictumab (monoclonal immunoglobulin G2, anti-frizzled receptor) having completed the first phase of clinical trial, hinders the Wnt signaling by blocking the binding of *WNT3A* ligand to frizzled receptors (*FZD1, FZD2, FZD5, FZD7, FZD8*),[347–349] Rosmantuzumab (monoclonal immunoglobulin G1 anti-human R-spondin 3) being on its clinical trial, targets Rspo3 to hinder its binding to the leucine-rich repeat-containing G-coupled receptors (*LGR*s) and prevent the activation of *RSPO-LGR* pathway thus inhibiting the proliferation of tumor cells in acute myeloid leukemia.[30,350] Foscenvivint/PRI724 (inhibiting receptor that targets β-catenin and hinders the transcriptive activity of cAMP response element-binding protein) effectively inhibits the Wnt signaling pathway by decreasing the level of β-catenin in pluripotent human embryonal carcinoma cisplatin-resistant germ cell tumor line,[351–353] and Zilovertamab/Cirmtuzumab (monoclonal immunoglobulin G1, anti-receptor tyrosine kinase like orphan receptor Ror-1) blocks the activity of *WNT5A* ligand and hinders cell-proliferation in chronic lymphocytic leukemia[354,355] amongst others, are overly suggested to be utilized in the clinic.

In skeletal disorders treatment, targeting the Wnt signaling pathway is validated to be clinically sufficient. Gene alterations encoding for porcupine inhibitors such as *WNT974* and *WNT-C59* (inhibitor for Wnt3a-mediated activation of T-cell factor binding site) are documented for their detrimental outcomes in decreasing bone ossification and increasing bone remodeling activity thereby causing various skeletal disorders[356] but with an efficacy of alendronate used in patients reported.

It is reported, through clinical observations, on the development of sclerostin moAbs (monoclonal antibodies) targeting and counterpoising sclerostin as an approving pharmacological method due to its abilities to enhance BMD, reduce bone-rupture risks and promote metabolic activity in mouse models.[293,357] It is intriguing that anti-sclerostin compounds increase bone formation while decreasing bone resorption in contrast to teriparatide, the only osteoanabolic agent currently approved in the USA for treating osteoporosis. Antisclerostin therapy has led to improved clinical outcomes with a favorable balance of benefits and risks, therefore this approach to osteoporosis treatment is now a welcomed addition to current options.

Utilizing other agents in targeting the Wnt signaling cascade has also shown effectiveness.

A flavonol found aplenty in herbs, named Quercetin (nonspecific protein kinase enzyme inhibitor), was shown in a recent study, functioning as an antioxidant agent enhancing the production of osteocytes and hindering the death of osteoblast cells amongst other functions. However, there are a few underlying negative results in bone coming from its utilization which require further investigation.[358] Recent report has demonstrated, Polydatin, also known as Piecid, a stilbenoid glucoside derived found in grape juice, to enhance the production of human bone marrow mesenchymal stem cells in osteogenic cells via the crosstalk between Wnt pathway and BMP2 pathway.[330] Another new report has

Table 3 The association of Wnt signals with various human skeletal disorders and their forms of treatment.

