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Neuropilin-1: Emerging roles in nerve-vessel-bone coupling during fracture repair (Review)

KEYUE ZHANG[1,2*], TONG LI[3*], HUAN LIU[1,2*], HUIBO TI[1,2*], ZHENYU ZHANG[1,2], ZHENGDONG YUAN[2], JUNJIE WU[2], XIELIN YAN[1,2], ZIXIN LUO[1,2], HAITING HU[1,2], XINGCHEN YAO[1,2], SHUWEN SHI[1,2], XIAN WU[1,2], FENG-LAI YUAN[1,2] and XIAO CHEN[1,3]

[1]Institute of Integrated Chinese and Western Medicine, Affiliated Hospital of Jiangnan University, Wuxi, Jiangsu 214122, P.R. China; [2]Wuxi Medical College, Jiangnan University, Wuxi, Jiangsu 214122, P.R. China; [3]Geriatrics Ward, Jiangsu Second Rongjun Hospital, Wuxi, Jiangsu 214062, P.R. China

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Abstract. Fracture healing is a sophisticated biological process orchestrated by the spatiotemporal coordination of neural, vascular and skeletal systems to sustain reparative homeostasis. However, this regulatory network is often disrupted in pathological conditions, such as osteoporosis, diabetes and aging, leading to impaired outcomes, such as delayed fracture healing and nonunion. Synthesizing available multidisciplinary evidence suggests that neuropilin-1 (NRP1), a transmembrane glycoprotein with pleiotropic functions, may serve as a potential mediator integrating multisystemic signals and participating in nerve-vessel-bone crosstalk during fracture repair. Endowed with distinctive structural domains, NRP1 selectively binds diverse ligands and has been observed to localize preferentially in active bone repair zones and critical cellular populations. There, it exhibits preliminary biological potential to assist in coordinating angiogenesis, modulating the function of bone-repair cells, and guiding nerve fibers. In systemic metabolic disorders or in localized, extreme inflammatory microenvironments, dysregulation of NRP1-mediated signaling may be associated with clinical complications, such as delayed fracture healing and nonunion. Therefore, a more in-depth exploration of the nerve-vessel-bone crosstalk and pathological networks potentially governed by NRP1 may provide preliminary mechanistic insight into the imbalances in bone repair. The present review summarizes the current understanding of the possible roles of NRP1 in physiological fracture healing and organizes reported specific dysregulation patterns across systemic high-risk diseases and distinct inflammatory osteolytic states. Finally, the present review discusses the translational potential of NRP1 as a candidate therapeutic target for delayed healing, explicitly highlighting current translational opportunities and significant preclinical barriers, in an aim to provide a preliminary theoretical framework for developing NRP1-targeted therapies for nonunion.

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1. Introduction

Fracture healing is a complex biological process that requires precise spatiotemporal coordination among the nervous, vascular and skeletal systems (1,2). With the accelerated aging of the global population, fractures have emerged as a major and growing public health challenge (3). The global burden is substantial, with 178 million new cases reported in 2019 alone, a 33.4% increase since 1990 (3). This significant incidence highlights the urgent clinical need to understand the fundamental biology of fracture repair, particularly when this intricate process fails (3).

The successful healing of these fractures is often critically impaired by underlying conditions, with osteoporosis being a

Correspondence to: Professor Feng-Lai Yuan or Dr Xiao Chen, Institute of Integrated Chinese and Western Medicine, Affiliated Hospital of Jiangnan University, 1000 Hefeng Road, Wuxi, Jiangsu 214122, P.R. China
E-mail: bjjq88@jiangnan.edu.cn
E-mail: cx427795916@126.com

*Contributed equally

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predominant risk factor that compromises bone quality (4,5). The repair process relies on a sophisticated crosstalk between systems. Specifically, the nervous system modulates bone metabolism through neuropeptides, such as calcitonin gene-related peptide (CGRP). In parallel, a specialized capillary subtype, known as type H vessels (the 'H' denotes high expression of both CD31 and endomucin), couples angiogenesis with osteogenesis via angiocrine factors, such as Wnt (wingless-related integration site) ligands (6-8). However, the molecular mechanisms that spatiotemporally integrate these multisystemic cues remain incompletely elucidated.

Initially recognized as a neuronal guidance cue and synaptogenesis regulator, neuropilin-1 (NRP1) has since been identified as a pleiotropic co-receptor for a wide spectrum of ligands, including vascular endothelial growth factor (VEGF), semaphorin 3A (SEMA3A) and TGF-β (9-12). Its potential role is further supported by research demonstrating that NRP1 mediates SEMA3A signaling to enhance osteogenesis and accelerate fracture repair (13). Emerging evidence currently suggests that NRP1 lies at the crossroads of nerve-vessel-bone crosstalk, functioning as a potential signaling node that coordinates the distribution of nerves, vascular patterning, and osteogenic differentiation during skeletal regeneration (14-18).

In the context of impaired fracture healing, as commonly observed in osteoporosis, NRP1 signaling is frequently disrupted, potentially leading to dysregulated bone remodeling (19). This is associated with poor repair outcomes, including dysregulated arterial capillary formation, abnormal axonal orientation and delayed callus mineralization (14,18,20). The present review summarizes the multifaceted functions of NRP1 in orchestrating the intercellular communication essential for bone repair. It discusses the preliminary translational potential of targeting NRP1 signaling as a candidate therapeutic strategy to recalibrate the impaired healing microenvironment and restore bone homeostasis.

2. Structure and distribution of NRP1

Structure of NRP1. NRP1 is a conserved transmembrane glycoprotein of ~130 kDa that is widely expressed across vertebrate species. It exhibits a modular three-domain structure consisting of a large extracellular domain, a single transmembrane helix and a short cytoplasmic tail (21,22).

Extracellular domain. The extracellular domain of NRP1 consists of five subdomains arranged sequentially from the N-terminus to the C-terminus, designated as a1, a2, b1, b2 and c domains, and this region serves as the core ligand-binding domain of NRP1 (23,24). Among these, the a1/a2 domains are mainly responsible for binding to SEMA3 family molecules (25). The b1/b2 domains are the primary binding sites for VEGF and exhibit a strong preference for the VEGFA165 isoform. This interaction provides a key basis for VEGF-mediated endothelial cell migration (26). In addition to the aforementioned classical interactions, the b1/b2 domains can also mediate the binding to C-end rule (CendR) peptides (27-29). The C domain mediates homodimerization of NRP1, a key step in the assembly of functional receptor complexes (30), as illustrated in Fig. 1.

Transmembrane domain. The hydrophobic transmembrane domain of NRP1 contains a GxxxG motif that promotes its homodimerization (31). NRP1 stabilizes its oligomerization state through the synergistic effect of the extracellular c domain and the GxxxG motif, providing the necessary structural framework for the formation of functional complexes with receptors such as VEGF receptor 2 (VEGFR2) and platelet-derived growth factor receptor β (PDGFRβ) (22,31-35), as illustrated in Fig. 1.

Cytoplasmic domain. The cytoplasmic domain of NRP1 is a short tail structure with no kinase activity; yet, it undertakes indispensable biological functions. A postsynaptic density protein-95/discs large/zonula occludens-1 (PDZ)-binding motif known as the SEA tripeptide sequence (Ser-Glu-Ala, the three C-terminal residues of the cytoplasmic tail) exists at its C-terminus (21,36). This motif can specifically bind to intracellular adaptor proteins containing PDZ domains, thereby regulating cell adhesion and cytoskeletal rearrangement. In addition, NRP1 acts as a coreceptor to enhance VEGFR2 phosphorylation, thereby amplifying downstream p38 MAPK and ERK1/2 signaling (37,38). Moreover, NRP1 can maintain its own stability through a Rab11A-dependent recycling pathway and undergo post-translational modifications, such as glycosaminoglycan and glycosylation modifications (39,40) (Fig. 1)

Soluble NRP1 (sNRP1). In addition to transmembrane NRP1, a soluble isoform of NRP1, sNRP1, also exists. sNRP1 is generated by alternative splicing or proteolytic cleavage and contains only the extracellular a and b domains (41,42). As a decoy receptor, sNRP1 can competitively bind to free VEGFA165 and prevent it from binding to the NRP1/VEGFR2 complex on the cell surface (41), as illustrated in Fig. 1.

Regional and cell-specific distribution of NRP1 in bone tissue. NRP1 exhibits regional and cell-specific distribution in bone tissue, which is highly consistent with its functions in osteogenesis, bone repair and neurovascular coupling (17,18,43). NRP1 is localized in bone tissue regions with active osteogenesis and bone repair, such as the metaphyses of long bones and trabecular bone margins. These regions are rich in osteoblasts and bone marrow endothelial cells, and this specific localization provides an anatomical basis for NRP1-mediated bone formation and bone repair signaling pathways (18,43). At the cellular level, NRP1 is expressed in osteoblasts, osteoclast precursors, chondrocytes, intraosseous vascular endothelial cells and sensory nerve endings in bone tissue. This expression pattern has been confirmed by relevant studies in both mouse and human bone tissues, further supporting its potential involvement in mediating bone metabolism and repair-related signaling pathways (6,14,17,44).

3. NRP1 regulates the functions of key vascular cells and structural homeostasis

NRP1 regulates the functions of key vascular cells. Precapillary arterioles and capillaries, composed of vascular endothelial cells and accompanying pericytes, are key vascular structures responsible for nutrient transport and metabolic exchange in bone tissue (6). Research on non-skeletal systems has confirmed that an abnormal NRP1 expression leads to vascular structural disorganization and impaired maturation (45).

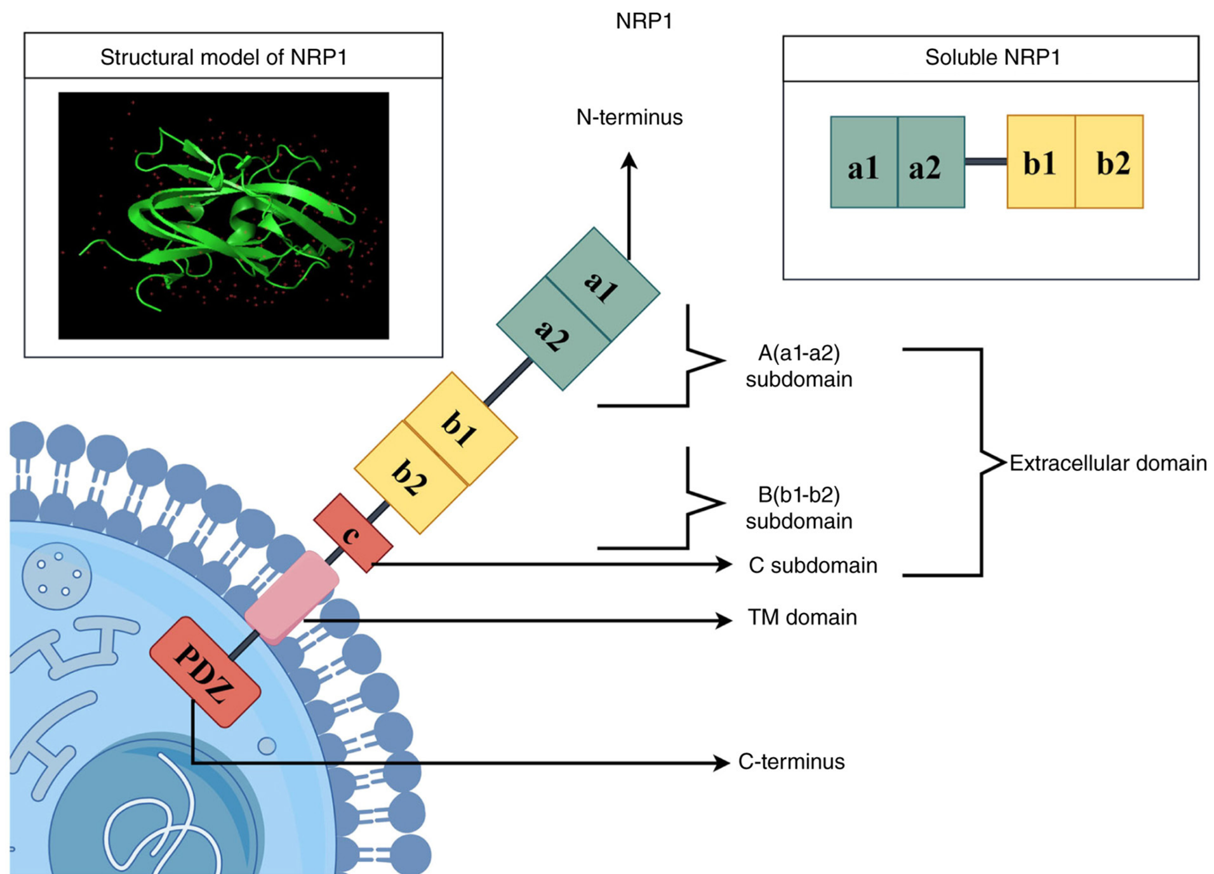

Figure 1. Systematic illustration of the molecular structures of both membrane-bound NRP1 and its soluble variant, sNRP1, and a structural model of NRP1. NRP1, neuropilin-1; sNRP1, soluble NRP1.

