Injury 52S2 (2021) S44–S48

Contents lists available at ScienceDirect

Injury

journal homepage: www.elsevier.com/locate/injury

Experimental agents to improve fracture healing: utilizing the WNT signaling pathway☆

Melanie Haffner-Luntzer

Institute of Orthopedic Research and Biomechanics, University Medical Center Ulm, Helmholtzstraße 14, 89081 Ulm, Germany

ARTICLE INFO

Article history:
Accepted 16 November 2020

Keywords:
Fracture healing
WNT signaling
bone repair

ABSTRACT

The process of bone healing largely recapitulates bone development in the embryo and ideally achieves complete restoration of bone shape and structure. However, because successful fracture healing requires tight interactions of numerous cell types and signaling molecules, any disruption of this highly coordinated processes can result in delayed healing or even non-union formation. The rate of fracture healing complications in orthopedic patients is reported to be 5–20%. Therefore, there is a need for new therapeutic strategies to improve fracture healing in patients with healing complications. One treatment strategy would include the easy and safe application of a pharmacological agent inducing osteoanabolic effects during fracture healing. One potential promising molecular target is the osteoanabolic WNT signaling pathway. This pathway plays an important role during embryonic bone development, homeostasis, mechanotransduction, development of osteoporosis and bone regeneration. This review focuses on preclinical studies targeting WNT signaling molecules to accelerate fracture healing. The three main investigated antagonists of the WNT signaling pathway, which can be blocked experimentally by antibodies, are Sclerostin, Dickkopf-1 and Midkine. Treating animals with antibodies against these proteins enhanced bone formation in the fracture callus. This indicates a therapeutic potential for these antibodies to accelerate fracture healing in patients with orthopedic complications.

Introduction

Bone tissue has the rare capability of scarless self-repair after injury. However, because successful fracture healing requires tight interactions of many cell types and signaling molecules, any disruption of this highly coordinated processes can result in delayed healing or even non-union formation [1]. The rate of fracture healing complications in orthopedic patients is reported to be 5–20% [2,3]. Reasons for impaired bone healing are manifold and include inappropriate mechanical stabilization, infection, impaired blood supply, comorbid diseases, advanced age, hormone and nutrition status of the patient, pharmacological therapy and genetic variation [4-6]. Therefore, there is a need for new therapeutic strategies to improve fracture healing especially in patients with healing complications. An ideal treatment strategy would include the easy and safe application of a pharmacological agent inducing osteoanabolic effects during fracture healing. One such potential molecular target is the osteoanabolic WNT signaling pathway. This pathway plays an important role during embryonic bone development, bone homeostasis, mechanotransduction in bone tissue and bone regeneration [7-10]. Therefore, this review focuses on preclinical studies targeting WNT signaling molecules to accelerate fracture healing.

Molecules of the WNT signaling pathway

WNT proteins are encoded by the large family of WNT genes of which the first, WNT1, was discovered more than three decades earlier in the mouse [11]. Currently, up to 19 different WNT genes are known in mammalian genomes [11]. Genes of the WNT family code for cysteine-rich secreted growth factor proteins, which act as signaling molecules [12]. WNT proteins are secreted with the help of the WNTless protein into the extracellular space, where they bind to cell-surface receptors of target cells. WNT proteins have been categorized into two groups based on whether they have the ability to induce the formation of a secondary body axis in *Xenopus laevis* embryos and to transform the cell shape of the mouse epithelial cell line C57MG [13]. WNT proteins with these characteristics were classified as canonical WNT poteins, while other WNT molecules were categorized as non-canonical [14]. The transforming ability of canonical WNTs was demonstrated to increase

☆ This paper is part of a supplement supported by the Osteosynthesis and Trauma Care Foundation (OTCF) through a research grant from Stryker.

E-mail address: melanie.haffner-luntzer@uni-ulm.de

https://doi.org/10.1016/j.injury.2020.11.051

the cellular levels of the transcriptional coactivator β–catenin [15]. By contrast, non-canonical WNTs ligands employ alternative β–catenin-independent signaling mechanisms, including intracellular calcium release and activation of protein kinase C, or the c-Jun N-terminal kinase. This review will focus on canonical WNT molecules, because most osteoanabolic agents target this part of the WNT signaling pathway.

