Role of NEL-like molecule-1 in osteogenesis/chondrogenesis (Review)

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Abstract. A dynamic balance exists between osteogenesis and osteoclastogenesis in bone tissue, which can lead to several bone diseases, such as osteoporosis, osteoarthritis, bone necrosis and bone defects, in cases of insufficient osteogenesis or excessive osteoclastogenesis. NEL-like molecule-1 (NELL-1) was first discovered in 1999 as an osteogenic factor that can prevent or treat bone diseases by increasing osteogenic levels. To date, research has identified multiple signaling pathways involved in improving osteogenic levels. Furthermore, to apply NELL-1 in clinical practice, researchers have optimized its osteogenic effect by combining it with other molecules, changing its molecular structure and performing bone tissue engineering. Currently, research on NELL-1 is gaining increasing attention. In the near future, it will definitely be applied in clinical practice to eliminate diseases. Thus, the present study provides a comprehensive review of NELL-1 in enhancing osteogenic levels from the perspectives of the molecular mechanism, interactions with other molecules/cells, molecular-level changes, applications in bone tissue engineering and its expression in tumors, providing a solid theoretical basis for its clinical application.

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Abbreviations: TMJOA, temporomandibular joint osteoarthritis; NELL-1, NEL-like molecule-1; TSP, thrombospondin protein; vWF, von Willebrand factor; EGF, epidermal growth factor; TGF, transforming growth factor; BMP, bone morphogenetic protein; JNK, Jun N-terminal kinases; PTHLH, parathyroid hormone-like hormone; MAPK, mitogen-activated protein kinase; BMSCs, bone marrow stromal cells; PSCs, perivascular stem cells; Pi, inorganic phosphate; Ihh, Indian Hedgehog; PTHrP, parathyroid hormone related protein; Nfatc, nuclear factor of activated T-cells; Sca-1, stem cell antigen-1; MPCs, mesenchymal progenitor cells; MSCs, mesenchymal stem cells; Cntnap4, contactin-associated protein-like 4; ASCs, adipose-derived stem cells; SAG, smoothened agonist; PPARγ, peroxisome proliferator-activated receptor gamma; PEG, polyethylene glycol; PLCL, poly lactide-co-caprolactone; TCP, tricalcium phosphate; HA, hydroxyapatite; Chi, chitosan; NNP, nanoparticles; PLGA, polylactic-co-glycolic acid; DBM, demineralized bone matrix; OPN, osteopontin; OCN, osteocalcin

Key words: NELL-1, bone diseases, osteogenesis, bone tissue engineering

Contents

1. Background

Disruption in the normal formation of bone and/or cartilage may cause a series of bone diseases, including but not limited to osteoporosis (1,2), osteoarthritis (3,4), osteonecrosis (5,6) and bone defects (7,8). Osteoporosis is the most common bone disease, with estimates indicating that >200 million individuals worldwide suffer from it (9). Osteoarthritis is a common musculoskeletal disease that affects >10% of the elderly population (10). Osteonecrosis of the jaw has an incidence of 1.3-10%, with mandibular osteonecrosis being more prominent than maxillary osteonecrosis (11). Approximately 5-10% of fractures eventually have delayed union or nonunion, leading to bone defect (12). Therefore, there is still a need to discover bone-specific osteogenic anabolic agents for the treatment of these conditions.

NEL-like molecule-1 (NELL-1), a neuroepidermal growth factor-like protein, was first discovered by Ting *et al* (13) in 1999 and found to be able treat osteoporotic bone loss (14). Of note, the protein was found in the cranial tissues of patients with unilateral coronal sclerosis and was isolated (13). The protein consists of the following domains: A thrombospondin protein (TSP)-1-like N-terminal domain, a coiled-coil domain,

four von Willebrand factor (vWF) C-type domains and six epidermal growth factor (EGF)-like domains (15).

The NELL-1 protein, encoded by the NELL-1 gene, contains 810 amino acids and has a molecular weight of ~90 kilodaltons (kDa) before N-terminal glycosylation and oligomerization. The secreted rat NELL-1 is a phosphorylated homotrimer with a molecular weight of >400 kDa (16). Of note, human recombinant NELL-1 shares 92.6% homology with rat NELL-1. However, the molecular weight of recombinant NELL-1 expressed in Chinese hamster ovarian cells was ~140 kDa under reducing conditions and >700 kDa under nonreducing conditions in sodium dodecyl sulfate gel electrophoresis, suggesting that NELL-1 may be secreted as a pentamer. Researchers have speculated that the crimp helical structure of the 5'-end of NELL-1's first vWF domain may cause oligomerization similar to that in the cartilage oligomeric matrix protein (17).