Human skeletal disease	Wnt-related molecules involved	Results of mutations	Existing therapies	Potential novel therapies	Clinical issues	Reference
Osteoporosis (OR)	*LRP5*, *WNT3A*a upregulation, *SOST* overexpression.	High bone resorption and low bone formation caused by agedness and reduced amount of estrogen.	Drugs like Raloxifene (also known as SERM), diphosphonates, and denosumab (monoclonal antibody therapy) hinder bone resorption.	Anti-Sost monoclonal antibody (Romosuzab, BPS804, *etc*) stimulating bone formation and suppressing bone resorption by enhancing osteoblast differentiation and OCIF/OPG production, treating HPP and OI.	Romosozumab treatment is linked to cardiovascular incidents.	289—302
			Teriparatide (parathyroid hormone PTH) an effective anabolic agent promotes bone remodeling.	Caveolin-3 serving as a biological indicator by enhancing ossification and inhibiting OR.	A mixture of antibodies that attack both *DKK1* and *SOST* has shown great possibilities to be effective in the enhancement of bone-rupture healing.	
Osteoarthritis (OA)	β-catenin activation, *WNT7B* overexpression and *SOST* expression.	Joint-related (Articular) cartilaginous tissue impairment, Development of bone spur (osteophyte), and solidification of the under-cartilaginous-bone (subchondral).	Treatment with P.O NSAIDs and HA joint shot (injection).	Sirolimus (Rapamycin) decreasing MMP13 gene expression and reducing cartilaginous demolition. Small Wnt pathway inhibitor molecules (like SM04690) protecting cartilaginous tissues from demolition. Mianserin, an anti-R-spondin 2 agent, inhibiting the abnormal functioning of Wnt pathway in OA.	The systematic hindering of Wnt signaling pathway has shown possibilities of affecting the bone remodeling process non-positively.	73,303—309
Rheumatoid Arthritis (RA)	*TNF*-α induction of *DKK1* expression and Sost expression; Increased *WNT5A* expression; Decreased *SFRP5* expression.	Bone resorption stimulation; Erosive Arthritis (Bone erosion); Pro-inflammatory cytokine expression enhancement; Cartilaginous demolition via MMPs production stimulation by Wnt5a ligand.	MTX treatment (a traditionally synthesized DMARD) and other biological DMARDs functioning as anti-inflammatory cytokines factors.	Possible treatment of OCD (easing the pain caused by cartilaginous demolition and inflammation) by hindering Wnt5A gene expression via the *WNT5A*/*ROR2* signaling pathway.	The monoclonal antibody treatment with Denosumab have no apparent premises on giving positive results in cartilaginous demolition.	310—323

Osteosarcoma/OS (Primary Bone Tumors)	*WIF-1; DKK3; SFRP2.*	Metastatic cell proliferation facilitation by both Wnt ligands and inhibitors.	Standardized treatment using MMT, combination CTx, RTx, and surgery.	GSK-3β treatment has shown great premises in suppressing the further-production of osteogenic sarcoma cells by activating the Wnt signaling pathway.	The inhibitory function of *GSK-3B* is done through a distinct Wnt-independent mechanism that calls for additional research in order for actual application to take place. Virus-like-particle-based anti-HER2/neu vaccine as well as CAR-T cells also show great premises for the future.	[324—331]
Multiple Myeloma (MM)	*SOST* overexpression.	Osteolytic lesions from the high concentration of *SOST*.	Treatment of osteolytic lesions with Zoledronic acid (also known as Zometa) and Denosumab (monoclonal antibody treatment).	Antibodies targeting Sost and suppressers targeting a protein complex (also known as proteasome inhibitor) integrated has displayed an acceptable method for treating osteolytic lesions and decrease the proliferation of metastatic cells.	There are concerns whether the usage of antibodies targeting *SOST* in MM patients aggravate the MM disease by increasing the proliferation of metastatic cells.	[332—335]
Bone Metastasis	*DKK1* overexpression	Osseous metastatic disease (bone metastasis) stimulation caused by osteoclastic cells high-differentiation.	Usage of Zoledronic acid and Denosumab to treat bone lesions.	The expression of *DKK1* gene serving as biological indicator to predict and prevent the osseous metastatic disease in the upcoming years.	Further research is needed when considering if bone metastasis and other tumors are biologically affined or not.	[336,337]

Notes: CAR-T, chimeric antigen receptor T cells; CTx, chemotherapy; DMARDs, disease-modifying antirheumatic drugs; HA, hyaluronic acid; HPP, hypophosphatasia; MMP13, matrix metallopeptidase 13; MMPs, matrix metalloproteinases; MMT, multi-modal therapy; MTX, methotrexate; NSAIDs, non-steroidal anti-inflammatory drugs; OCD, osteochondritis dissecans; OCIF, osteoclastogenesis inhibitory factor; OI, osteogenesis imperfecta, P.O, per Os (oral administration); OPG: osteoprotegerin; RA, rheumatoid arthritis; RANKL, receptor activator of nuclear factor kappa-B ligand; SERM, selective estrogen receptor modulators; RTx, radiotherapy; HER2/neu, human epidermal growth factor receptor 2.

shown the significance of a WIOTM (Wnt-induced osteogenic tissue model) "band-aid" capable of being transplanted in maintaining human skeletal stem cells and repairing bone defects in mice thus demonstrating the therapeutical significance of Wnt signaling manipulation in clinic.[359]