The central signaling molecule of the WNT/β–catenin pathway is the transcriptional co-activator β–catenin, the stability of which is tightly controlled. In the absence of a WNT ligand, cytoplasmic β–catenin is bound to a multi-protein complex consisting of the scaffolding protein Axin, the tumor suppressor Adenomatous polyposis coli (APC) and two serine-threonine kinases (Glycogen synthase kinase 3, GSK3α/β and Casein kinase 1 α/β, CK1α/β). Both these kinases phosphorylate β–catenin at N-terminal serine/threonine residues. Phosphorylated β–catenin is then ubiquitinated and thereby targeted for degradation by the proteasome pathway [16]. Therefore, in the absence of a WNT signal, only very low levels of β–catenin are present in the cytoplasm. WNT/β–catenin signaling is initiated by binding of a WNT ligand to a receptor of the Frizzled (FZD) family and its co-receptors low density lipoprotein receptor-related protein 5 or 6 (LRP5/6). Subsequently, the scaffolding protein Dishevelled (DVL) is recruited to the receptor complex and LRP5/6 is phosphorylated by the combined action of Gsk3β and Ck1γ. Phosphorylated LRP5/6 then serves as a docking site for the recruitment of the cytoplasmic sprotein Axin; this inhibits phosphorylation and thereby degradation of β–catenin. As a consequence, newly synthesized β–catenin accumulates in the cytoplasm and translocates to the nucleus. Binding of nuclear β–catenin to TCF/LEF proteins and the recruitment of additional cofactors initiates transcription of WNT target genes [16]. Many target genes are cell-type specific. Because of its pivotal role in development and tissue homeostasis, it is not surprising that the β–catenin pathway activity is tightly regulated by a wide range of modifiers. These effectors might act in a promoting or antagonizing manner at different levels in the signaling cascade. In bone tissue, the most important and best investigated WNT modifiers are Kremen, Frizzled-related proteins (sFRP), Dickkopf (DKK), Midkine (MDK) and Sclerostin (SCL). All of these proteins antagonize canonical WNT signaling. In general, the canonical WNT/β–catenin pathway is known for its osteoanabolic action, because humans or mice lacking one or more components of this signaling cascade display dramatic osteoporotic bone phenotypes [17-21].

Utilizing the WNT signaling pathway to enhance fracture healing by experimental agents

Because of its osteoanabolic action, activation of the canonical WNT/β–catenin pathway is also critical for bone formation during fracture healing. Confirming this, transgenic mice lacking important components of the signaling cascade or overexpressing WNT inhibitors frequently display impaired bone healing. For example, LRP5$^{-/-}$ mice, LRP6$^{-/+}$ mice and mice overexpressing the LRP5/6-antagonist Kremen2 specifically in osteoblasts showed reduced bone formation and mechanical strength in the fracture callus because of reduced β–catenin signaling [22,23]. By contrast, transgenic mice lacking WNT inhibitors or overexpressing WNT molecules displayed accelerated fracture healing: SCL$^{-/-}$ mice display increased bone formation and callus size and strength during fracture healing because of increased activation of β–catenin in osteoblasts in the fracture callus [24-26]. Inducible WNT7b expression during fracture healing promoted callus mineralization and accelerated bone healing [27]. These are just some examples from studies demonstrating the power of the WNT signaling pathway to modify fracture healing by using genetically modified mouse models. However, in the light of a possible clinically relevant treatment option, pharmacological inhibitors of Wnt antagonists, for example, monoclonal antibodies, are more relevant [28].

Search strategy for studies investigating experimental agents to target the Wnt signaling pathway during fracture healing

A literature research was performed in Pubmed in March 2020 using the keywords search term “Wnt” AND “antibody” AND “fracture healing”. This search resulted in 25 publications. The by far most used antibodies for targeting the WNT signaling pathway are against SCL, DKK1 and MDK. Therefore, this review will focus on these three main investigated antagonists of the WNT signaling pathway, which can be blocked experimentally by antibodies.