Endothelial cells. Existing evidence suggests that NRP1 may regulate endothelial cell function and play a potential role in angiogenesis during bone regeneration. *In vivo* research using a rabbit ischemic bone defect regeneration model confirmed that the local sustained release of NRP1 via a 3D-printed magnesium alloy composite scaffold significantly promoted neovascularization in the defect area (46). *In vitro* experiments have further clarified its molecular mechanism: As a co-receptor of VEGFR2, NRP1 forms a stable ternary complex with VEGFA and VEGFR2 on the surface of endothelial cells, thereby enhancing signal transduction efficiency and activating the phosphatidylinositol 3-kinase-protein kinase B (PI3K-AKT) pathway. This ultimately upregulates the expression of VEGFA, fibroblast growth factor 2 and the nuclear receptor Nr4a1 in endothelial cells, thereby regulating endothelial cell migration and proliferation (46), as demonstrated in Fig. 2.

Although the macroscopic pro-angiogenic effect has been confirmed in bone regeneration, the in-depth analysis of the multidimensional mechanistic network of NRP1 in endothelial cells still requires drawing on studies in non-skeletal systems. The following mechanisms, including ligand competition, directional vascular sprouting and the maintenance of endothelial barrier homeostasis, have not yet been directly verified in bone regeneration models, but can provide a potential reference framework for the study of bone repair mechanisms.

During the initiation phase of angiogenesis, NRP1 regulates the directional sprouting of blood vessels. *In vitro* experiments have confirmed that SEMA3A selectively inhibits the VEGFA165-induced phosphorylation of focal adhesion kinase and Src in endothelial cells via NRP1, thereby blocking the downstream angiogenic signals of VEGF (47). *In vivo* angiogenesis models have further confirmed that VEGFA165 and SEMA3A exert functional antagonism through NRP1, jointly regulating the intensity and scope of vascular sprouting (47). This ligand-competition mechanism was first directly verified by Miao *et al* (48) through biochemical and functional experiments: On the surface of NRP1-expressing cells, SEMA3A and VEGFA165 share overlapping binding sites in the b1/b2 domains of NRP1 and can competitively inhibit each other's binding to NRP1 (48). The aforementioned studies on non-skeletal systems suggest that NRP1 itself does not preset a pro-angiogenic or anti-angiogenic direction, and its functional output may depend on the relative concentrations and temporal expression of the two ligands in the microenvironment, rather than being linearly determined by a single signaling pathway. The applicability of this regulatory mode in angiogenesis during fracture healing remains to be verified. Clinical studies on rheumatoid arthritis have provided pathological evidence for this ligand competition mechanism. Clinical studies have found that synovial vascular density is significantly higher in patients with rheumatoid arthritis with VEGFA165-positive expression than in those with a negative expression (49). Subsequent research has demonstrated that Sema3A expression in the synovial lining cells of joints in patients with rheumatoid arthritis is significantly decreased.

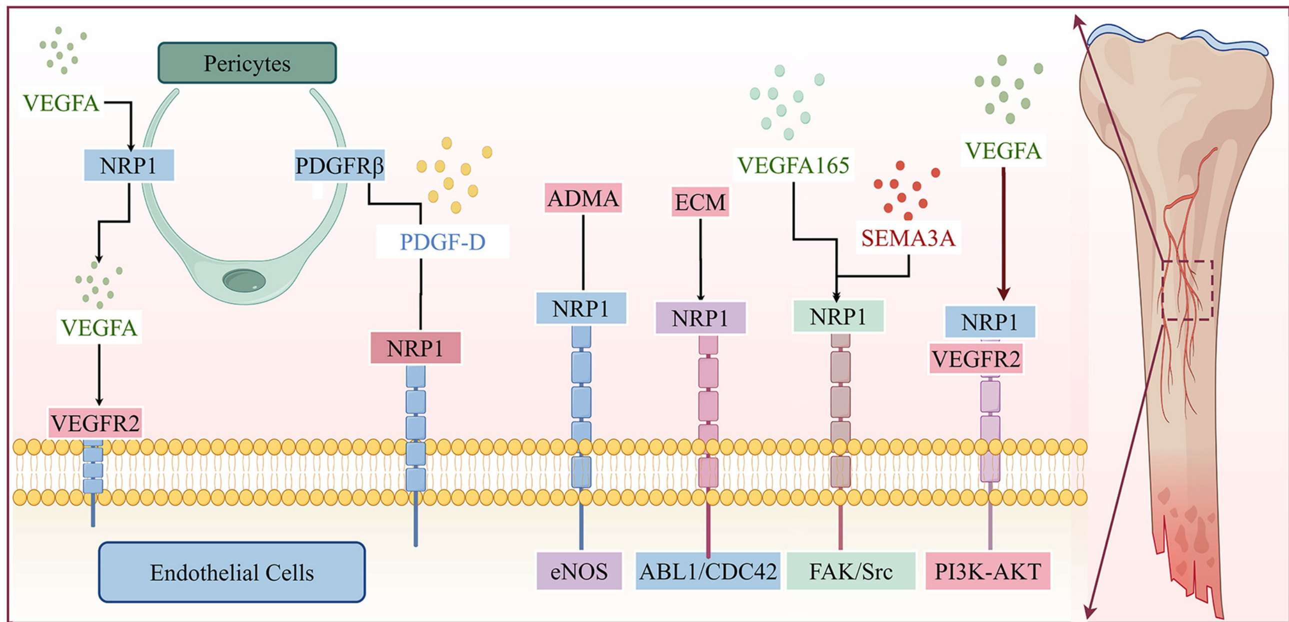

Figure 2. Schematic illustration of the molecular mechanisms by which NRP1, as a multiligand co-receptor, regulates angiogenesis and vascular homeostasis maintenance. At the endothelial cell level, VEGFA forms a ternary complex with NRP1 and VEGFR2 to activate the PI3K-AKT pathway, promoting neovascularization in bone defect areas; this mechanism has been directly validated in bone regeneration models. The extracellular matrix and VEGFA165/SEMA3A mediate the ABL1/CDC42 and FAK/Src pathways, respectively, via NRP1 to regulate vascular sprouting and branching. NRP1 also maintains endothelial cell survival and barrier function through the eNOS pathway. In terms of endothelial-pericyte crosstalk, pericyte NRP1 can capture VEGFA and present it to endothelial VEGFR2 to form a transcellular complex. At the same time, PDGF-D simultaneously binds to endothelial NRP1 and pericyte PDGFRβ to form a ternary complex, jointly regulating vascular permeability and pericyte coverage. NRP1, neuropilin-1; VEGF, vascular endothelial growth factor; SEMA3A, semaphorin 3A; eNOS, extracellular nitric oxide synthase; PDGF, platelet-derived growth factor receptor.

When Sema3A expression is insufficient, the dominant occupancy of NRP1 on the surface of synovial endothelial cells by VEGFA enhances pro-angiogenic signals and drives the formation of pathological pannus (50), as illustrated in Fig. 2.

Vascular sprouting is a crucial step in blood vessel formation. During vascular sprouting, NRP1 is highly expressed in endothelial tip cells. It mediates the activation of the Abelson murine leukemia viral oncogene homolog 1/cell division cycle 42 (ABL1/CDC42) pathway by the extracellular matrix, thereby promoting actin remodeling and filopodia formation, which are necessary for the directional migration of tip cells and normal vascular branching (51), as demonstrated in Fig. 2. Moreover, *in vitro* experiments using human umbilical artery endothelial cells have shown that NRP1 specifically binds to the endocytic adaptor protein GAIP-interacting protein C terminus 1 via the conserved SEA motif at the C-terminus of its cytoplasmic domain. It selectively mediates the endocytosis of the active conformation of integrin α5β1 at the cell membrane into Rab5-positive early endosomes, followed by their subsequent recycling to adhesion sites, ultimately enhancing endothelial cell adhesion to fibronectin (37).

Following blood vessel formation, NRP1 also plays a critical role in endothelial cell survival and in maintaining endothelial barrier homeostasis. In a previous study, *in vitro* functional experiments using human umbilical vein endothelial cells (HUVECs) and *in vivo* analyses using endothelial-specific NRP1 knockout mice confirmed that NRP1 maintains dimethylarginine dimethylaminohydrolase 1 (DDAH1) expression in endothelial cells via a post-transcriptional regulatory mechanism (52). DDAH1 has been identified as the key enzyme responsible for degrading asymmetric dimethylarginine (ADMA) *in vivo*, an endogenous inhibitor of nitric oxide (NO) synthase (NOS). By eliminating intracellular ADMA, it relieves its inhibitory effect on NOS and restores NO bioavailability in endothelial cells (53). A previous study using a mouse hindlimb ischemia model further validated the endothelial protective effects of NRP1 (54). NRP1 carried by mesenchymal stem cell-derived extracellular vesicles was shown to act on endothelial cells in ischemic tissues, significantly promoting nitric oxide production and inhibiting endothelial cell apoptosis by activating the endothelial NOS pathway, thereby exerting endothelial protection (54) (Fig. 2).

Furthermore, research using an *in vitro* HUVEC model confirmed that endothelial NRP1 is a component of adherens junctions. It stabilizes endothelial adherens junctions by promoting the binding of vascular endothelial cadherin (VE-cadherin) to p120 catenin, thereby maintaining the integrity and homeostasis of the endothelial barrier (55). In addition to the aforementioned regulatory mechanisms, NRP1 also modulates endothelial barrier function through a mechanical force-dependent pathway. Studies have demonstrated that the synthetic NRP1-specific agonist RCαβ activates the NRP1-MET proto-oncogene, hepatocyte growth factor receptor (NRP1-MET) signaling axis, which in turn upregulates Ras homolog family member A (RhoA) activity, promotes the assembly of actin stress fibers in endothelial cells, and increases cell stiffness. Furthermore, this NRP1-mediated signaling drives the redistribution of VE-cadherin from continuous intercellular junctions to punctate focal adherens junctions, recruits vinculin to enhance intercellular mechanical force transmission, and ultimately increases endothelial permeability (56,57). It should be noted that this effect is

caused by the activation of the MET signaling axis by the synthetic NRP1-specific agonist RCαβ. It is not contradictory to the aforementioned function of NRP1 in maintaining barrier homeostasis under physiological conditions, but rather reflects the dependence of the downstream functional output of NRP1 on ligand type.

Taken together, the prominent effect of NRP1 as a co-receptor of VEGFR2 in driving endothelial angiogenesis has been directly verified in a rabbit ischemic bone defect regeneration model (46). However, its multidimensional regulatory mechanisms in upstream ligand competition, directional vascular sprouting, and the maintenance of endothelial barrier homeostasis are currently mainly derived from studies in non-skeletal systems, which provide a conceptual framework for deciphering the temporal regulatory patterns of NRP1 on blood vessels during fracture healing (33,58-60).

Pericytes. Historically, the role of NRP1 in the vascular network has been centered on endothelial cells; however, accumulating evidence indicates that it also exerts multi-faceted regulatory functions in pericytes. High-resolution confocal imaging has confirmed that NRP1 is expressed on pericytes covering the vascular wall (61), providing a foundation for subsequent functional studies. It should be noted that all regulatory mechanisms of NRP1 in pericytes identified to date are derived from studies in non-skeletal systems. These findings provide potential research directions for understanding impaired vascular maturation during bone repair. Yet, there is no direct evidence from fracture models supporting its role in intraosseous pericytes, which remains a critical gap in this field.

Research on non-skeletal systems has shown that NRP1 is involved throughout the pericyte life cycle, from differentiation to the execution of physiological functions. An *in vitro* tumor microenvironment model first confirmed that PDGF-BB secreted by tumor cells directly interacts with NRP1 on the surface of mouse embryonic mesenchymal stem cells, significantly upregulating the expression of pericyte-specific markers, such as α-smooth muscle actin and desmin, and driving the transdifferentiation of mesenchymal stem cells into a pericyte phenotype (58). After pericytes mature, NRP1 becomes a crucial foundation for maintaining their functions. Research using pericyte-specific gene knockout has confirmed that neuron-glial antigen 2/PDGFRβ-positive pericytes in the kidneys of adult mice highly express NRP1 (59). Although NRP1 deficiency does not affect the number of surviving pericytes, it disrupts basement membrane protein homeostasis, impairs glomerular basement membrane integrity, and leads to microscopic hematuria and glomerular hyperfiltration (59).