NELL-1 has been considered a TSP-1-like molecule because of the presence of an N-terminal TSP-1 phospholipid-binding domain (16). However, NELL-1 lacks some major TSP-1 motifs, including type I and III TSP repeats, Arg-Gly-Asp binding domains and C-terminal domains. Phospholipid binding is a typical biochemistry feature of NELL-1. EGF repeats in NELL-1 are key components that allow its binding to protein kinase C subunits, and this interaction between EGF repeats and their corresponding factors is considered a new type of binding mode (18). The lack of a fifth EGF repeat NELL-1 shearing isomer may affect this EGF binding by regulating the binding with calcium. The vWF domain is also thought to contribute to NELL-1 oligomerization and mediate cell adhesion (17). Of note, TSP-1 can bind and activate the potential form of transforming growth factor (TGF)-β1 (19). However, to date, no study has confirmed the ability of NELL-1 to bind to TGF-β1; however, the existence of a consensus repeat junction domain indicates that NELL-1 can bind with bone morphogenetic protein (BMP) members of the TGF-β superfamily (20).

An analysis of the NELL-1 gene using the GeneCards platform (https://www.genecards.org/) performed as part of the present study revealed a three-dimensional structure in AlphaFold (predicted) (Fig. 1A) and a three-dimensional structure in the protein databank (representative) (Fig. 1B). In addition, Gene Ontology (GO) (Table I) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses for NELL1 were performed. However, no related genes were successfully enriched in the KEGG analysis.

To study the interaction between NELL-1 and other molecules, the Pathway Commons (https://www.pathwaycommons.org/) and BioGRID (https://thebiogrid.org/) databases were used to obtain a network diagram centered on NELL-1 (Fig. 1C and D).

In recent years, research on NELL-1 in bone and cartilage has become increasingly popular (21-30). These articles or reviews have elaborated on the interaction between NELL-1 and bone/cartilage from different perspectives. However, most of these studies have only considered NELL-1 in their research, which is different from the approach of the current review. A PubMed search using the key word 'NELL-1' identified several recent manuscripts (Fig. 1E), which confirm NELL-1's close association with bone formation (31,32). In line with this, the current study reviews the available literature on NELL-1, focusing on its molecular mechanism, interactions with other molecules/cells, molecular-level changes, applications in bone tissue engineering and its expression in tumors. In other words, this comprehensive review focused on two aspects: Theoretical study and clinical application. The theoretical study was conducted on some star molecules in the signaling pathway that have been extensively studied, such as BMP, RUNX family transcription factor 2 (RUNX2), Hedgehog, nuclear factor of activated T-cells (Nfatc) and Wnt. Clinical applications were explored based on interactions of NELL-1 with other molecules/cells, molecular-level changes and applications in bone tissue engineering. Finally, the expression of NELL-1 was assessed in tumors. In conclusion, NELL-1 has a biological role in treating bone tissue diseases through complex signaling pathways and its expression can be optimized to amplify its biological functions. Thus, the present review aimed to summarize these studies and provide a theoretical basis for the early widespread clinical application of NELL-1.

2. Molecular mechanisms of NELL-1 in osteogenesis: Theoretical study

This section elaborates on how NELL-1 plays an osteogenic role at the theoretical level. In other words, it is described how NELL-1 exerts its osteogenic effect through the activation and inhibition of a series of signaling pathways. A literature search revealed that certain key molecules in the signaling pathway have been extensively studied, such as BMP, RUNX2, Hedgehog, Nfatc and Wnt. Therefore, the chapters below elaborate on key molecules in these signaling pathways to make it easy for the reader to understand the related mechanisms.

NELL-1 exerts its osteogenic effects through BMP. The interaction and differences between NELL-1 and BMP were first explored in the present review. NELL-1, which is regulated by BMP-9, participates in the regulation of biological processes related to osteoblast differentiation and may play an important role in the healing of cranial sutures (33). During the process of bone formation stimulated by NELL-1, microRNA (miR)-370-3p can target BMP-2 and interfere with the expression of parathyroid hormone (PTH)-like hormone (34). During bone regeneration and repair, growth factor NELL-1 significantly attenuates or completely reverses BMP-2-induced inflammation, perhaps due to NF-κB. This is caused by the reduction in transcriptional activity or reactive oxygen species production (35). NELL-1 and BMP-2 synergistically enhance osteogenic differentiation of myoblasts and phosphorylate the Jun N-terminal kinase (JNK)-mitogen-activated protein kinase (MAPK) pathway (36).

However, notable differences exist between NELL-1 and BMP-2. Of note, NELL-1 may promote bone defect healing through endogenous cell recruitment and angiogenesis induction, which differs from BMP-2's mechanism of action (37). Unlike BMP-2, NELL-1 cannot initiate ectopic bone formation in muscle tissue but can induce bone marrow stromal cells (BMSCs) to form bone in a mouse muscle bag model, highlighting the specificity of BMP deficiency (38). While recombinant BMP-2 increases bone formation *in vivo*, it also