Sclerostin

SCL is a secreted WNT antagonist produced by articular chondrocytes and osteocytes [29]. SCL interacts with the extracellular domain of the LRP5/6 receptor to prevent binding of WNT ligands (Fig. 1) [30]. Evidence from humans carrying missense mutations in the Sclerostin gene (van Buchem disease) suggests SCL as a potential target for the treatment of low bone mineral density (BMD) and bone mass, because these patients display dramatically increased BMD [30]. Indeed, clinical trials testing fully humanized anti-SCL antibodies show promising results as an anti-osteoporotic drug in increasing BMD and reducing fracture risk in postmenopausal women [31-33]. Furthermore, the effects of an anti-SCL treatment strategy by using monoclonal antibodies during fracture healing have been demonstrated in many preclinical studies. Mice and rats which underwent anti-SCL therapy during fracture healing displayed increased radiographic density, bone mass and strength in the fracture callus [30,34-36]. Furthermore, SCL-antibody (SCL-Ab) treatment was also effective in accelerating bony union in cynomolgus monkeys undergoing fibular osteotomy [36]. The underlying mechanism is the inhibition of the negative effects of SCL on osteoblastogenesis. Furthermore, SCL was shown to stimulate RANKL secretion from osteocytes, thereby inducing osteoclastogenesis [37]. However, the effects of SCL-Ab on mechanotransduction during fracture healing remain unclear, because SCL-Ab treatment did not accelerate fracture healing in a mechanically induced delayed healing model [38].

Dickkopf-1

DKK1 is also a secreted WNT antagonist interacting with the binding of WNT ligands to LRP5/6 (Fig. 1). This molecule is expressed by several cell types in the bone, including osteoblasts, osteocytes, preadipocytes and endothelial cells [30,39]. Furthermore, DKK1 was recently described as a pro-inflammatory ligand in inflammatory conditions [40]. DKK1$^{+/-}$ mice display increased bone mass and bone strength. By contrast, osteoblast-specific DKK1-overexpressing mice exhibit reduced BMD and bone mass, highlighting the negative effects of DKK1 on bone formation [30]. Effects of anti-DKK1 antibodies on fracture repair were tested in several rodent models. DKK1-Ab-treated rats and mice display increased BMD, strength and earlier bony union in the fracture callus [30,41]. Underlying mechanisms were described as a positive effect on MSC osteoblast differentiation mediated by DKK1-Ab, however, molecular mechanisms have not been studied in detail [41,42]. Furthermore, the influence of DKK1-Ab on the early inflammatory phase of fracture is unclear. Recently, a bispecific antibody targeting both SCL and DKK1 was tested during fracture healing in mice. Simultaneous inhibition of SCL and DKK1 led to more rapid fracture healing than administration of SCL-Ab or DKK1-Ab alone [43].