Beyond its intracellular regulation within single cells, NRP1 plays unique roles in communications between pericytes and endothelial cells. Pericytes can capture extracellular VEGFA in a paracrine manner via NRP1 and present it to VEGFR2 on endothelial cells, forming a transcellular complex. This complex transcellularly regulates the endothelial VEGFR2 pathway and vascular permeability homeostasis by delaying VEGFR2 endocytosis and enhancing the Y949-Src-VE-cadherin signaling axis (60). Muhl *et al* (33) further confirmed that NRP1 can mediate transcellular signaling between endothelial cells and pericytes. PDGF-D simultaneously binds to NRP1 on endothelial cells and PDGFRβ on adjacent pericytes to form a ternary complex. This complex accumulates at cell contact protrusions, thereby forming a local signaling hub. This unique signaling mode enables PDGF-D to maintain vascular pericyte coverage and to mediate contact-dependent pericyte migration, ultimately indirectly regulating vascular homeostasis (33), as illustrated in Fig. 2.

In addition, the function of NRP1 in pericytes is not linearly determined by a single signaling pathway, but rather exhibits a high degree of context dependence. A previous study on skin microvessels in gene knockout mouse models demonstrated that the regulatory effect of NRP1 on vascular permeability depends on the expression ratio of NRP1 in pericytes and endothelial cells (60). When NRP1 is highly expressed in pericytes, endothelial NRP1/VEGFR2 *cis*-complexes maintain moderate permeability; if endothelial NRP1 is deficient, pericyte NRP1 forms trans-complexes with endothelial VEGFR2, significantly enhancing permeability. When NRP1 expression is low in pericytes, vascular permeability is always dominated by endothelial NRP1/VEGFR2 cis-complexes (60). This further confirms that the function of NRP1 depends on its cellular expression pattern rather than on linear regulation by a single signaling pathway.

Taken together, the regulatory functions of NRP1 in pericyte differentiation, transcellular communication and vascular permeability have been preliminarily verified in non-skeletal systems such as the kidney and skin. There is currently no direct evidence from fracture models supporting the aforementioned roles of NRP1 in intraosseous pericytes, which remains an urgent gap in the field of vascular maturation during bone repair. The functional regulation of intraosseous pericytes may follow similar principles, but the expression dynamics and functions of NRP1 in pericytes during fracture healing still require direct verification.

NRP1 and type H vessels. Type H vessels are a subtype of capillaries characterized by a high expression of cluster of differentiation 31 (CD31) and endomucin. They form a network through vertically arranged vascular columns connected by vascular loops or arches. They are mainly distributed in osteogenically active regions such as the metaphyses of long bones and the endosteum. They are considered the key structural carriers of bone-vessel coupling (6,62,63).

Studies on bone development models have demonstrated that VEGF is a key regulatory factor of skeletal angiogenesis and participates in the angiogenesis-osteogenesis coupling process mediated by type H vessels (6,64,65). Further studies on bone homeostasis have indicated that osteoclast precursors can directly promote the growth of type H vessels by secreting PDGF-BB (66-68). These findings have established that type H vessels depend on VEGF and PDGF signaling.

In the field of systemic vasculature, NRP1 has been identified as a co-receptor for both the VEGF and PDGF signaling axes. It is involved in endothelial-pericyte crosstalk and in vascular maturation and stabilization (33). This dual co-receptor property biochemically demonstrate that NRP1 possesses the molecular basis to simultaneously integrate VEGF and PDGF signals, suggesting that it may participate in the regulation of intraosseous type H vessels. However, there is currently no direct research evidence regarding the expression

and function of NRP1 in type H vessels of bone tissue in bone repair models. Its regulatory role in type H vessels remains an important hypothesis to be verified in the field of bone-vessel coupling. Future studies can first detect the expression and distribution of NRP1 in intraosseous type H vessels, and then use fracture models with endothelial cell- or pericyte-specific NRP1 knockout to examine its function in the formation and maintenance of type H vessels. Further studies are required to confirm that NRP1 regulates type H vessels, as this may provide a new perspective on the coupling mechanism between intraosseous angiogenesis and osteogenesis.

As summarized above, there are critical differences in the sufficiency of research evidence supporting the distinct mechanisms of NRP1 in the regulation of bone tissue vasculature. Among these, the role of NRP1 as a co-receptor of VEGFR2 in promoting neovascularization in bone defect areas has been fully validated through animal experiments. It represents the most clearly established core function to date. By contrast, the functions of NRP1 in guiding the direction of angiogenic sprouting, mediating signal communication between pericytes and endothelial cells and regulating the formation of type H vessels in bone have not yet been directly investigated in fracture models. Existing studies on non-skeletal systems suggest that the intensity and extent of angiogenic sprouting are co-regulated by the relative concentrations of VEGFA165 and SEMA3A ligands in the local microenvironment; however, whether this regulatory principle applies to the unique microenvironment of fracture healing remains to be definitively determined by experimental evidence.

Interaction of NRP1 with two angiogenic factors. The pro-angiogenic function of NRP1 is highly dependent on its specific interactions with the two major signaling molecule families, VEGF and PDGF. At present, the elucidation of the relevant mechanisms is mainly derived from studies on non-skeletal systems, and direct validation in the fracture microenvironment remains very limited (6,69-71).

Among the VEGF family, VEGFR1 and VEGFR2 are key receptors that regulate endothelial cell function and are also the main targets for interaction with NRP1 (72,73).

The pro-angiogenic effect of the NRP1-VEGFR2 signaling axis has been directly verified in a rabbit ischemic bone defect model through the synergistic action of 3D-printed magnesium alloy scaffolds (46). This model also confirmed that Mg^{2+} can further enhance the signal transduction efficiency of the NRP1-VEGFR2 ternary complex on the surface of endothelial cells by upregulating VEGFA expression in bone marrow mesenchymal stem cells (BMSCs) (46).

However, the majority of the additional potential molecular mechanisms underlying the interaction between NRP1 and VEGF are derived primarily from *in vitro* studies conducted on non-bone tissues (74-76). *In vitro* studies on receptor kinetics and endothelial cell function have biochemically elucidated the interaction patterns between NRP1 and members of the VEGFR family on endothelial cell surfaces (74). In the ligand-free resting state, VEGFR1 and NRP1 form a constitutive stable complex. This complex regulates the intracellular trafficking and membrane distribution homeostasis of both receptors, and it is a consensus in the field that it can buffer aberrant VEGFR2 activation under basal conditions (74). Upon VEGFA binding, the formation of stable complexes between NRP1 and membrane-localized VEGFR2 is significantly enhanced. This process increases the phosphorylation and activation of VEGFR2 and its downstream ERK signaling, thereby synergistically amplifying VEGFA-mediated endothelial cell signal transduction and angiogenic sprouting (75,76). In addition to binding membrane receptors, NRP1 on endothelial cells can bind soluble VEGFR1, thereby finely regulating endothelial cell adhesion and migration (10).

Notably, similar amplification mechanisms have also been identified in other pathological models. In tumor and liver fibrosis models, VEGFA165 can enhance the stability of NRP1/VEGFR2 complexes on the surface of vascular endothelial cells and liver sinusoidal endothelial cells, respectively, amplify downstream intracellular signaling pathways, including ERK and PI3K-Akt, and drive pathological angiogenesis (77,78). On the other hand, the abnormal inhibition of this signaling axis will also lead to severe vascular network defects. For example, in a previous study, in a mouse model of neonatal exposure to diethylstilbestrol, the downregulation of VEGFR2 and its co-receptor NRP1 can directly cause uterine pathological angiogenic dysfunction and abnormal vascular maturation (79).

In the aforementioned bone defect, tumor and liver fibrosis models, endothelial NRP1 amplifies downstream signaling by stabilizing the VEGFA/VEGFR2 complex, and this core biochemical mechanism is highly conserved across models. However, the vascular phenotypes driven by this mechanism differ: It manifests as ordered reparative angiogenesis in bone defects, but as disordered pathological angiogenesis in tumors. This discrepancy suggests that the final functional output of the NRP1-VEGFR2 signaling axis is not linearly determined by a single mechanism, but may depend on the intensity and duration of signals in the local microenvironment. Specifically, the temporal expression of VEGFA in bone defects may contribute to the formation of a reparative vascular network, whereas persistent ligand stimulation in tumors may drive abnormal vascular proliferation. At present, the effect of this axis in mediating reparative angiogenesis in bone defects has been directly verified. Still, its functional switching mechanism under different pathological microenvironments remains to be further clarified in the context of fracture.

In addition to the VEGF signaling axis, the PDGF signaling axis plays an important role in mediating intercellular communication, pericyte recruitment, and maintenance of blood vessel homeostasis. Although all direct experimental evidence for the regulation of this signaling axis by NRP1 currently comes from non-skeletal systems, these findings provide a potential theoretical framework for elucidating the mechanisms of vascular maturation disorders during bone repair.

At the level of cellular behavior regulation, NRP1 is an important auxiliary molecule for mesenchymal stem cells (MSCs) to respond to PDGF signals. It is an essential condition for the complete phosphorylation of PDGFRα homodimers induced by PDGF-AA. It can partially enhance the activation level of PDGF-BB-mediated PDGFRβ homodimers, thereby regulating the migration and proliferation of MSCs (71). In addition, NRP1/PDGF also plays a crucial role in intercellular communications. NRP1 can function as a specific coreceptor

for PDGF-D, mediating trans-signaling between endothelial cells and pericytes to maintain pericyte coverage and, indirectly, regulate vascular homeostasis (33).

Taken together, NRP1 provides the molecular basis for regulating the entire angiogenesis process by integrating the VEGF and PDGF signaling axes. However, the spatiotemporal expression patterns of VEGF and PDGF and the modes of intercellular communication in the bone repair microenvironment are fundamentally different from those in existing non-skeletal system studies. Therefore, whether NRP1 can replicate these regulatory patterns in the *in situ* bone defect area to achieve orderly coordination of angiogenesis and maturation remains a key gap in this field. A summary of the strength and classification of evidence for NRP1-mediated bone angiogenesis regulation is presented in Table I.

4. Functions of NRP1 in regulating key cells involved in bone repair

Fracture repair relies on the synergistic action of osteoblasts, osteoclasts, chondrocytes and BMSCs. NRP1 is considered to participate in the signaling regulation of this cellular network potentially and to affect the proliferation, differentiation and maintenance of homeostasis of the aforementioned cells through direct and indirect pathways (17,44,80,81).

Osteoblasts. Osteoblasts are the primary executors of new bone formation, and existing studies suggest that NRP1 may regulate osteogenic differentiation and bone regeneration (17,82). Early *in vivo* research demonstrated that in the developing bones of mice and chick embryos, NRP1 was localized to osteoblasts migrating along blood vessels in the metaphyses, to osteoblasts at the edge of trabeculae in the marrow cavity, and to endothelial cells in bone tissue, which also widely expressed NRP1 (43). This expression pattern indicates that NRP1 may participate in skeletal development through dual pathways: Regulating osteoblast function and mediating angiogenesis (43).

In vitro experiments on BMSCs have shown that SEMA3A binds to NRP1 on the cell surface, promoting osteogenic differentiation, while inhibiting adipogenic differentiation by activating the canonical Wnt/β-catenin signaling pathway. This effect was further verified in a mouse cortical bone defect model, where the local application of recombinant SEMA3A significantly increased the proportion of osteoblasts in the defect area and accelerated bone regeneration and repair (17) (Fig. 3).

In addition to the canonical pathway mediated by SEMA3A, NRP1 can also regulate the osteogenic process by interacting with the collagen receptor discoidin domain receptor 2 (DDR2). *In vitro* preosteoblast models have demonstrated that NRP1 overexpression can significantly upregulate the mRNA and protein levels of osteogenic markers, enhance alkaline phosphatase activity and promote mineralized nodule formation. The underlying mechanism is that NRP1 co-localizes with DDR2 on the cell membrane and prolongs its half-life, thereby amplifying the downstream ERK1/2-Runt-related transcription factor 2 (Runx2) signaling cascade (82,83).