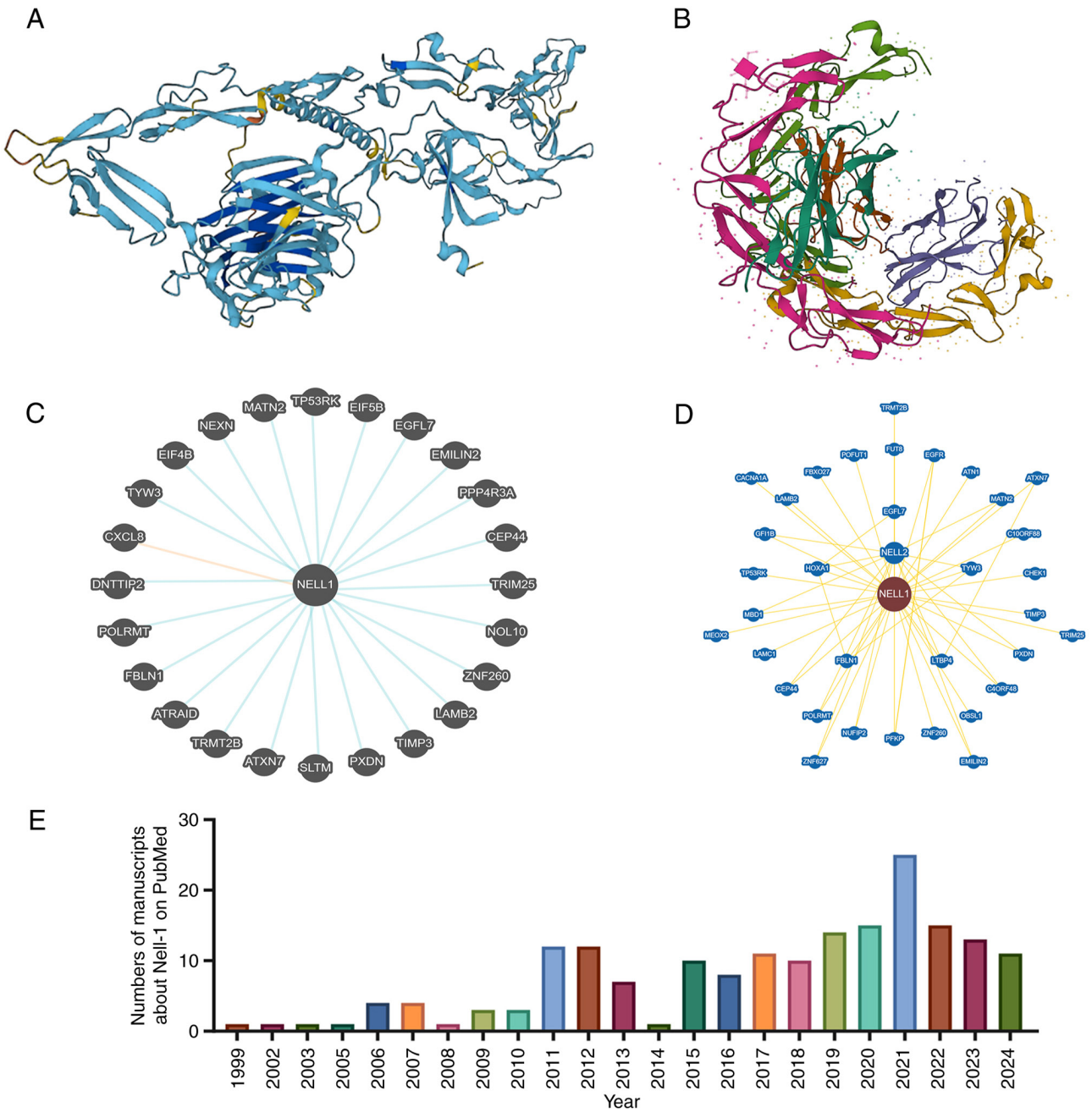

Figure 1. Three-dimensional structure and network diagrams of NELL. (A) Three-dimensional structure from AlphaFold (predicted) for the NELL-1 gene. (B) Three-dimensional structure from PDB (representative) for the NELL-1 gene. (C) Network diagram centered on NELL-1 in Pathway Commons. (D) Network diagram centered on NELL-1 in BioGRID. (E) Number of recent manuscripts on NELL-1. NELL-1, NEL-like molecule-1.

leads to a large number of adipogenic reactions. By contrast, NELL-1 selectively enhances bone formation. NELL-1 is a candidate growth factor that can induce osteogenesis of human perivascular stem cells (PSCs) (39). Fibroblast growth factor-2 and TGF-β1 can stimulate NELL-1 expression, but BMP-2 has no direct effect (40). Although BMP-2 induced a greater bone mass, the central cavity of the bone was filled with adipose bone marrow tissue. Despite the lower bone mass induced by NELL-1, histologic analysis through immunohistochemistry of type X collagen confirmed that it was similar to newly formed mixed cartilaginous bone found in an area of the trabecular bone. This difference indicates that NELL-1 has potential clinical advantages in bone tissue engineering and regeneration (41). Recombinant human NELL-1 can increase matrix mineralization and inorganic phosphate (Pi) influx in the cell line MC3T3-E1, which is closely associated with the activation of Pi transporter-1 and -2 channels, with the activation of the latter being more obvious. Pi transporters induced by recombinant human BMP-2 are only associated with Pi transporter-1 activation, indicating the fundamental difference between NELL-1 and BMP-2 signals (42). Chondrocytes in the proliferative zone of the growth plate produce factors involved in cartilage metabolism and bone formation. One study found that the expression of BMP-1, -2 and -5-7, as well as insulin-like growth factor 1, growth differentiation factor 5 and osteoclast stimulating factor 1 was considerably high, whereas the

Table I. Functional enrichment analysis for NEL-like molecule-1-related genes.