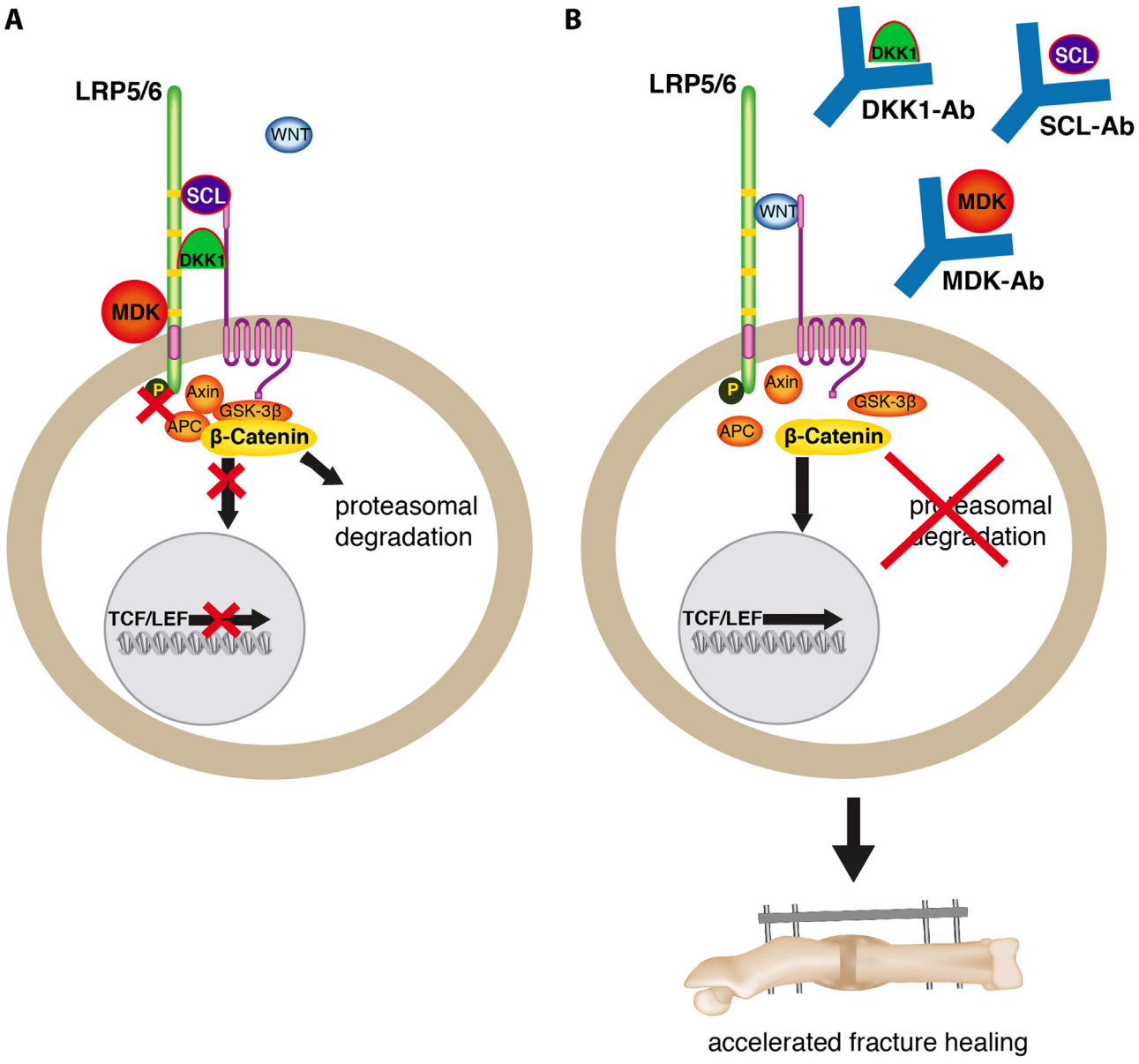

Fig. 1. Mode of action of WNT signaling antagonists and antibodies against these antagonists. A) Inactive Wnt/β-catenin signaling in the presence of WNT antagonists like Midkine (MDK), Sclerostin (SCL) and Dickkopf-1 (DKK1). B) Active Wnt/β-catenin signaling after administration of anti-MDK, anti-SCL or anti-DKK1 antibodies led to accelerated fracture healing.

Midkine

MDK is a WNT signaling antagonist which can act both extra- and intracellularly by binding to different receptors dependent on the involved cell type, including LRP6 (Fig. 1) [44-46]. MDK expression is almost absent in the healthy adult organism; its expression is tightly restricted to brain, kidney and long bones [47-49]. However, during inflammatory conditions like an acute fracture, MDK expression is upregulated locally in the injured tissue and circulating MDK levels are also increased. During inflammatory processes, MDK promotes cell migration of neutrophils and macrophages [50]. MDK is expressed during all phases of fracture healing in different cell types in the fracture callus, including macrophages, chondrocytes and osteoblasts [46]. Preclinical studies demonstrated that intracellular MDK expression in chondrocytes is important for cartilaginous callus formation during endochondral fracture healing [51]. However, blocking extracellular MDK by anti-MDK antibody administration led to increased bone formation and strength in the fracture callus both in middle-aged and osteoporotic mice [52,53]. An underlying mechanism might be increased osteoblast activity through elevated WNT/β-catenin signaling during osteoblastogenesis in the fracture callus. Furthermore, MDK-Ab treatment had a significant effect on inflammatory cytokines and cells in the early fracture hematoma. Systemic MDK-Ab administration normalized interleukin-6 expression and the presence of neutrophils in the fracture callus in osteoporotic mice [54]. The first clinical data demonstrated that circulating MDK levels are increased after fracture in humans and that postmenopausal women displayed further increased serum MDK levels compared to male fracture patients [55]. This indicates a therapeutic potential for humanized MDK-Ab to accelerate fracture healing, particularly in postmenopausal women. However, further research is needed to clarify the effects of MDK-Ab on the entire organism during regeneration after orthopedic trauma.