The aforementioned osteogenic regulatory mechanisms, which are of utmost significance under physiological conditions, are equally important under pathological conditions. NRP1 also exerts a protective effect in counteracting metabolic osteogenic inhibition. *In vitro* and *in vivo* research using diabetic pathological models has demonstrated that supplementation with SEMA3A can restore osteoblast activity via NRP1 and increase bone mineral density and bone mass (20). In addition, in *in vitro* osteogenic models of glucocorticoid-induced inhibition, the NRP1 pathway can antagonize glucocorticoid inhibition of osteoblasts (84).

Osteoclasts. Osteoclasts are the key functional cells responsible for bone resorption in the skeletal system, and their excessive activation is the central cause of pathological bone loss (85,86).

In a previous study using a mouse cortical bone defect model, tje local application of recombinant SEMA3A significantly reduced the proportion of osteoclasts in the defect area and inhibited bone resorption activity (17). *In vitro* experiments using primary bone marrow mononuclear cells further elucidated the molecular mechanism underlying this effect. Upon binding to NRP1 on the cell surface, SEMA3A was shown to reverse the receptor activator of nuclear factor κB ligand (RANKL)-induced downregulation of NRP1 expression. It promoted the formation of a stable complex between NRP1 and Plexin-A1 (17). This complex competitively blocked the interaction between Plexin-A1 and the triggering receptor expressed on myeloid cells 2-DNAX activation protein of 12 kDa (TREM2-DAP12) signaling complex, thereby inhibiting downstream immunoreceptor tyrosine-based activation motif (ITAM) signaling and nuclear factor of activated T-cells cytoplasmic 1 (NFATc1) transcriptional activation. Moreover, the SEMA3A-NRP1-Plexin-A1 pathway also inhibited RhoA GTPase activity and blocks the directional migration of osteoclast precursors to bone resorption sites. The synergistic action of these two independent pathways ultimately inhibits the maturation, differentiation and bone-resorptive function of osteoclasts (17), as illustrated in Fig. 3.

In addition to the canonical SEMA3A-mediated pathway that has been verified in bone defect models, the negative regulatory effect of NRP1 on osteoclasts has been further supported by *in vitro* research in other non-fracture models. At the functional level, small molecules such as wedelolactone have been confirmed to inhibit osteoclast differentiation via the NRP1-Plexin-A1 pathway (87). sNRP1 secreted by osteocytes can also directly inhibit osteoclast differentiation through a paracrine pathway (88). The research results from the aforementioned non-fracture models collectively suggest that NRP1 is a potential negative regulator of osteoclast differentiation and function and may play an important role in maintaining bone homeostasis. However, the specific role of this negative regulatory effect in the physiological bone remodeling process during fracture healing remains to be verified.

Chondrocytes. Chondrocytes are the key components that maintain the structural integrity of the callus and play a critical role in fracture healing. To date, to the best of our knowledge, there are no direct *in vivo* studies on the regulation of chondrocyte function by NRP1 during fracture healing.

In terms of non-fracture acute mechanical stress defense, a previous *in vitro* study using a high-magnitude cyclic

Table I. Strength and classification of evidence for NRP1-mediated bone angiogenesis regulation.

Evidence level	Model type	Cell type	Regulatory mechanism	Functional effect
Bone regeneration model	*In vitro* bone cell model + *in vivo* bone repair model	Endothelial cells	NRP1 acts as a VEGFR2 coreceptor, forming a VEGFA-NRP1-VEGFR2 ternary complex andactivating the PI3K-AKT pathway	Promotes endothelial migration and proliferation, accelerating angiogenesis in bone defect areas
Non-skeletal system	*In vitro* non-bone cell model + *In vivo* non-skeletal model + clinical research	Endothelial cells	SEMA3A competitively binds to NRP1 with VEGF165, inhibiting FAK/Src phosphorylation	Antagonistically regulates vascular sprouting; drives pathological angiogenesis in rheumatoid arthritis
Non-skeletal system	*In vitro* non-bone cell model	Endothelial tip cells	NRP1 mediates ECM-induced activation of the ABL1/ CDC42 pathway	Promotes directional migration of tip cells and vascular branching
Non-skeletal system	*In vitro* non-bone cell model	Endothelial cells	NRP1 binds to GIPC1, mediating the endocytic recycling of integrin α5β1	Enhances endothelial cell adhesion to fibronectin
Non-skeletal system	*In vitro* non-bone cell model + *in vivo* non-skeletal model	Endothelial cells	NRP1 maintains DDAH1 expression and activates the eNOS pathway	Inhibits endothelial cell apoptosis
Non-skeletal system	*In vitro* non-bone cell model	Endothelial cells	NRP1 promotes the binding of VE-cadherin to p120 catenin	Stabilizes endothelial adherens junctions and maintains barrier integrity
Non-skeletal system	*In vitro* non-bone cell model	Endothelial cells	RCαβ activates the NRP1-MET-RhoA signaling axis	Increases endothelial cell permeability
Non-skeletal system	*In vitro* non-bone cell model	Mesenchymal stem cells/Pericytes	PDGF-BB directly binds to NRP1 on the surface of mesenchymal stem cells	Drives the differentiation of mesenchymal stem cells into pericytes
Non-skeletal system	*In vivo* non-skeletal model	Pericytes	NRP1 deficiency disrupts basement membrane protein homeostasis	Impairs the integrity of the glomerular basement membrane
Non-skeletal system	*In vitro* non-bone cell model	Pericytes + Endothelial cells	Pericyte NRP1 captures VEGFA and presents it to endothelial VEGFR2, forming a transcellular complex	Regulates the homeostasis of vascular permeability
Non-skeletal system	*In vitro* non-bone cell model	Pericytes + Endothelial cells	PDGF-D binds to endothelial NRP1 and pericyte PDGFRβ to form a ternary complex	Maintains vascular pericyte coverage and regulates vascular homeostasis
Non-skeletal system	*In vivo* non-skeletal model	Pericytes + Endothelial cells	Vascular permeability depends on the ratio of NRP1 expression between pericytes and endothelial cells	Bidirectionally regulates vascular permeability
Non-skeletal system	*In vitro* non-bone cell model	Endothelial cells	VEGF-A enhances the stability of the NRP1-VEGFR2 complex and amplifies VEGFR2-Erk signaling	Promotes vascular sprouting
Non-skeletal system	*In vitro* non-bone cell model	Endothelial cells	NRP1 binds to soluble VEGFR1	Finely regulates endothelial cell adhesion and migration

Table I. Continued.

Evidence level	Model type	Cell type	Regulatory mechanism	Functional effect
Non-skeletal system	*In vivo* non-skeletal model	Endothelial cells	VEGF-A165 enhances the stability of the NRP1/VEGFR2 complex, amplifying downstream signaling	Drives pathological angiogenesis
Non-skeletal system	*In vivo* non-skeletal model	Endothelial cells	Downregulation of VEGFR2 and NRP1 expression	Leads to impaired uterine angiogenesis and abnormal vascular maturation

NRP1, neuropilin-1; SEMA3A, semaphorin 3A; VEGFA, vascular endothelial growth factor A; VEGFR2, VEGF receptor 2; ECM, extracellular matrix; PDGR, platelet-derived growth factor.

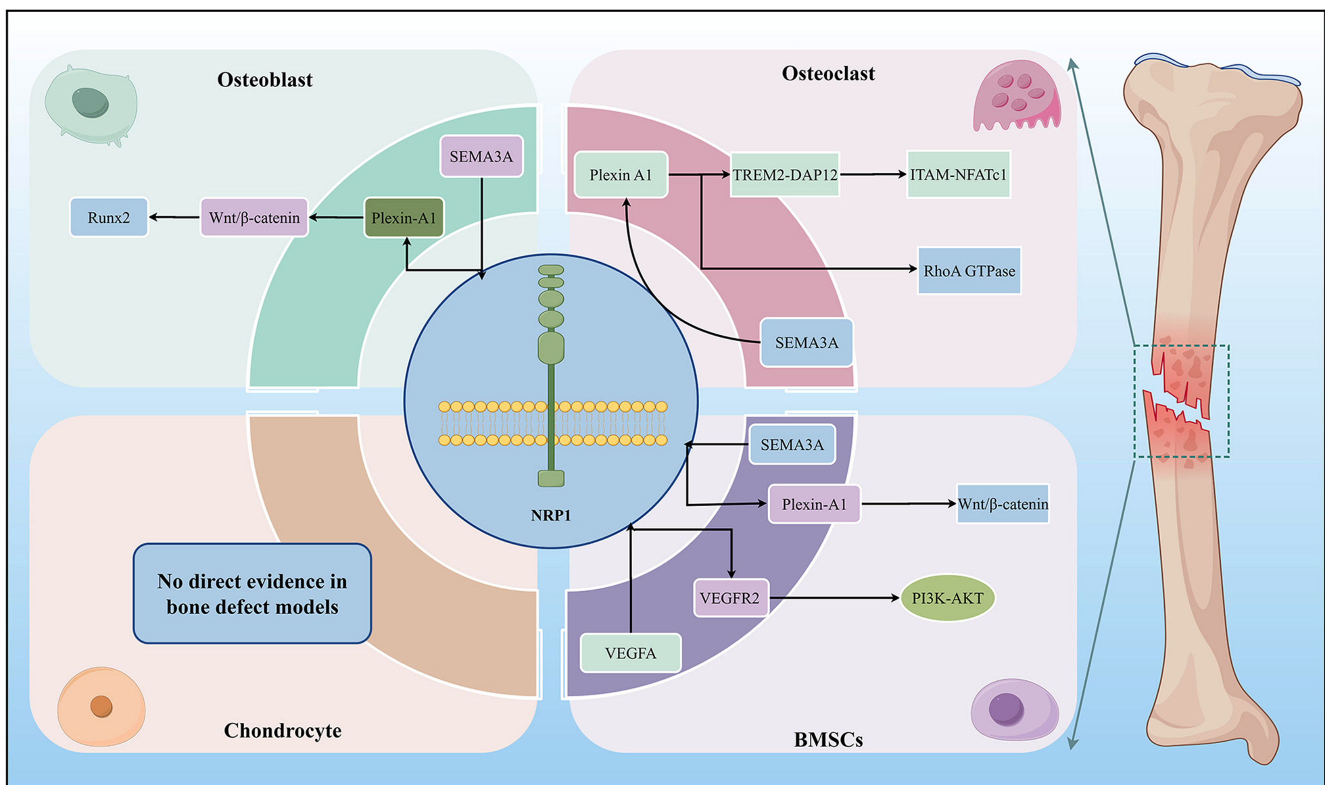

Figure 3. Schematic illustration of the molecular mechanisms by which NRP1, as a core signaling node, regulates the functions of osteoblasts, osteoclasts, BMSCs and chondrocytes during bone repair. At the osteoblast level, SEMA3A binds to the NRP1-Plexin-A1 receptor complex, activates the Wnt/β-catenin pathway, and upregulates Runx2 expression, promoting osteogenic differentiation and inhibiting adipogenic differentiation. At the osteoclast level, SEMA3A simultaneously blocks two independent downstream pathways through the NRP1-Plexin-A1 complex: it inhibits the TREM2-DAP12-ITAM-NFATc1 signaling axis to reduce osteoclast differentiation and maturation. It suppresses RhoA GTPase activity to block the directional migration of osteoclast precursors, ultimately inhibiting bone resorption. At the BMSC level, the SEMA3A-NRP1-Plexin-A1 pathway activates Wnt/β-catenin signaling to promote osteogenic differentiation; VEGFA forms a ternary complex with NRP1 and VEGFR2, activating the PI3K-AKT pathway to promote BMSC migration. All the above regulatory mechanisms in osteoblasts, osteoclasts, and BMSCs have been directly validated in bone repair models. There is currently no direct experimental evidence in bone repair models for NRP1-mediated functional regulation in chondrocytes. NRP1, neuropilin-1; BMSCs, bone marrow mesenchymal stem cells; SEMA3A, semaphorin 3A; VEGFA, vascular endothelial growth factor A; VEGFR2, VEGF receptor 2; Runx, runt-related transcription factor 2; TREM2, triggering receptor expressed on myeloid cells 2; DAP12, DNAX activation protein of 12 kDa; ITAM, immunoreceptor tyrosine-based activation motif; NFATc1, nuclear factor of activated T cells cytoplasmic 1.

tensile strain model of ATDC5 chondrocytes suggested that excessive mechanical stress downregulated endogenous SEMA3A expression in chondrocytes, while, as a compensation, it upregulated its co-receptor NRP1 and signaling receptor Plexin-A1 on the chondrocyte surface (89). The exogenous supplementation of SEMA3A was found to bind to the aforementioned upregulated receptor complex, inhibit the excessive activation of the AKT, ERK and NF-κB pathways, and reduce the production of pro-inflammatory cytokines and matrix-degrading enzymes, thereby protecting the chondrocyte

extracellular matrix (89). The results from this *in vitro* acute mechanical stress model indicate that the SEMA3A-NRP1 signaling axis exerts a chondroprotective effect under acute mechanical stress, providing a reference framework for understanding the mechanism by which early fracture cartilaginous callus resists abnormal stress. However, given the differences in phenotype and mechanical microenvironment between the ATDC5 cell line and *in vivo* cartilaginous callus chondrocytes, the actual role of this mechanism in fracture healing remains to be directly verified *in vivo*.