Summary and perspective

Bone well-development and homeostasis is vital for health maintenance, and it is highly regulated by the Wnt signaling pathway known to orchestrate the osteoblast differentiation, bone growth and repair. This review coordinates the studies on the critical role of Wnt signaling pathway for the attainment of normal bone mass and structure and their significance in generating the energy in osteoblasts-lineage cells. The identification of specific and well-conserved regulatory proteins and different signaling pathways involved at various steps of this pathway designate that the Wnt-related processes are as tightly regulated as they are highly predisposed to gain alterations and be linked to pathological processes and development of numerous abnormal skeletal conditions. Supplementary research is highly necessary to further inquest the contributions of Wnt signaling pathway on osteoblast metabolism, bone homeostasis and the energy balance of the body. The compelling evidence emerging from newly reported studies will be highly embraced and utilized for novel therapeutics design to treat or alleviate skeletal disorders or bone diseases in the clinic. In the upcoming years, researchers dedicated to carcinogenic therapies and bone disorder operation should gain a better understanding on bone-related-affairs and further supply with relevant and applicable pre-emptive inventions. In spite of all that, aspiration, and expectation for the expansion of accurate understanding and execution in this discipline and especially on Wnt signaling pathway, will always remain increasingly serviceable to human beings.

Author contributions

RV: data analysis and literature formation. GC, MW, and XZ: supervision and grant holder.

Conflict of interests

The authors declare no conflict of interest.

Funding

This work was supported by grants by Zhejiang Qianjiang Talent Program (No. 21040040-E), the Department of Sci-Tech of Zhejiang Province (No. LGF19H140002), a startup grant from Zhejiang Sci-Tech University (No. 18042290-Y, and 2021Q031), funds from National Natural Science Foundation of China (No. 81900806, and 81400489), the basic Public Welfare Planning Project of Zhejiang Province (No. LGD20C040001) and Jiaxing Science Technology Foundation (No. 2020AY10001) and Jiaxing Key Laboratory of Animal Model Generation and Precise Synthesis of New Drug Leads.

Abbreviations

BMD	Bone mineral density
BMP	Bone morphogenetic protein
CAD	Coronary artery disease
CAR-T	Chimeric antigen receptor T cells
COL1A1/COL2A1	Collagen, type I/type II, alpha 1
CGOF	Conditional gain of function
CTx	Chemotherapy
DMARDs	Disease-modifying antirheumatic drugs
DMP1	Dentin matrix acidic phosphoprotein 1
E2A/TCF3	Transcription factor 3/ E2A immunoglobulin enhancer-binding factors E12/E47
FGF	Fibroblast growth factor
GLI	Glioma-associated oncogene
GOF	Gain of function
HA	Hyaluronic acid
HER2/neu	Human epidermal growth factor receptor 2
HPP	Hypophosphatasia
Ihh	Indian hedgehog
LOF	Loss of function
MLST8	mTOR associated protein, LST8 homolog
MMPs	Matrix metalloproteinases
MMP13	Matrix metallopeptidase 13
MMT	Multi-modal therapy
MSCs	Meristematic stem cells
MST1/2	Macrophage-stimulating protein
MSX2	Homeobox protein MSX-2
MTX	Methotrexate
NSAIDs	Non-steroidal anti-inflammatory drugs
OC	Osteocalcin
OCD	Osteochondritis dissecans
OCIF	Osteoclastogenesis inhibitory factor
OE	Overexpression
OI	Osteogenesis imperfecta
OMIM	Online Mendelian inheritance in man
OPG	Osteoprotegerin
PDGF	Platelet-derived growth factor
PNM	Perinatal mortality
P.O	Per Os (oral administration)
PRX1	Peroxiredoxin 1
RA	Rheumatoid arthritis
RANKL	Receptor activator of nuclear factor kappa-B ligand
RAP	RAS/RAP GTP-binding protein
RIC	Resistance to inhibitors of cholinesterase
RARβ	Retinoic acid receptor beta
RTx	Radiotherapy
SERM	Selective estrogen receptor modulators
SIN1	Stress activated protein kinase interacting protein 1
TAZ	Transcription adaptor putative zinc finger
Tb.N	Trabecular number
Tb.Sp	Trabecular separation
TGF-β	Transforming growth factor-β
TMD	Tissue mineral density
TSC1/2	Tuberous sclerosis proteins 1 and 2
YAP	Yes-associated protein

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