Conclusion

Experimental agents which increase WNT/β-catenin signaling during fracture repair showed promising results in accelerating bone formation in the fracture callus and, therefore, boosting fracture healing success. Namely, antibodies against SCL, DKK1 and MDK were tested successfully in preclinical models. In future, clinical studies are needed to establish a safe and efficient treatment strategy using these targets to improve fracture patient outcome. However, the first two clinical trials testing an anti-SCL antibody failed to show an improvement of tibial and hip fracture healing [56,57]. This might be due to intrinsic differences between animal models and the human situation, like differences in bone metabolism and remodeling or the different loading situation. Also, dosage and treatment regime might differ between preclinical and clinical studies. Often, therapeutic antibodies are applied in much higher concentrations in the animal model compared to patients. Furthermore, it might be necessary to test antibodies targeting WNT antagonists in patient cohorts with a higher risk for fracture healing complications to see stronger effects. More research is needed to test whether results from preclinical fracture healing studies can be verified also in the clinical setting.

Declaration of Competing Interest

I hereby declare that I have no conflict of interest regarding this manuscript.

Acknowledgements

This work was supported by the Collaborative Research Centre CRC1149 (funded by the Deutsche Forschungsgemeinschaft, German Research Foundation).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.injury.2020.11.051.

References

[1] Claes L, Recknagel S, Ignatius A. Fracture healing under healthy and inflammatory conditions. Nature reviews Rheumatology 2012;8:133–43.
[2] Bhandari M, Tornetta P, 3rd Sprague S, Najibi S, Petrisor B, Griffith L, et al. Predictors of reoperation following operative management of fractures of the tibial shaft. J Orthop Trauma 2003;17:353–61.
[3] Cheung WH, Miclau T, Chow SK, Yang FF, Alt V. Fracture healing in osteoporotic bone. Injury 2016;47(Suppl 2):S21–6.
[4] Einhorn TA. Enhancement of fracture-healing. The Journal of bone and joint surgery American volume 1995;77:940–56.
[5] Einhorn TA. The cell and molecular biology of fracture healing. Clinical orthopaedics and related research 1998:S7–21.
[6] Einhorn TA, Gerstenfeld LC. Fracture healing: mechanisms and interventions. Nature reviews Rheumatology 2015;11:45–54.
[7] Chen Y, Alman BA. Wnt pathway, an essential role in bone regeneration. Journal of cellular biochemistry 2009;106:353–62.
[8] Gregory CA, Green A, Lee N, Rao A, Gunn W. The promise of canonical Wnt signaling modulators in enhancing bone repair. Drug News Perspect 2006;19:445–52.
[9] Kim JB, Leucht P, Lam K, Luppen C, Ten Berge D, Nusse R, et al. Bone regeneration is regulated by wnt signaling. J Bone Miner Res 2007;22:1913–23.
[10] Rawadi G, Roman-Roman S. Wnt signalling pathway: a new target for the treatment of osteoporosis. Expert Opin Ther Targets 2005;9:1063–77.
[11] Nusse R, Varmus H. Three decades of Wnts: a personal perspective on how a scientific field developed. EMBO J 2012;31:2670–84.
[12] Holstein TW. The evolution of the Wnt pathway. Cold Spring Harb Perspect Biol 2012;4:a007922.
[13] McMahon AP, Moon RT. Ectopic expression of the proto-oncogene int-1 in Xenopus embryos leads to duplication of the embryonic axis. Cell 1989;58:1075–84.
[14] Du SJ, Purcell SM, Christian JL, McGrew LL, Moon RT. Identification of distinct classes and functional domains of Wnts through expression of wild-type and chimeric proteins in Xenopus embryos. Mol Cell Biol 1995;15:2625–34.
[15] Shimizu H, Julius MA, Giarre M, Zheng Z, Brown AM, Kitajewski J. Transformation by Wnt family proteins correlates with regulation of beta-catenin. Cell Growth Differ 1997;8:1349–58.
[16] Nusse R, Clevers H. Wnt/beta-Catenin Signaling, Disease, and Emerging Therapeutic Modalities. Cell. 2017;169:985–99.
[17] Lu Y, Ren X, Wang Y, Bardai G, Sturm M, Dai Y, et al. Novel WNT1 mutations in children with osteogenesis imperfecta: Clinical and functional characterization. Bone 2018;114:144–9.
[18] Macsai CE, Foster BK, Xian CJ. Roles of Wnt signalling in bone growth, remodelling, skeletal disorders and fracture repair. J Cell Physiol 2008;215:578–87.
[19] Panigrahi I, Didel S, Kirpal H, Bellampalli R, Miyanath S, Mullapudi N, et al. Novel mutation in a family with WNT1-related osteoporosis. Eur J Med Genet 2018;61:369–71.