A, GO-Biological Process terms

GO ID	Qualified GO term	PubMed ID
GO:0007399	Involved in nervous system development	8975702
GO:0010468	Involved in regulation of gene expression	21723284
GO:0030154	Involved in cell differentiation	-
GO:0030501	Involved in positive regulation of bone mineralization	21723284
GO:0033689	Involved in negative regulation of osteoblast proliferation	21723284
GO:0042177	Involved in negative regulation of protein catabolic process	21723284
GO:0045669	Involved in positive regulation of osteoblast differentiation	21723284

B, GO-Cellular Component terms

GO ID	Qualified GO term	PubMed ID
GO:0005576	Located in extracellular region	-
GO:0005634	Nucleus	-
GO:0005635	Located in nuclear envelope	21723284
GO:0005737	Located in cytoplasm	21723284
GO:004847	Located in perinuclear region of cytoplasm	21723284

C, GO-Molecular Function terms

GO ID	Qualified GO term	PubMed ID
GO:0005080	Enables protein kinase C binding	21873635
GO:0005509	Enables calcium ion binding	-
GO:000551535	Enables protein binding	21723284
GO:000820135	Enables heparin binding	21873635

The GO analysis results were directly obtained from the GeneCards database (https://www.genecards.org/cgi-bin/carddisp.pl?gene=NELL1&keywords=nell-1)'. GO, Gene Ontology.

mRNA levels of BMP-3, BMP-4 and NELL-1 were exceedingly low (43). In a study involving *in vitro* rat organ culture, although BMP-7 and NELL-1 induced similar bone formation in the stretch suture, the mechanisms by which they achieved this differed. Accordingly, BMP-7 induced chondrocyte proliferation and differentiation, whereas NELL-1 accelerated chondrocyte hypertrophy and endochondral bone formation (Fig. 2A) (44).

NELL-1 exerts its osteogenic effect through RUNX2. The interactions between NELL-1 and RUNX2 in the nucleus and cytoplasm are described in this chapter.

NELL-1 and RUNX2 are closely related in the nucleus, with NELL-1 being a key downstream target of RUNX2. RUNX2 directly combines with osteoblast-specific binding elements 2 and transactivates the human NELL-1 promoter (45). Under the direct transcriptional control of RUNX2, NELL-1 is preferentially expressed in osteoblasts and well-regulated during bone development (38). NELL-1 is a key downstream functional mediator of RUNX2. RUNX2-regulated NELL-1 promotes osteoblast differentiation by activating MAPK and enhancing RUNX2 phosphorylation. When NELL-1 is blocked or deleted, RUNX2 activity is significantly reduced (46). However, during cartilage formation, the biological potential of NELL-1 remains unaffected by RUNX2's nuclear introduction and DNA binding (47).

Osterix is a direct transcriptional regulator that inhibits the expression of the NELL-1 gene, which helps regulate the delicate balance between NELL-1 transcription and RUNX2 regulation and may have a key role in the differentiation and mineralization of osteoblasts (48). Activating NELL-1 expression enhances implant osseointegration by upregulating the RUNX2/osterix axis, highlighting the potential of the BMSC lamellar implant complex in gene therapy (49). In NELL-1-deficient mice, osteoblast markers, including RUNX2, were generally reduced, whereas early proliferative Sox9 was enriched. NELL-1 is an important growth factor that regulates osteochondral differentiation by controlling the expression of RUNX2 and Sox9 in the skull (50). In a rat model, NELL-1 has demonstrated its potential as an effective osteoinductive molecule. In addition, the regulation of NELL-1 by RUNX2 indicates that NELL-1's role in osteoblasts is more specific compared to that of BMP, which influences multiple cell types (Fig. 2B) (51).