By sharp contrast, NRP1 exerts an opposite regulatory effect in chronic inflammatory microenvironments of cartilage degeneration, such as osteoarthritis. SEMA3A and NRP1 mRNA are synchronously upregulated in knee chondrocytes from patients with osteoarthritis, and their expression levels are closely associated with the progression of cartilage degeneration (44,80). Further *in vitro* studies using inflammatory chondrocyte models have demonstrated that interleukin (IL)-1β and TNF-α coordinately upregulate SEMA3A and NRP1 expression in chondrocytes via the CCAAT/enhancer-binding protein β transcriptional pathway. Overactivated SEMA3A-NRP1 signaling inhibits the PI3K-AKT pro-survival pathway in an NRP1-dependent manner, driving chondrocytes to shift toward a catabolic phenotype and increasing apoptosis (80). Moreover, this signaling axis specifically upregulates matrix metalloproteinase 13, a key enzyme in cartilage matrix degradation and accelerates type II collagen degradation (44). *In vitro* intervention studies have further confirmed this pro-degenerative effect: exosomal miR-485-3p derived from synovial mesenchymal stem cells can target and silence NRP1 mRNA in chondrocytes, correct the abnormality of the PI3K-AKT pathway, reduce chondrocyte apoptosis, and restore the expression of anabolic markers (90).

The research results from the aforementioned *in vitro* acute mechanical stress model and chronic inflammatory model of osteoarthritis reveal a microenvironment-dependent bidirectional regulatory pattern of chondrocytes in non-fracture scenarios: NRP1 exerts a chondroprotective effect under acute mechanical stress, whereas it may drive cartilage degeneration in a chronic inflammatory microenvironment. Since both effects are derived from non-fracture models, they cannot be directly extrapolated to the cartilaginous callus during fracture healing. Therefore, targeted interventions against NRP1 may carry the risk of opposite effects, and this functional uncertainty is a key issue that urgently needs to be addressed in research on NRP1 in bone repair.

BMSCs. In an ischemic bone defect model, exogenous NRP1 loaded on 3D-printed magnesium alloy scaffolds can form a synergistic regulatory axis with sustained-release magnesium ions and exert effects through two independent pathways: In addition to activating the endothelial NRP1-VEGFR2-PI3K-AKT pathway to promote angiogenesis as described above, it can also significantly enhance the migration ability of BMSCs through the same signaling axis (46). Beyond VEGF signaling, NRP1 can also function as a functional co-receptor for SEMA3A to regulate the differentiation fate of BMSCs through a completely distinct downstream pathway. Hayashi *et al* (17) confirmed that this signaling axis exerts dual bone-protective effects, promoting osteogenic differentiation. It inhibits adipogenic differentiation in BMSCs and suppresses the differentiation and function of osteoclast precursors. The promoting effect on bone regeneration was further verified in a mouse cortical bone defect model (17) (Fig. 3).

In addition to the regulation of angiogenesis and osteogenic differentiation, which have been verified in the aforementioned bone defect models, the majority of the other functions of NRP1 in BMSCs and their upstream regulatory mechanisms are derived from studies outside the fracture-healing system. At the molecular mechanism level of osteogenic differentiation, Shi *et al* (13) further refined the SEMA3A-NRP1-Wnt pathway through *in vitro* experiments using mouse BMSCs. They found that activated Wnt/β-catenin can, in turn, transcriptionally upregulate NRP1 expression, forming a 'SEMA3A-NRP1-Wnt/β-catenin-NRP1' positive feedback loop that continuously amplifies the osteogenic effect (13). At the level of maintaining aging-related functions, research using a mouse model of aging-related bone loss demonstrated that pulsed electromagnetic fields can induce sensory nerves to secrete SEMA3A. After binding to NRP1 on the surface of $LepR^+$ BMSCs, SEMA3A restores their osteogenic potential and antagonizes cellular senescence (91).

In addition, NRP1 expression is also finely regulated by the non-coding RNA network. In models of periodontitis, miR-148a can target and silence NRP1 in periodontal ligament stem cells, thereby inhibiting their osteogenic differentiation (92). In models of osteoporosis, long non-coding RNA (lncRNA) MALAT1 indirectly upregulates NRP1 expression in BMSCs by competitively sponging miR-320a, thereby promoting their osteogenic differentiation (93).

Collectively, only two effects of NRP1 in BMSCs, namely vascular migration and osteogenic differentiation, have been directly verified in bone defect models. The remaining regulatory mechanisms, including the Wnt-NRP1 positive feedback loop, the anti-aging effect of neurogenic SEMA3A, and the post-transcriptional regulation by non-coding RNAs, are all derived from *in vitro* cell experiments or non-bone injury systems, such as periodontitis and osteoporosis, and their mode of action in fracture healing remains unclear. The strength and classification of evidence for the NRP1 regulation of key bone repair cell functions is summarized in Table II.

5. NRP1-mediated neuro-bone crosstalk in bone tissue

The mammalian skeleton is a highly innervated, dynamic organ. The nervous system not only mediates skeletal nociception, but also serves as a key regulator of skeletal development, injury repair and homeostasis maintenance (94-97). Of note, the majority of current studies on the role of NRP1 in the aforementioned neuro-bone regulation are derived from neural development models and bone homeostasis maintenance models, and no direct *in vivo* functional verification has been obtained in the context of fracture healing, at least to the best of our knowledge.

It has been elucidated in the field of neurobiology that SEMA3A can induce growth cone collapse via its primary receptor, NRP1, forcing axons to avoid SEMA3A-enriched regions, which constitutes the core molecular basis for its regulation of precise neural projection (16,98-100). The underlying regulatory mechanism has been clarified in *in vitro*

Table II. Strength and classification of evidence for NRP1 regulation of key bone repair cell functions.

Evidence level	Model type	Cell type	Regulatory mechanism	Functional effect
Bone repair model	*In vitro* bone cell model + *in vivo* bone repair model	Osteoblasts	SEMA3A-NRP1 activates Wnt/β-catenin pathway	Promotes osteogenesis, inhibits adipogenesis, and accelerates bone regeneration
Bone repair model	*In vitro* bone cell model + *in vivo* bone repair model	Osteoclasts	SEMA3A-NRP1-Plexin-A1 blocks TREM2-DAP12-NFATc1 pathway	Inhibits osteoclast differentiation and bone resorption
Bone repair model	*In vitro* bone cell model + *in vivo* bone repair model	Osteoclasts	SEMA3A-NRP1-Plexin-A1 inhibits RhoA GTPase activity	Inhibits the directional migration of osteoclast precursors
Bone repair model	*In vitro* bone cell model + *in vivo* bone repair model	BMSCs	NRP1 synergizes with magnesium ions to amplify the VEGFA-VEGFR2-PI3K-AKT pathway	Promotes BMSC migration and drives angiogenesis
Bone repair model	*In vitro* bone cell model + *in vivo* bone repair model	BMSCs	SEMA3A-NRP1 activates Wnt/β-catenin pathway	Promotes BMSC osteogenesis, inhibits adipogenesis, and provides dual bone protection
Bone biology field	*In vitro* bone cell model	Osteoblasts	NRP1 extends DDR2 half-life and amplifies the ERK1/2-Runx2 pathway	Enhances osteogenic differentiation and mineralization
Bone biology field	*In vitro* bone cell experiment + *in vivo* non-bone-repair model	Osteoblasts	SEMA3A mediates signaling through NRP1	Restores osteogenic activity and antagonizes metabolic osteogenic inhibition
Bone biology field	*In vitro* bone cell model	Osteoblasts	Activation of NRP1-related signaling pathways	Antagonizes glucocorticoid-induced osteogenic inhibition
Bone biology field	*In vitro* bone cell model	Osteoclasts	Wedelolactone acts through the NRP1-PlexinA1 pathway	Inhibits osteoclast differentiation
Bone biology field	*In vitro* bone cell model	Osteoclasts	Soluble NRP1 exerts a paracrine effect on osteoclast precursors	Directly inhibits osteoclast differentiation
Bone biology field	*In vitro* bone cell model	Chondrocytes	Exogenous SEMA3A binds to NRP1/Plexin-A1, inhibiting AKT/ERK/NF-κB pathways	Reduces matrix degradation and protects cartilage matrix
Bone biology field	*In vitro* bone cell model + Clinical research	Chondrocytes	IL-1β/TNF-α upregulates SEMA3A/NRP1, inhibits PI3K/AKT, and upregulates MMP13	Promotes chondrocyte apoptosis and matrix degradation, driving cartilage degeneration
Bone biology field	*In vitro* bone cell model	Chondrocytes	miR-485-3p targets and silences NRP1 in chondrocytes	Reduces chondrocyte apoptosis
Bone biology field	*In vitro* bone cell model	BMSCs	Forms a SEMA3A-NRP1-Wnt/β-catenin-NRP1 positive feedback loop	Continuously amplifies the osteogenic effect
Bone biology field	*In vitro* bone cell model	BMSCs	Neurogenic SEMA3A binds to NRP1 on the surface of LepR+ BMSCs	Restores BMSC osteogenic potential and antagonizes cellular senescence
Bone biology field	*In vitro* bone cell model	BMSCs	miR-148a targets and silences NRP1 in periodontal ligament stem cells (PDLSCs)	Inhibits osteogenic differentiation of periodontal ligament stem cells

Table II. Continued.

Evidence level	Model type	Cell type	Regulatory mechanism	Functional effect
Bone biology field	*In vitro* bone cell model	BMSCs	lncRNA MALAT1 sponges miR-320a, indirectly upregulating NRP1	Promotes BMSC osteogenic differentiation

NRP1, neuropilin-1; SEMA3A, semaphorin 3A; VEGFA, vascular endothelial growth factor A; VEGFR2, VEGF receptor 2; ECM, extracellular matrix; PDGR, platelet-derived growth factor.

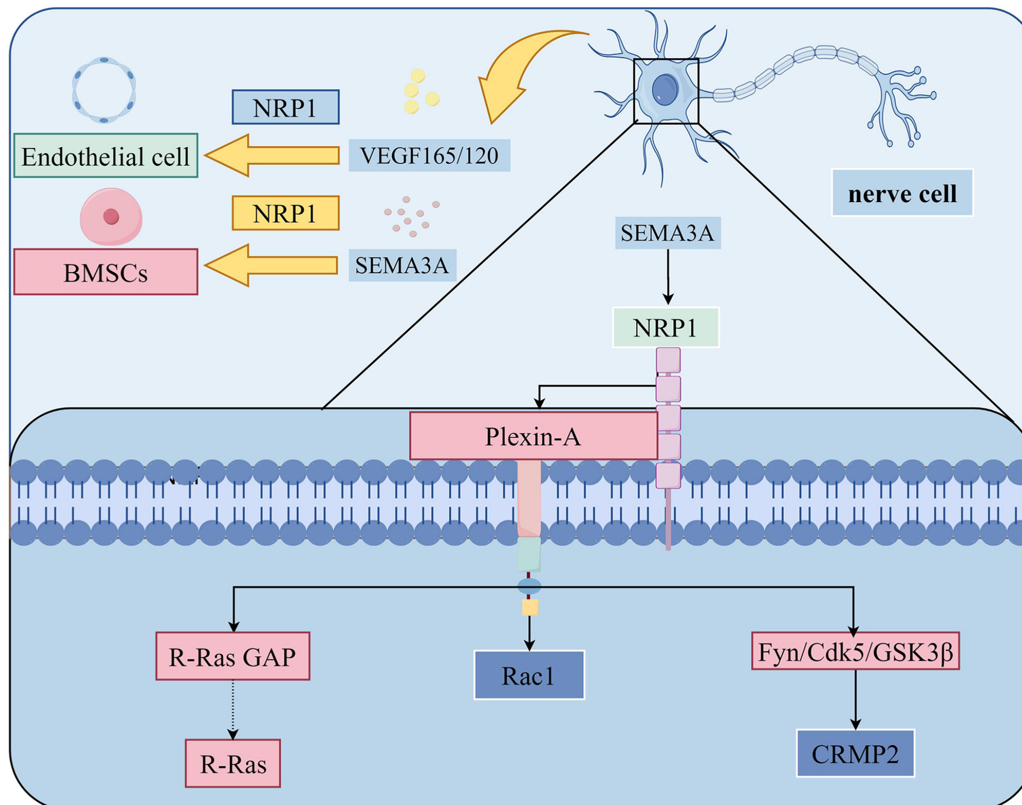

Figure 4. Schematic illustration of the potential molecular mechanisms by which NRP1, as a key co-receptor of SEMA3A, mediates neuro-osteogenic crosstalk and axon guidance during fracture repair. Sensory neurons secrete two classes of regulatory factors: SEMA3A acts on BMSCs through the Wnt/β-catenin/NRP1 positive feedback loop to promote their osteogenic differentiation, while VEGF165/120 induces endothelial cells to express NRP1 and guides blood vessels to grow along nerve fibers. In terms of axon guidance, SEMA3A binds to the NRP1-Plexin-A receptor complex on the surface of neuronal growth cones and activates three independent downstream pathways: Rac1-mediated plasma membrane endocytosis and actin cytoskeleton rearrangement induce growth cone collapse; Fyn-Cdk5-GSK3β cascade-mediated phosphorylation of CRMP2 inhibits microtubule assembly; and R-Ras GAP activity-mediated inhibition of integrin adhesion enhances axonal repulsion. NRP1, neuropilin-1; BMSCs, bone marrow mesenchymal stem cells; SEMA3A, semaphorin 3A; CRMP2; collapsin response mediator protein 2; R-Ras GAP, R-Ras GTPase-activating protein.