[20] Albers J, Schulze J, Beil FT, Gebauer M, Baranowsky A, Keller J, et al. Control of bone formation by the serpentine receptor Frizzled-9. The Journal of cell biology 2011;192:1057–72.
[21] Baron R, Kneissel M. WNT signaling in bone homeostasis and disease: from human mutations to treatments. Nat Med 2013;19:179–92.
[22] Liedert A, Rontgen V, Schinke T, Benisch P, Ebert R, Jakob F, et al. Osteoblast-specific Krm2 overexpression and Lrp5 deficiency have different effects on fracture healing in mice. PLoS One 2014;9:e103250.
[23] Burgers TA, Vivanco JF, Zahatnansky J, Moren AJ, Mason JJ, Williams BO. Mice with a heterozygous Lrp6 deletion have impaired fracture healing. Bone Res 2016;4:16025.
[24] Li C, Ominsky MS, Tan HL, Barrero M, Niu QT, Asuncion FJ, et al. Increased callus mass and enhanced strength during fracture healing in mice lacking the sclerostin gene. Bone 2011;49:1178–85.
[25] McGee-Lawrence ME, Ryan ZC, Carpio LR, Kakar S, Westendorf JJ, Kumar R. Sclerostin deficient mice rapidly heal bone defects by activating beta-catenin and increasing intramembranous ossification. Biochem Biophys Res Commun 2013;441:886–90.
[26] Morse A, Yu NY, Peacock L, Mikulec K, Kramer I, Kneissel M, et al. Endochondral fracture healing with external fixation in the Sost knockout mouse results in earlier fibrocartilage callus removal and increased bone volume fraction and strength. Bone 2015;71:155–63.
[27] Song D, He G, Song F, Wang Z, Liu X, Liao L, et al. Inducible expression of Wnt7b promotes bone formation in aged mice and enhances fracture healing. Bone Res 2020;8:4.
[28] Houschyar KS, Tapking C, Borrelli MR, Popp D, Duscher D, Maan ZN, et al. Wnt Pathway in Bone Repair and Regeneration - What Do We Know So Far. Front Cell Dev Biol 2018;6:170.
[29] Lewiecki EM. Role of sclerostin in bone and cartilage and its potential as a therapeutic target in bone diseases. Ther Adv Musculoskelet Dis 2014;6:48–57.
[30] Ke HZ, Richards WG, Li X, Ominsky MS. Sclerostin and Dickkopf-1 as therapeutic targets in bone diseases. Endocr Rev 2012;33:747–83.
[31] McClung MR, Grauer A, Boonen S, Bolognese MA, Brown JP, Diez-Perez A, et al. Romosozumab in postmenopausal women with low bone mineral density. N Engl J Med 2014;370:412–20.
[32] Saag KG, Petersen J, Brandi ML, Karaplis AC, Lorentzon M, Thomas T, et al. Romosozumab or Alendronate for Fracture Prevention in Women with Osteoporosis. N Engl J Med 2017;377:1417–27.
[33] Cosman F, Crittenden DB, Adachi JD, Binkley N, Czerwinski E, Ferrari S, et al. Romosozumab Treatment in Postmenopausal Women with Osteoporosis. N Engl J Med 2016;375:1532–43.
[34] Jawad MU, Fritton KE, Ma T, Ren PG, Goodman SB, Ke HZ, et al. Effects of sclerostin antibody on healing of a non-critical size femoral bone defect. Journal of orthopaedic research : official publication of the Orthopaedic Research Society 2013;31:155–63.
[35] Yee CS, Xie L, Hatsell S, Hum N, Murugesh D, Economides AN, et al. Sclerostin antibody treatment improves fracture outcomes in a Type I diabetic mouse model. Bone 2016;82:122–34.
[36] Ominsky MS, Li C, Li X, Tan HL, Lee E, Barrero M, et al. Inhibition of sclerostin by monoclonal antibody enhances bone healing and improves bone density and strength of nonfractured bones. J Bone Miner Res 2011;26:1012–21.
[37] Suen PK, Sclerostin Qin L. an emerging therapeutic target for treating osteoporosis and osteoporotic fracture: A general review. J Orthop Translat 2016;4:1–13.
[38] Kruck B, Zimmermann EA, Damerow S, Figge C, Julien C, Wulsten D, et al. Sclerostin Neutralizing Antibody Treatment Enhances Bone Formation but Does Not Rescue Mechanically Induced Delayed Healing. J Bone Miner Res 2018;33:1686–97.
[39] Pinzone JJ, Hall BM, Thudi NK, Vonau M, Qiang YW, Rosol TJ, et al. The role of Dickkopf-1 in bone development, homeostasis, and disease. Blood 2009;113:517–25.
[40] Chae WJ, Dickkopf1 Bothwell ALM. An immunomodulatory ligand and Wnt antagonist in pathological inflammation. Differentiation 2019;108:33–9.
[41] Li X, Grisanti M, Fan W, Asuncion FJ, Tan HL, Dwyer D, et al. Dickkopf-1 regulates bone formation in young growing rodents and upon traumatic injury. J Bone Miner Res 2011;26:2610–21.
[42] Hankenson KD, Gagne K, Shaughnessy M. Extracellular signaling molecules to promote fracture healing and bone regeneration. Adv Drug Deliv Rev 2015;94:3–12.
[43] 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.