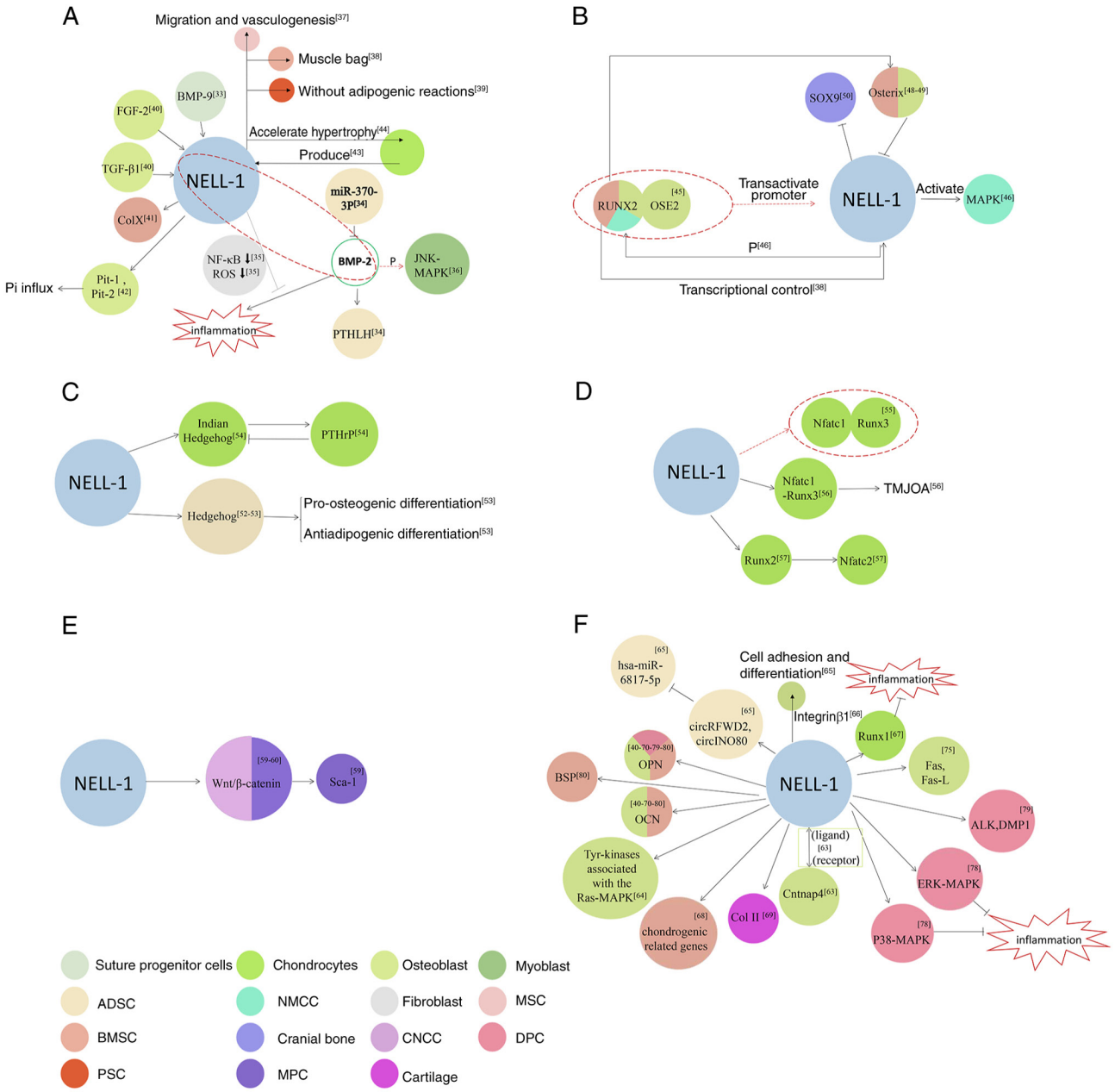

Figure 2. Molecular mechanism of NELL-1 acting on osteogenesis. (A) Molecular mechanisms of NELL-1 and BMP. (B) Molecular mechanisms of NELL-1 and RUNX2. (C) Molecular mechanisms of NELL-1 and Hedgehog. (D) Molecular mechanisms of NELL-1 and Nfatc. (E) Molecular mechanisms of NELL-1 and Wnt. (F) Molecular mechanisms of NELL-1 and other proteins. NELL-1, NEL-like molecule-1; BMP, bone morphogenetic protein; Nfatc, nuclear factor of activated T-cells; FGF, fibroblast growth factor; miR, microRNA; OPG, osteoprotegerin; OPN, osteopontin; OCN, osteocalcin; BMSCs, bone marrow stromal cells; PSCs, perivascular stem cells; Pi, inorganic phosphate; PTHrP, parathyroid hormone related protein; TGF, transforming growth factor; colX, type X collagen; JNK, Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; ROS, reactive oxygen species; PTHLH, parathyroid hormone-like hormone; RUNX2, RUNX family transcription factor 2; OSE, osteoblast specific binding elements; SOX9, SRY-related high-mobility group box 9; DMP1, dentin matrix protein 1; BSP, bone sialoprotein.

NELL-1 exerts its osteogenic effect through Hedgehog. Cells treated with NELL-1 have shown increased expression of the Hedgehog signaling pathway and the combined application of the smoothened antagonist cyclopamine reverses the osteogenic effect of NELL-1 (52). The Hedgehog signal was analyzed as a potential downstream target of the NELL-1 signal in regulating excessive osteogenic fat differentiation. NELL-1 is an effective anti-fat agent. In addition, NELL-1 signaling may inhibit fat differentiation through a Hedgehog-dependent mechanism (53). NELL-1 is a key regulator of epiphyseal homeostasis and endochondral ossification. Chondrocyte-specific NELL-1 inactivation significantly impedes bone development and leads to dwarfism and premature osteoporosis by inhibiting Indian Hedgehog (Ihh) signal transduction and changing the Ihh-PTH-related protein feedback circuit (Fig. 2C) (54).