axon guidance models: NRP1 must form a functional receptor complex with Plexin-A, and the intracellular domain of Plexin-A transduces downstream signals (99). This complex induces repulsive responses mainly through three synergistic pathways: i) It activates Rac1 to drive growth cone plasma membrane endocytosis and actin cytoskeleton rearrangement, directly inducing growth cone collapse; ii) it phosphorylates collapsin response mediator protein 2 through the Fyn-Cdk5-GSK3β kinase cascade, inhibiting microtubule assembly to block axon extension; third, it inhibits integrin-mediated cell adhesion via the R-Ras GAP activity of Plexin-A, synergistically enhancing the repulsive effect (99) as illustrated in Fig. 4.

Studies on classical bone development and bone homeostasis have confirmed that neuronal paracrine SEMA3A is a key functional isoform that maintains bone homeostasis under physiological conditions, and that its effects are mediated through NRP1, a key receptor (14,98). The study by Fukuda *et al* (14), first elucidated the central role of NRP1 in this regulation. Neuron-derived SEMA3A specifically regulates the projection and innervation density of CGRP-positive sensory nerves by binding to the NRP1-Plexin-A4 complex on intraosseous sensory nerve endings, thereby maintaining normal intraosseous innervation and indirectly regulating bone homeostasis (14,101). CGRP-positive sensory nerves

have been further confirmed to participate in the regulation of bone repair by secreting CGRP (102).

In addition to the indirect regulation of innervation, a recent study (13) on bone homeostasis have further revealed the direct regulatory effect of neurogenic SEMA3A on BMSCs via NRP1. Specifically, in that study, in a mouse tibial denervation model, the loss of sensory innervation led to the synchronous downregulation of SEMA3A and NRP1 in bone, accompanied by the impaired osteogenic capacity of BMSCs. *In vitro* co-culture experiments further confirmed that neurogenic SEMA3A secreted by dorsal root ganglia directly promoted the osteogenic differentiation of BMSCs through the SEMA3A-NRP1-Wnt/β-catenin pathway elucidated above (13). Previous research on disuse osteoporosis in rats further validated this mechanism *in vivo*. Following sciatic nerve transection, the expression of Sema3A and NRP1 in bone tissue was synchronously downregulated, which inhibited the activity of Wnt/β-catenin signaling, resulting in decreased osteogenic activity and abnormal osteoclast activation, and subsequently led to bone loss and destruction of bone microstructure (103) (Fig. 4).

In addition to directly regulating the function of BMSCs, nerves may indirectly regulate skeletal homeostasis by guiding vascular differentiation and spatial distribution. Research has confirmed that peripheral nerves serve as a template for the differentiation and spatial distribution of cutaneous arteries (104). Sensory neurons and Schwann cells can secrete VEGF164/120, induce endothelial cells to express arterial-specific markers, such as NRP1 and ephrinB2, and guide blood vessels to grow along nerve branches. The loss of sensory nerves or Schwann cells prevents cutaneous small blood vessels from inducing the expression of arterial markers, such as NRP1, resulting in significantly impaired arterial differentiation (104). Given that bone tissue is also densely populated with sensory nerve fibers, this 'neuro-vascular' template mechanism may also apply to the spatial configuration of the intraosseous vascular network. Still, its actual role in bone tissue and fracture healing remains to be experimentally verified (Fig. 4). The strength and classification of evidence for NRP1-mediated neuro-osseous crosstalk is summarized in Table III.

6. Dysregulated expression of NRP1 in pathological states of bone tissue

Systemic pathological conditions directly associated with impaired fracture healing. Diabetes mellitus and osteoporosis included in this section are both clinically recognized independent high-risk factors for delayed fracture union and nonunion, and belong to systemic pathological conditions directly related to impaired fracture healing. Existing experimental evidence suggests that the dysregulated expression and function of NRP1, driven by systemic pathological states, may be a potential mechanism mediating the imbalance in osteoclast-osteoblast homeostasis and poor fracture healing. However, the causal association between the dysregulated expression of NRP1 in the aforementioned pathological states and delayed fracture union has not yet been established.

Diabetes mellitus. Diabetes mellitus is an independent high risk factor for delayed fracture union and nonunion. Its core pathological mechanisms involve the accumulation of advanced glycation end products induced by hyperglycemia, the excessive production of reactive oxygen species, amplified chronic inflammation and abnormal insulin signaling. These factors collectively lead to increased apoptosis and inhibited osteoblast differentiation, excessive osteoclast activation, and impaired angiogenesis and innervation (105). Recent studies (106,20) have shown that the SEMA3A/NRP1 signaling axis may be a potential regulator of the aforementioned pathological processes.

Qiao *et al* (106) found that, in a rat model of type 2 diabetes mellitus, endogenous SEMA3A expression in BMSCs was significantly decreased, and exogenous supplementation with recombinant SEMA3A upregulated osteogenesis-related gene expression and restored the mineralization capacity of the cells (106). Subsequent the in-depth study focusing on osteoblasts by Zhang *et al* (20) further elucidated the molecular mechanisms underlying osteogenic inhibition in a high glucose environment. Specifically, *in vitro* experiments on osteoblasts by Zhang *et al* (20) confirmed that high glucose can downregulate the expression of SEMA3A and NRP1 in osteoblasts in a concentration-dependent manner, and inhibit the expression of core osteogenic markers, such as ALP and Runx2, as well as the formation of mineralized nodules. Exogenous supplementation with SEMA3A can significantly reverse the high-glucose-induced inhibition of osteogenic differentiation via the SEMA3A-NRP1-Wnt/β-catenin pathway. *In vivo* experiments using mice with streptozotocin-induced diabetes further verified that the downregulation of the SEMA3A/NRP1 axis was accompanied by bone loss, destruction of trabecular bone microstructure, and imbalance of bone turnover homeostasis, and exogenous SEMA3A intervention effectively ameliorated the abnormalities in bone mineral density, bone microstructure, and bone metabolic markers in mice (20).

Collectively, the inhibition of the SEMA3A/NRP1 signaling axis by a high-glucose environment is one of the potential regulatory mechanisms of diabetic bone loss, and restoring its activity can improve osteogenic function and bone structure in mice with diabetes. However, whether this mechanism mediates the impaired healing of fractures complicated by diabetes mellitus and whether targeted intervention can promote the healing of diabetic fractures remains to be further verified.

Osteoporosis. A previous study demonstrated that, in the bone tissue of ovariectomized rats with osteoporosis, the expression of lncRNA MALAT1 was significantly downregulated, while the expression of miR-320a was abnormally elevated (93). Under physiological conditions, MALAT1 in BMSCs sequestered miR-320a via the competing endogenous RNA (ceRNA) mechanism, relieving its inhibitory effect on the SEMA3A-NRP1-Wnt/β-catenin pathway. This regulatory balance was shown to be disrupted in osteoporosis, leading to the excessive activation of miR-320a, which ultimately impeded the osteogenic differentiation of BMSCs and resulted in insufficient bone formation (93).

In osteoclastogenesis, miR-148a in osteoclast precursor cells is a key positive regulator of osteoclast differentiation, and its expression is significantly upregulated in the serum of patients with postmenopausal osteoporosis and in the bone tissue of ovariectomized mice with osteoporosis. miR-148a

Table III. Strength and classification of evidence for NRP1-mediated neuro-osseous crosstalk.

Evidence level	Model type	Tissue and cell type	Regulatory mechanism	Functional effect
Bone biology field	*In vivo* non-bone-repair model	Intraosseous sensory nerve terminals	Neuronal SEMA3A binds to intraosseous sensory nerve NRP1-Plexin-A4	Regulates the projection density of intraosseous sensory nerves, indirectly maintaining bone homeostasis
Bone biology field	*In vivo* bone cell model + *in vivo* non-bone-repair model	BMSCs	Sensory nerve SEMA3A acts on BMSCs through the Wnt/β-catenin/ NRP1 positive feedback loop	Promotes BMSC osteogenic differentiation
Non-skeletal system	*In vivo* non-bone cell model + *in vivo* non-skeletal model	Neuronal axonal growth cones	SEMA3A-NRP1-Plexin-A activates Rac1, driving plasma membrane endocytosis and actin rearrangement	Directly induces growth cone collapse
Non-skeletal system	*In vivo* non-bone cell model + *in vivo* non-skeletal model	Neuronal axonal growth cones	SEMA3A-NRP1-Plexin-A phosphorylates CRMP2 through Fyn-Cdk5-GSK3β	Inhibits microtubule assembly and blocks axon extension
Non-skeletal system	*In vivo* non-bone cell model + *in vivo* non-skeletal model	Neuronal axonal growth cones	SEMA3A-NRP1-Plexin-A inhibits integrin adhesion through R-Ras GAP activity	Enhances the axonal repulsion effect and regulates precise neural projection
Non-skeletal system	*In vivo* non-skeletal model	Sensory nerves	Sensory neurons/Schwann cells secrete VEGF164/120, inducing endothelial expression of arterial markers such as NRP1	Guides blood vessels to grow along nerves, regulating the differentiation and distribution of skin arteries

NRP1, neuropilin-1; SEMA3A, semaphorin 3A; VEGFA, vascular endothelial growth factor A; VEGFR2, VEGF receptor 2; ECM, extracellular matrix; PDGR, platelet-derived growth factor.

can directly target and bind to the 3' untranslated region of NRP1 and inhibit its protein expression, thereby relieving the negative regulation of NRP1 on the NFATc1/c-Fos signaling axis, further excessively promoting the differentiation and maturation of osteoclasts and their bone resorption function, and ultimately exacerbating estrogen deficiency-induced bone loss (19).

In addition, increased osteocyte apoptosis is another pathological feature of postmenopausal and age-related osteoporosis, and NRP1 on the surface of mature osteocytes plays a key regulatory role in this process (107). Under physiological conditions, estrogen upregulates SEMA3A expression by inhibiting miR-497/195. After binding to NRP1 on the surface of osteocytes, SEMA3A activates the mature osteocyte-specific soluble guanylate cyclase-cyclic guanosine monophosphate-protein kinase G pathway and inhibits osteocyte apoptosis. Estrogen deficiency leads to the inactivation of this protective axis, increased osteocyte apoptosis and aggravated bone loss (107).

All the aforementioned regulatory associations are based on simple models of osteoporosis, revealing the role of NRP1 in regulating bone homeostasis through multicellular pathways. However, to the best of our knowledge, there is currently no evidence to indicate that it exerts the same effect in the complex scenario of osteoporosis complicated with fracture, and whether it mediates impaired fracture healing in such patients remains to be verified.

Other bone metabolic disorders with mechanistic reference significance. The following pathological states differ significantly from fracture nonunion in terms of pathogenesis and pathological nature. Relevant studies cannot be used directly as evidence of impaired fracture healing; however, they can provide a reference for understanding the functional complexity of NRP1 in regulating bone metabolism.