[44] Kadomatsu K, Kishida S, Tsubota S. The heparin-binding growth factor midkine: the biological activities and candidate receptors. J Biochem 2013;153:511–21.
[45] Nakamoto M, Matsubara S, Miyauchi T, Obama H, Ozawa M, Muramatsu T. A new family of heparin binding growth/differentiation factors: differential expression of the midkine (MK) and HB-GAM genes during mouse development. J Biochem 1992;112:346–9.
[46] Haffner-Luntzer M, Heilmann A, Rapp AE, Roessler R, Schinke T, Amling M, et al. Antagonizing midkine accelerates fracture healing in mice by enhanced bone formation in the fracture callus. Br J Pharmacol 2016;173:2237–49.
[47] Diamond-Stanic MK, Romero-Aleshire MJ, Hoyer PB, Greer K, Hoying JB, Brooks HL. Midkine, a heparin-binding protein, is increased in the diabetic mouse kidney postmenopause. Am J Physiol Renal Physiol 2011;300:F139–46.
[48] Tsutsui J, Uehara K, Kadomatsu K, Matsubara S, Muramatsu T. A new family of heparin-binding factors: strong conservation of midkine (MK) sequences between the human and the mouse. Biochem Biophys Res Commun 1991;176:792–7.
[49] Liedert A, Schinke T, Ignatius A, Amling M. The role of midkine in skeletal remodelling. Br J Pharmacol 2014;171:870–8.
[50] Weckbach LT, Muramatsu T, Walzog B. Midkine in inflammation. ScientificWorldJournal 2011;11:2491–505.
[51] Haffner-Luntzer M, Heilmann A, Rapp AE, Beie S, Schinke T, Amling M, et al. Midkine-Deficiency Delays Chondrogenesis during the Early Phase of Fracture Healing in Mice. Plos One 2014:9.
[52] Haffner-Luntzer M, Heilmann A, Rapp AE, Roessler R, Schinke T, Amling M, et al. Antagonizing midkine accelerates fracture healing in mice by enhanced bone formation in the fracture callus. Brit J Pharmacol 2016;173:2237–49.
[53] Haffner-Luntzer M, Kemmler J, Heidler V, Prystaz K, Schinke T, Amling M, et al. Inhibition of Midkine Augments Osteoporotic Fracture Healing. PLoS One 2016;11:e0159278.
[54] Haffner-Luntzer M, Fischer V, Prystaz K, Liedert A, Ignatius A. The inflammatory phase of fracture healing is influenced by oestrogen status in mice. Eur J Med Res 2017;22:23.
[55] Fischer V, Kalbitz M, Muller-Graf F, Gebhard F, Ignatius A, Liedert A, et al. Influence of Menopause on Inflammatory Cytokines during Murine and Human Bone Fracture Healing. Int J Mol Sci 2018:19.
[56] Bhandari M, Schemitsch EH, Karachalios T, Sancheti P, Poolman RW, Caminis J, et al. Romosozumab in Skeletally Mature Adults with a Fresh Unilateral Tibial Diaphyseal Fracture: A Randomized Phase-2 Study. The Journal of bone and joint surgery American volume 2020.
[57] Schemitsch EH, Miclau T, Karachalios T, Nowak LL, Sancheti P, Poolman RW, et al. A Randomized, Placebo-Controlled Study of Romosozumab for the Treatment of Hip Fractures. The Journal of bone and joint surgery American volume 2020;102:693–702.