NELL-1 exerts its osteogenic effect through Nfatc. Nfatc1 is a key transcription factor that mediates NELL-1-RUNX3 signal transduction. When NELL-1 is used for processing, Nfatc1 is combined with the promoter 833-810 region of RUNX3 (55). The Nfatc1-RUNX3 signaling pathway may be involved in osteochondral injury caused by temporomandibular joint

osteoarthritis (TMJOA) (56). Nfatc2 may play an important role in NELL-1-mediated osteochondral differentiation *in vivo* and *in vitro*. Studies have also found that Nfatc2 is the main response gene of NELL-1, whereas RUNX2 is the intermediary between NELL-1 and Nfatc2 (Fig. 2D) (57).

NELL-1 exerts its osteogenic effect through Wnt. The newly discovered ability of NELL-1 to stimulate Wnt signal transduction and inhibit fat production may represent a new method for treating bone loss in osteoporosis (58). Recombinant human NELL-1 induces stem cell antigen-1 (Sca-1) transcription in mesenchymal progenitor cells (MPCs), which requires complete Wnt/β-catenin signal conduction (59). NELL-1 is a key regulator of craniofacial nerve crest cells and the mandible. It also activates Wnt/β-catenin access (Fig. 2E) (60).

NELL-1 exerts its osteogenic effect through other molecules. This chapter explores the interaction between NELL-1 and other molecules in different parts of bones, craniofacial bones and teeth.

At the cellular level, the expression of NELL-1 may regulate osteoblast differentiation and is sufficient and necessary (61). Apart from NELL-1, nerve growth factor, Notum, prostaglandin signaling and the activator protein-1 family can effectively restore the mechanical reactivity of aging bone (62). In the process of osteogenesis, NELL-1 exhibits a ligand-receptor-like association with contactin-associated protein-like 4 (Cntnap4), which indicates that Cntnap4 may be the cell surface-specific receptor for NELL-1 (63). A previous study also indicated that roundabout guidance receptor 2 (Robo2) serves as a receptor for NELL-1 (64). When binding to specific receptors, NELL-1 transmits osteogenic signals by activating some Tyr kinases associated with the Ras-MAPK cascade, ultimately leading to osteogenic differentiation (65). NELL-1 induces the osteogenic differentiation of MC3T3-E1 by inducing late markers [osteopontin (OPN) and osteocalcin (OCN)] (40). During the osteogenic differentiation of human adipose-derived stem cells (ASCs) induced by NELL-1, circular RNA (circRNA) of COP1 E3 ubiquitin ligase and circRNA of INO80 complex ATPase subunit upregulate and inhibit the expression of hsa-miR-6817-5p, respectively, affecting the positive effects of NELL-1 on osteogenesis (66). The adhesion of NELL-1 to the cell surface depends on integrin β1, the cell surface target of NELL-1 that plays an important role in promoting cell adhesion and osteogenic differentiation of NELL-1 (67). NELL-1 can bind to all-trans retinoic acid-induced differentiation factor (APR3) on the cell surface, after which APR3 can inhibit osteogenic proliferation but promote osteogenic differentiation (68). NELL-1 can effectively inhibit the expression of inflammatory cytokines and their downstream cartilage catabolic enzymes by upregulating the expression of RUNX1 in articular cartilage chondrocytes. Therefore, NELL-1 is a promising candidate for a disease-modifying osteoarthritis drug, as it promotes chondrogenesis and inhibits inflammation, thereby preventing and reducing cartilage injury associated with arthritis (69). In addition, NELL-1 specifically promotes chondrogenesis and differentiation of human BMSCs *in vitro* by increasing the expression of chondrogenesis-related genes and proteins (70). During the process of ameliorating cartilage loss, NELL-1 enhances alcian blue and saffron-O staining and increases the deposition of type II collagen (71). In terms of femoral fracture treatment using NELL-1, data have also shown an increase in the immunostaining of the bone differentiation markers OPN and OCN. Therefore, NELL-1 effectively enhances *in situ* osteogenesis in the bone marrow (72). The response of canine PSCs to bone induction signals from NELL-1 is similar to that of human PSCs (73). NELL-1 gene polymorphisms have also been associated with osteoporosis (74,75). Only a few studies have been conducted on the relationship between NELL-1 and osteoclastogenesis. In one such study, NELL-1 could increase the osteoprotegerin/RANK ligand expression ratio in BMSCs, thereby inhibiting osteoclastogenesis (49).

In the craniofacial bone, the expression of NELL-1 affects bone metabolism. While normal NELL-1 expression regulates the differentiation and apoptosis of osteoblasts, NELL-1 overexpression deisrupts these pathways, resulting in craniofacial abnormalities, such as the premature closure of sutures. NELL-1-induced apoptosis was only observed in osteoblasts but not in NIH3T3 or primary fibroblasts (76). NELL-1 overexpression was reported to induce considerable apoptosis of skull osteoblasts through increased Fas and Fas ligand production (77).

In teeth, NELL-1 can promote bone formation in a concentration- and time-dependent manner (78,79). NELL-1 can inhibit lipopolysaccharide-induced inflammation of human dental pulp cells, which may be mediated by the p38-MAPK and extracellular signal-regulated kinase-MAPK signaling pathways rather than the JNK-MAPK signaling pathway (80). HrNELL-1 can increase the activity of alkaline phosphatase and enhance the expression of important odontogenic markers in human dental pulp cells, including OPN and dentin matrix protein 1, thereby promoting odontogenic differentiation and dentin formation of human dental pulp cells (81). Under NELL-1 induction, the expression of bone sialoprotein and OPN increases during the intermediate stage, whereas OCN expression increases at the later stage (the entire process ranges from 0-21 days). Alkaline phosphatase activity and the number of calcium nodules were highest in the NELL-1 group (Fig. 2F) (82).