Infectious osteolysis. In *Porphyromonas gingivalis* lipopolysaccharide (P-LPS)-induced infectious osteolysis, the Toll-like receptor 4 pathway on the surface of osteoclast

precursors and osteoblast precursors is specifically activated by P-LPS. This pathway can significantly downregulate the expression of the SEMA3A/NRP1 axis in both cell types. This regulatory imbalance disrupts bone homeostasis in a bidirectional manner. It not only promotes the differentiation and the activation of osteoclasts and their bone-resorptive function, but also inhibits the proliferation, differentiation and mineralization of osteoblasts. These changes eventually induce progressive bone loss. Exogenous supplementation with recombinant SEMA3A can simultaneously reverse the excessive osteoclast activation and osteogenic dysfunction described above and significantly alleviate P-LPS-mediated osteolysis (108). It should be clearly stated that this infection model is characterized by chronic, persistent inflammation-driven pathological bone destruction, which differs fundamentally from the temporally controlled physiological bone remodeling process during fracture healing. Its core reference value lies in suggesting that NRP1 may play a bone-protective role in inflammation-driven bone loss. This also provides a potential research direction for the subsequent exploration of the molecular mechanism of delayed fracture healing under infectious conditions.

Genetic association between NRP1 gene variants and bone metabolic homeostasis. At the genetic level, research on the Chinese Han population has demonstrated that mutations in the NRP1 rs2070296 and rs180868035 loci are significantly associated with increased osteoporosis risk and decreased bone mineral density. Among these, the missense mutation NRP1 rs180868035 can directly disrupt SEMA3A binding to NRP1 and disrupt bone metabolic homeostasis by inhibiting osteoblast proliferation and differentiation and promoting osteoclast activation (109). This genetic finding provides population-level evidence of the role of NRP1 in maintaining bone homeostasis. However, the association between these findings and fracture healing remains speculative, and there is currently no research data available (to the best of our knowledge) on the association between these gene loci and fracture-healing phenotypes. Therefore, this finding can only provide population-level reference for the study of bone repair mechanisms, and cannot be directly used as evidence for the association between NRP1 dysregulation and impaired fracture healing.

Periprosthetic osteolysis (PPO) and the microenvironment-dependent functional difference phenomenon. Clinical specimens and *in vitro* experiments have shown that multinucleated osteoclast-like cells phagocytosing wear particles in PPO lesions exhibit a high expression of NRP1 and its ligand SEMA3A (110). During the process of wear particle-induced osteoclast differentiation, the expression of NRP1 is continuously upregulated with cell maturation, and wear particles can further amplify this effect (110). In the aforementioned pathological environment of PPO, the highly expressed NRP1 fails to effectively inhibit osteoclast bone resorption, leading instead to persistent pathological bone destruction. This result constitutes a clear functional difference from the aforementioned conclusion that NRP1 negatively regulates osteoclast differentiation in bone homeostasis models (110).

Combined with the known characteristics of NRP1 signaling pathways, the aforementioned phenomenon may be mediated by dual mechanisms. First, the chronic foreign-body inflammatory environment may specifically downregulate Plexin-A1 expression in osteoclast precursor cells. By contrast, the osteoclast inhibitory effect of SEMA3A depends on the functional complex formed by NRP1 and Plexin-A1 (17). Therefore, the high expression of NRP1 alone may not effectively transduce inhibitory signals. Second, a large amount of VEGFA165 and PDGF-BB produced under the stimulation of wear particles can competitively occupy the b1/b2 binding sites of NRP1 (48), which not only blocks the binding of SEMA3A, but also may turn to activate pro-osteoclast signals. All the aforementioned mechanisms are reasonable speculations based on existing evidence and remain to be directly verified by subsequent experiments.

It can be seen that the dissociation between NRP1 expression and function in PPO is not a mere experimental discrepancy, but directly reflects the high microenvironment-dependent nature of NRP1 function. Whether it ultimately exerts a bone-protective or bone-destructive effect may be jointly determined by the microenvironmental signal background, such as the type of local inflammation, the co-receptor expression profile and the abundance of competitive ligands, rather than a simple linear correlation with its expression level. This is precisely a key scientific issue worthy of in-depth exploration in the current field of NRP1 in bone metabolism. The strength and classification of evidence for NRP1 dysregulation in pathological bone states is summarized in Table IV.

7. Translational prospects and challenges of NRP1 as a therapeutic target for bone repair

NRP1 is involved in the interactive regulation of nerves, blood vessels and bone, and has potential mediating value in maintaining bone homeostasis and repairing injuries. Given the widespread expression of NRP1 across multiple systemic tissues, systemic intervention may entail substantial off-target risks and an uncertain safety profile; thus, it is critical to explore effective and precise intervention strategies (111-113).

Intervention strategies validated in bone regeneration-related models

Targeted activation strategy of the SEMA3A-NRP1 signaling axis. As the canonical ligand of NRP1, the targeted activation strategy of SEMA3A has been directly validated in mouse cortical bone defect models, which simultaneously achieve dual bone-protective effects by inhibiting bone resorption and promoting bone formation (17). Mechanistically, its osteoclast-inhibitory effect has been clarified through the dual mechanisms described above. Moreover, it can promote osteogenic differentiation and block the adipogenic transformation of MSCs via the FARP2-Rac1-Wnt/β-catenin pathway, and all effects are strictly dependent on the Sema-binding domain of NRP1 (17). Both SEMA3A knockout mice and NRP1-SEMA3A-binding site mutant knock-in mice exhibit severe bone mass reduction phenotypes, genetically confirming that this axis is a key regulatory pathway for maintaining bone homeostasis. The local or systemic administration of recombinant SEMA3A can significantly accelerate the repair of mouse cortical bone defects and reverse postmenopausal bone loss, providing direct *in vivo* evidence for the application of NRP1 agonist-based interventions in bone regeneration (17).

Table IV. Strength and classification of evidence for NRP1 dysregulation in pathological bone states.

Pathological state classification	Pathology type	Evidence level	Model type	Molecular mechanism	Degree of association with fracture healing
Systemic pathological states directly related to bone repair impairment	Diabetes	Bone biology field	*In vitro* bone cell experiment + *in vivo* non-bone-repair model	High glucose inhibits the SEMA3A/NRP1-Wnt/β-catenin axis, suppressing osteogenesis and promoting osteoclastogenesis	High-risk factor for fracture
Systemic pathological states directly related to bone repair impairment	Osteoporosis	Bone biology field	*In vitro* bone cell experiment + *in vivo* non-bone-repair model	BMSCs: miR-320a upregulation inhibits NRP1/β-catenin, impeding osteogenesis; osteoclast precursors: miR-148a upregulation silences NRP1, promoting osteoclastogenesis; osteocytes: Estrogen deficiency inactivates the SEMA3A/NRP1 pathway, increasing osteocyte apoptosis	High-risk factor for fracture
Bone metabolic disorders with mechanistic reference significance	Infectious osteolysis	Bone biology field	*In vitro* bone cell experiment + *in vivo* non-bone-repair model	P-LPS activates the TLR4 pathway, downregulating the SEMA3A/NRP1 axis in osteoblasts/osteoclasts, bidirectionally disrupting bone homeostasis	Different pathological nature, provides mechanistic reference only
Bone metabolic disorders with mechanistic reference significance	NRP1 gene mutation	Bone biology field	*In vitro* bone cell experiment + clinical samples	Mutations like rs180868035 disrupt SEMA3A-NRP1 binding, inhibiting osteogenesis and promoting osteoclastogenesis	Different pathological nature, provides mechanistic reference only
Bone metabolic disorders with mechanistic reference significance	Periprosthetic osteolysis (PPO)	Bone biology field	*In vitro* bone cell experiment + clinical samples	Wear particles induce high expression of NRP1 in osteoclast-like cells, which lose their osteoclast-inhibitory function and paradoxically promote bone destruction	Different pathological nature, provides mechanistic reference only

NRP1, neuropilin-1; SEMA3A, semaphorin 3A; VEGFA, vascular endothelial growth factor A; VEGFR2, VEGF receptor 2; P-LPS, *Porphyromonas gingivalis* lipopolysaccharide; TLR4, Toll-like receptor 4.

However, the study by Hayashi *et al* (17) still has certain limitations. The core experiments are all based on mouse models. Although the effects have been preliminarily verified in human-derived cells, there is a lack of long-term efficacy and safety data from large animals. Recombinant SEMA3A has a short half-life and poor bone targeting, and systemic administration may cause off-target effects. In addition, the study only evaluated the short-term administration effects and lacked long-term safety monitoring data.

Local delivery of NRP1 based on functionalized scaffolds. To address the bottlenecks of the widespread systemic expression of NRP1, leading to off-target effects and the easy degradation of recombinant proteins, the local delivery system of liposome-encapsulated NRP1 loaded onto 3D-printed

magnesium alloy scaffolds has been fully validated in ischemic bone defect models. It is currently one of the research strategies with preliminary exploratory value in bone regeneration. This system achieves stable, sustained release of NRP1 via a composite coating and synergizes with the osteogenic effect of magnesium ions to enhance local angiogenesis via the NRP1-VEGFR2-PI3K-AKT pathway, ultimately realizing synergistic repair of the angiogenesis-osteogenesis coupling (46). This strategy can improve the local bioavailability of NRP1 without obvious systemic adverse reactions, effectively avoiding the off-target risks of systemic interventions, and has potential translational value in clinical scenarios such as ischemic bone injury and fracture nonunion.

However, the study described above (46) still has certain limitations. The animal experiments are based solely on rabbit ischemic bone defect models and lack long-term efficacy and safety verification in large animals. In addition, this strategy conducted only a 12-week short-term safety monitoring period, and the long-term systemic safety and metabolic effects of degradation products remain to be investigated.

Intervention strategies with biological rationality but not yet validated in fracture models. The following intervention strategies have all been mechanistically validated in non-fracture systems, and their efficacy in bone repair remains to be confirmed *in vivo*.

Targeted strategy of the miR-148a-NRP1 axis. The inhibition of osteogenic differentiation by the inflammatory microenvironment is a potential pathological mechanism underlying impaired repair of inflammatory bone defects. In a model of LPS-induced osteogenic dysfunction of periodontal ligament stem cells, it was found that the upregulated expression of miR-148a negatively regulates NRP1 expression by directly targeting the 3' untranslated region of NRP1 Mrna (19), thereby impairing the osteogenic capacity of cells. The inhibition of miR-148a or the overexpression of NRP1 can significantly reverse this inflammation-mediated osteogenic dysfunction, whereas the knockdown of NRP1 can completely abrogate the osteogenic-promoting effect of miR-148a inhibitors (92). The negative regulatory relationship between miR-148a and NRP1 has also been verified in clinical periodontitis tissues and in derived stem cells (92). It should be clarified that although the miR-148a-NRP1 axis exhibits potential to regulate osteogenic differentiation in the context of periodontitis, the evidence presented above is derived solely from *in vitro* cell models. Whether this axis exerts the same efficacy in the complex *in vivo* environment of fracture healing remains to be directly verified.

Based on findings in periodontitis models, the miR-148a-NRP1 axis may be a potential target for intervening in osteogenic dysfunction in inflammatory microenvironments. Given that inflammation is also a key inducer of impaired fracture healing, this strategy has only theoretical potential application in inflammatory fracture-healing disorders. Currently, it lacks *in vivo* intervention evidence from fracture animal models.

Regulatory potential of natural small molecules. Previous studies have confirmed that natural products, such as flavonoids, saponins and polyphenols, can promote angiogenesis and bone repair (114-117). Recent studies on luteolin and naringin have found that NRP1 and its mediated signaling pathways are also potential molecular targets for these naturally active ingredients to regulate bone homeostasis (84,103).

In vitro experiments using primary osteoblasts have shown that luteolin can significantly upregulate mRNA and the protein expression of the SEMA3A/NRP1/Plexin-A1 pathway in glucocorticoid-injured rat primary calvarial osteoblasts, promote osteoblast proliferation and inhibit abnormal apoptosis (84). In a previous study, naringin was shown to exert a potent *in vivo* bone-protective effect in a rat model of disuse osteoporosis (103). It upregulated NRP1 expression in bone tissue in a dose-dependent manner, simultaneously promoting bone formation and inhibiting bone resorption through the SEMA3A-NRP1-Wnt/β-catenin pathway, and effectively maintaining bone mass and bone microstructure (103).

The aforementioned studies collectively indicate that the SEMA3A-NRP1 axis is a potential molecular target for traditional Chinese medicine active ingredients to regulate bone metabolism. Luteolin provides a new intervention direction for glucocorticoid-induced osteoblast dysfunction, while naringin further verifies the feasibility of targeting this pathway to prevent and treat bone metabolic disorders *in vivo*. However, it should be noted that luteolin has undergone only preliminary mechanistic exploration *in vitro* in primary osteoblasts and lacks *in vivo* verification in glucocorticoid-induced osteoporosis and fracture-healing animal models. Naringin has also not been studied in the context of fracture repair. The clinical translational value and optimal administration regimens of both still require further in-depth exploration.