3. Treatment optimization plan: Clinical application

This section focuses on how the therapeutic effect can be enhanced from the perspective of clinical application by improving the osteogenic role of NELL-1. Undeniably, NELL-1 on its own has osteogenic effects, but they can be improved through modification. In other words, it was explored how clinical therapeutic outcomes can be optimized through a series of physical or chemical changes that lead to increased activity of NELL-1. Through a literature search, it was found that the current approaches to optimize the efficacy of NELL-1 mainly include the following: Enhancement of the osteogenic effects of NELL-1 through interactions with other molecules/cells, through molecular-level changes and through bone tissue engineering. Therefore, these optimization strategies were summarized below.

Enhancement of the osteogenic effects of NELL-1 through interactions with other molecules/cells

Enhancement of the osteogenic effect of NELL-1 through interactions with BMP. Combined treatment with NELL-1 and BMP exerts synergistic osteogenic effects, which may be due to the obvious difference in their signaling pathways, and has a mutually reinforcing role (38). Combining NELL-1 with BMP-2 can improve clinical bone regeneration and exert a mechanism for typical Wnt pathway activity during NELL-1 and BMP-2 osteogenesis (58). Given that BMP-2 and NELL-1 enhance each other, the simultaneous delivery of both agents can significantly improve bone healing following tibial distraction osteogenesis (83). Compared to BMP-2 alone at a lower dosage, the combination of NELL-1 and BMP-2 showed more mature and complete bone defect healing, as evidenced by high-resolution micro-computed tomography (CT) and histological analysis (84). The combined effects of NELL-1 and BMP-2 in the controlled-release vector may eventually be applied in clinical practice to avoid the common adverse reactions of conventional BMP-2 alone. One study reported that using NELL-1 in controlled-release carriers can improve spinal fusion rates in clinical settings (85). The synergistic delivery of BMP-2 and NELL-1, an osteochondral-specific signal transduction protein, has potential therapeutic effects and is of great clinical significance (36). BMP-2 and NELL-1 genes exhibit synergistic effects on the osteogenic differentiation of BMSCs, with one study showing that BMP-2- and NELL-1-modified BMSCs can promote the formation and maturation of new bone in a rabbit maxillary sinus model (86). Applying NELL-1 and BMP-2 on dental pulp can induce dentin tubule repair and dentin formation and reduce inflammatory cell reaction. These results show that the combination of NELL-1 and BMP-2 can actively regulate pulp repair (87). Self-assembled polyelectrolyte complexes have been prepared to better control the delivery of BMP-2/NELL-1 through heparin binding and further enhance the biological activity of growth factors by enhancing their stability *in vivo* (88). Liposuction-derived human PSCs have been identified as a new and rich source of mesenchymal stem cells (MSCs) for cartilage regeneration, with one study showing that NELL-1, TGF-β3 and BMP-6 combined with human PSCs can significantly enhance and accelerate cartilage repair (89).

Enhancement of the osteogenic effect of NELL-1 through interaction with Hedgehog. After combined treatment with the Hedgehog signal agonist smoothened agonist (SAG) and NELL-1, new bone formation significantly increased, along with increased defect vascularization. As a new treatment strategy, NELL-1 plus SAG shows promise for treating critical-size bone defects. Future research will focus on optimizing the dosage and delivery strategies for SAG and NELL-1 combination products (90). The combined application of Hedgehog and NELL-1 has an additive effect on promoting bone differentiation and antilipogenic differentiation of ASCs. As such, the combination of cytokine Sonic Hedgehog-N and NELL-1 may be a feasible future approach for inducing osteogenic differentiation of MSCs (52).

Enhancement of the osteogenic effect of NELL-1 through interactions with other molecules. Incorporating peroxisome proliferator-activated receptor (PPAR)γ inhibitors with NELL-1 treatment can enhance bone formation by promoting anabolic processes, without affecting NELL-1's ability to inhibit osteoclast activity and adipogenesis. The combination of PPARγ inhibitors and therapeutic NELL-1 can be further developed as a new strategy to reverse bone loss in age-related osteoporosis and reduce bone marrow obesity (91). NELL-1 combined with zoledronic acid for the treatment of traumatic osteonecrosis in rats can promote osteoblast activity, inhibit osteoclast activity and preserve the bone mass and shape of the femoral head, indicating its significant role in preventing the collapse of femoral head osteonecrosis. This strategy is expected to reverse the course of osteonecrosis (92).