Exploratory strategies with insufficient evidence. sNRP1 can be stably detected in serum and has been confirmed as an independent biomarker for a poor prognosis in various malignant tumors, including breast cancer, ovarian cancer and hepatocellular carcinoma, in cancer research. Its high expression is significantly associated with a shorter patient survival, and the association between its level and the efficacy of anti-angiogenic therapy has been reported (118-122). It should be strictly clarified that all the aforementioned evidence is derived entirely from the field of oncology, and there are currently (to the best of our knowledge) no *in vitro* or *in vivo* studies available on fracture healing that directly examine the association between sNRP1 and the healing process. In addition, malignant tumors are a chronic, continuously progressive and uncontrollable process of pathological angiogenesis. By contrast, angiogenesis in fracture healing is acute, self-limiting and precisely regulated by physiological repair programs. Whether sNRP1 has similar clinical significance across these two distinct biological backgrounds remains an open question. Given the established regulatory functions of NRP1 in bone tissue injury and repair, and the application of sNRP1 as a biomarker in other diseases, exploring its association with fracture-healing phenotypes has only pure theoretical value. Positioning it as a potential biomarker for fracture healing still lacks clinical or preclinical data to support this claim.

Opportunities in translational research and existing barriers

Translational opportunities. NRP1 may serve as a potential nexus of neural, vascular and skeletal signals, providing a unique opportunity to develop pleiotropic bone repair strategies.

i) Multi-pathway regulatory effect. Unlike traditional bone repair therapies that target a single cytokine, the modulation of NRP1 can simultaneously engage multiple important pathways, including VEGF-mediated angiogenesis, SEMA3A-mediated osteogenic differentiation and osteoclast inhibition (17,46). In addition, based on the regulatory roles of NRP1 in the projection of intraosseous sensory nerves and in promoting BMSC osteogenic differentiation via neurogenic SEMA3A, it theoretically has the potential to integrate neuro-osseous coupling repair. Still, to date, to the best of our knowledge, no direct evidence has been obtained in fracture models (13,14). If this multi-pathway synergistic effect is fully confirmed, it is expected to yield new insight into the clinical problem of the limited efficacy of single-target therapies.

ii) The rise of smart biomaterials. Technological breakthroughs in smart biomaterials have enabled novel, targeted and precise delivery solutions for NRP1 and the exertion of its functions. Recent research has confirmed that the local delivery system based on 3D-printed biodegradable magnesium alloy scaffolds can achieve spatiotemporally synergistic controlled release of NRP1 and Mg^{2+}, providing the first verifiable technical solution to the problem of systemic off-target effects of NRP1 (46). This strategy provides potential research and translational ideas worth exploring to address the clinically intractable ischemic osteogenic disorder and promote bone defect repair.

Existing barriers. Despite its promising prospects, translating NRP1 research from the laboratory to clinical application remains challenging, particularly due to biological complexity and clinical safety concerns.

Spatial specificity and systemic off-target risks. NRP1 is widely expressed across multiple tissues, and its function is highly microenvironment-dependent. This characteristic indicates that systemic intervention is highly likely to simultaneously disrupt the normal physiological functions of NRP1 across the cardiovascular, nervous, and immune systems, raising fundamental questions about the feasibility of systemic therapeutic modulation (112). This characteristic also directly indicates that the systemic therapeutic regulation viewpoint proposed in this paper remains impractical at present. How to achieve precise intervention at the fracture site, while avoiding the disruption of the physiological functions of NRP1 in the cardiovascular, nervous and immune systems is a critical safety challenge that needs to be overcome for the clinical translation of its targeting strategies. Although the current local delivery systems have shown no systemic adverse reactions in the short term, their safety in the context of long-term intervention is completely unknown.

General lack of large animal models and clinical evidence. Currently, the functional evidence for NRP1 in bone repair is primarily based on mouse models and *in vitro* systems. Due to critical differences between the human skeletal system and rodents in metabolic rate, mechanical loading patterns, and healing time course, the therapeutic efficacy of NRP1 remains unverified in standardized large-animal models and human clinical trials. This gap is not a skippable developmental step, but a critical link that must be completed in the logical chain of target validation.

Incomplete elucidation of the spatiotemporal and context-dependent nature of the mechanism of action. As stated multiple times in the present review, the function of NRP1 is not fixed. NRP1 undergoes bidirectional functional switching across different pathological microenvironments, and the threshold for switching between its protective and destructive effects has not yet been elucidated, thereby preventing the determination of the time window and direction of targeted intervention. For example, in the chronic foreign-body inflammatory environment of periprosthetic osteolysis, NRP1 exhibits an abnormally high expression, but loses its proper bone-protective function, leading instead to progressive bone destruction (110). This bidirectional regulatory capacity means that the optimal intervention window for NRP1 remains unclear during the dynamic process of fracture healing, as the microenvironment is constantly changing. Until these basic parameters are elucidated, NRP1 intervention strategies will lack a fundamental basis.

8. Summary and prospects

The present review article systematically summarizes the research progress on the regulatory mechanisms of NRP1-mediated bone repair. The functions that have been directly validated in bone defect models to date include: NRP1 functioning as a co-receptor of VEGFR2 to drive endothelial angiogenesis, enhancing the migration and paracrine function of BMSCs via the NRP1-VEGFR2 axis, and synchronously promoting osteogenesis and inhibiting osteoclastogenesis through the SEMA3A axis (17,46).

However, significant gaps remain in the regulatory network of NRP1 in bone repair. At the cellular and tissue levels, the regulation of NRP1 in pericytes, type H vessels and chondrocytes is derived from non-skeletal systems, and its role in fracture healing remains unclear. At the signaling network level, the function of the NRP1-PDGF axis in vascular maturation and the regulatory mechanisms of neuro-osseous crosstalk have not been directly verified in fracture models. At the pathological and translational levels, the causal association between NRP1 dysregulation and impaired healing has not been established, and multiple intervention strategies have not yet been translated from bone homeostasis models to fracture models.

In bone defect models that have been directly validated, the effects mediated by NRP1, including promoting osteogenesis, inhibiting osteoclastogenesis and regulating the osteogenic differentiation of BMSCs, mainly depend on the binding of SEMA3A. However, the regulatory mode of competitive binding between VEGFA165 and SEMA3A during the initiation of vascular sprouting is currently mainly derived from studies on non-skeletal systems, suggesting that its functional outcome may be determined by the local concentration ratio of the two ligands. Furthermore, the function of NRP1 exhibits cell-type specificity: It has been confirmed in bone defect models that NRP1 can drive angiogenesis in endothelial cells; studies in non-skeletal systems further indicate that it can also guide vascular sprouting and maintain barrier homeostasis in endothelial cells, and regulate vascular permeability and maturation in pericytes. It can promote differentiation and mineralization, counteract metabolic osteogenic inhibition in osteoblasts, inhibit maturation, differentiation and bone resorption in osteoclast precursor cells, enhance migration

and paracrine function, and regulate the balance between osteogenic and adipogenic differentiation in bone marrow mesenchymal stem cells.

In addition, NRP1 activation exerets significant context-dependent bidirectional effects in chondrocytes and osteoclasts. In chondrocytes, under acute mechanical stress, NRP1 can inhibit excessive activation of the AKT, ERK and NF-κB pathways, thereby protecting the cartilage matrix. By contrast, chronic inflammation in osteoarthritis can inhibit the PI3K-AKT pro-survival pathway, driving chondrocyte apoptosis and matrix degradation. In the osteoclast lineage, under physiological conditions, NRP1 inhibits bone resorption through SEMA3A signaling; in diabetes and osteoporosis, the downregulated expression of NRP1 is accompanied by enhanced bone resorption; while in periprosthetic osteolysis, a paradoxical phenomenon of abnormally high NRP1 expression coexisting with bone destruction is observed. This functional switch from protection to destruction suggests that the pros and cons of NRP1 activation are not determined solely by the pathway itself, but are jointly shaped by the local microenvironment's inflammatory state and the cellular context.

It is precisely this high microenvironment-dependent nature of function that constitutes the core challenge of current NRP1 research and also points the way for future research. First, elucidating the molecular switches that govern the directional conversion of NRP1 function, particularly the inflammatory threshold and signaling nodes at which it shifts from protection to destruction, will directly determine the direction of targeting strategies. Second, there is an urgent need to systematically validate the regulatory functions of NRP1 in pericytes, type H vessels, chondrocytes and neuro-osseous crosstalk in fracture models. At the technical level, although the existing local delivery strategy based on 3D-printed magnesium alloy scaffolds has shown preliminary efficacy, transitioning from sustained release to precise regulation within a specific time window remains a barrier to clinical applicability. In the longer term, NRP1 may become a promising research target for bone repair, and its spatiotemporal regulation at specific stages of fracture healing is a key area for interdisciplinary collaboration among materials science, bone biology and pharmacology.

Core scientific issues and future research directions. Based on current research on NRP1 in bone repair, a series of key scientific issues remain to be elucidated, and future research can be prioritized and pursued sequentially based on evidence. The most prominent problem in current research is the severe lack of evidence for key mechanisms. The role of NRP1 in type H vessels, intraosseous pericytes, and fracture callus formation and remodeling remains poorly verified in fracture models. The molecular switches underlying its ligand dependence and cell specificity have also not been clearly elucidated. Moreover, the mechanisms underlying the bidirectional effects of NRP1 across different pathological scenarios remain unclear. This molecule is downregulated in osteoporosis and diabetes, but is abnormally upregulated in periprosthetic osteolysis. The key threshold and core signaling mechanism underlying its functional shift from bone protection to bone destruction remain unclear, which makes it difficult to determine the direction of targeted interventions. At the translational research level, most existing intervention strategies have been validated only in small-animal bone defect models, lacking support from clinically relevant composite pathological models such as diabetes and aging. Furthermore, the optimal spatiotemporal window for local delivery of NRP1 has not yet been established; thus, it is difficult to support the development of precise and effective targeted interventions.

Future research is required to prioritize addressing the core shortcomings outlined above, such as the following: First, to systematically validate the regulatory effects of NRP1 on type H vessels, pericytes and chondrocytes in standard fracture healing models, elucidate the molecular mechanism of its bidirectional functional conversion, and consolidate the core theoretical foundation. Second, to construct composite animal models of diabetes and osteoporosis complicated by fractures, clarify the direct causal association between dysregulated NRP1 expression and delayed fracture union or nonunion, and fill a key evidence gap in the pathological mechanisms underlying these conditions. At the translational application level, focus should be on developing local delivery systems for NRP1 with spatiotemporally controlled release, reducing systemic off-target risks, while ensuring repair efficacy, and improving the safety and clinical applicability of targeted interventions.

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Authors' contributions

KZ was involved in the conceptualization of the study, in the writing of the original draft of the manuscript, and in literature curation. TL was involved in the critical literature analysis

and synthesis, visualization, and in the writing reviewing and editing of the manuscript. HL was involved in the validation of data from the literature, in the writing of the original draft of the manuscript, and in the writing reviewing and editing of the manuscript. HT was involved in literature curation and validation, and in the writing reviewing and editing of the manuscript. ZZ was involved in literature curation, provision of academic resources (institutional database subscriptions, scientific visualization software licenses) and in the writing reviewing and editing of the manuscript. ZY was involved in the formal analysis, validation of data from the literature, and in the writing reviewing and editing of the manuscript. JW, XYan, HH and XYao were involved in literature curation and validation, and in the writing reviewing and editing of the manuscript. ZL was involved in literature curation, in critical literature analysis and synthesis, and in the writing reviewing and editing of the manuscript. SS was involved in formal analysis, validation of data from the literature, and in the writing reviewing and editing of the manuscript. XW provided resources, and was involved in the validation of data from the literature, in the writing reviewing and editing of the manuscript. FLY was involved in funding acquisition, study supervision and correspondence. XC was involved in the conceptualization of the study, in study supervision and correspondence. This work is presented as a narrative review. All cited literature and original materials for figures and tables have been thoroughly reviewed by KZ and XC, who jointly confirm the accuracy and objectivity of the narrative synthesis presented in this review. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

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Patient consent for publication

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Competing interests

The authors declare that they have no competing interests.

Use of artificial intelligence tools

During the preparation of this work, AI tools were used to improve the readability and language of the manuscript or to generate images, and subsequently, the authors revised and edited the content produced by the AI tools as necessary, taking full responsibility for the ultimate content of the present manuscript.

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