Enhancement of the osteogenic effect of NELL-1 through interactions with cells. The combination of human PSCs and NELL-1 has a significant additive effect on the angiogenesis and bone formation of the implant, with studies simultaneously observing the expression of angiogenic growth factor *in vitro*. This combined treatment can improve vascularized bone regeneration (93). NELL-1 significantly increases the osteogenic potential of human PSCs in osteoporotic and nonosteoporotic donors. Accordingly, one study showed that the combination of human PSCs and NELL-1 can synergistically enhance spinal fusion in osteoporotic rats (94). Radiographic images and quantitative analysis from another study in which a mouse osteonecrosis model was treated with adipose tissue-derived pericytes and NELL-1 found that this group had the largest bone formation among the treatment groups, with histomorphology analysis showing strong bone and vascular formation (95). Pericytes are a potential new source of cells for future research in bone regeneration medicine. NELL-1 can significantly induce the proliferation of pericytes and has been found to promote angiogenesis *in vivo* and *in vitro*. NELL-1 is a candidate growth factor that can induce osteogenic differentiation of pericytes. A study showed that the combination of purified human pericytes with NELL-1 has osteogenic potential (Table II) (96). Overall, the combined treatment of cells and NELL-1 can be used as a new treatment approach to improve bone regeneration and treat osteonecrosis and osteoporosis.

Enhancement of the osteogenic effect of NELL-1 through molecular-level changes. The following chapter focuses on how the osteogenic ability of NELL-1 can be enhanced through changes at the molecular level. A literature search unveiled that when the osteogenic role of NELL-1 is examined, changes at the molecular level can make the osteogenic effect more significant. Currently, these changes mainly include polyethylene glycol (PEG)ylation of NELL-1 and NELL-1570. Therefore, these two modifications were summarized to outline the treatment approaches.

Enhancement of the osteogenic effect of NELL-1 through molecular-level changes of PEGylation. The PEGylation of NELL-1 significantly improves its thermal stability while maintaining its biological activity *in vitro*. Furthermore, PEGylation can significantly increase the elimination half-life of NELL-1 (5.5-15.5 h) and increase its content in bone tissues (femur, tibia, spine and skull) by >2-3 times (97). Furthermore, evidence has shown that PEGylation increases the half-life of NELL-1 in a mouse model without hampering its osteogenic potential, thereby improving the pharmacokinetics of its systemic delivery. Weekly injections of NELL-PEG via the

Table II. Combination with other molecules/cells.

Model	Combination with other molecules/cells	Outcome	(Refs.)
Mouse muscle pouch model	BMP	Synergistic osteogenic effects	(38)
Osteoporosis	BMP-2	Improving bone regeneration	(58)
Bone formation in DO	BMP-2	Improving bony healing	(83)
Rat calvarial defects model	BMP-2	Mature and complete defect healing	(84)
Rat posterolateral spinal fusion model	BMP-2	Increasing fusion rate, avoiding adverse effects associated with BMP-2	(85)
Nude mouse model	BMP-2	Osteochondral specificity, potential therapeutic effects	(36)
Rabbit maxillary sinus floor elevation model	BMP-2	Promoting new bone formation and maturation	(86)
Rat pulp repair	BMP-2	Inducing dentin formation, reducing inflammatory cell response, regulating pulp repair	(87)
Reconstructive bone surgeries	BMP-2	Better control, enhancing stability, potentiating bioactivity	(88)
Osteoarthritis	BMP-6	Enhancing and accelerating cartilage repair	(89)
Parietal bone defect in CD-1 mice	Hedgehog signal activator SAG	Increasing new bone formation, increasing defect vascularization, healing of bone defects	(90)
hASCs	Sonic Hedgehog-N	Pro-osteogenic and antiadipogenic differentiation of ASCs	(52)
Osteoporosis	PPARγ suppression	Upregulating anabolic processes, reversing bone loss, decreasing marrow adiposity	(91)
Osteonecrosis	Zoledronate	Active osteoblast activity, reduced osteoclast activity, decreasing the femoral head deformity, stimulating bone formation, reversing osteonecrosis	(92)
Intramuscular ectopic bone model	hPSC	Vascularized bone regeneration	(93)
Osteoporosis	hPSC	Increasing osteogenic potential, enhancing spinal fusion	(94)
Osteonecrosis	AD pericytes	Robust bone and vessel formation	(95)
Nude mouse muscle pouch model	Pericytes	Inducing pericyte proliferation, increasing osteogenic differentiation, pro-angiogenic effects	(96)

BMP, bone morphogenetic protein; SAG, Smoothened agonist; PPARγ, peroxisome proliferator-activated receptor γ; PSCs, perivascular stem cells; ASCs, adipose-derived stem cells; AD pericytes, adipose tissue-derived pericytes.

intravenous or intraperitoneal route successfully improved overall bone quality (98). Compared to unmodified NELL-1, all three PEGylation conjugates (three monomer PEG sizes: 5, 20 and 40 kDa) showed enhanced thermal stability and prolonged circulation time *in vivo*. In addition, PEGylated NELL-1 maintains its osteogenic activity without any obvious cytotoxicity (99). NELL-PEG injection significantly enhances bone regeneration by promoting high bone turnover, bone formation and mineral adhesion rates. Immunohistochemical results have also confirmed that the NELL-PEG treatment group had superior bone remodeling activity (100). Different types of triblock PEG injectable hydrogels can reach a stable gel state at 37˚C and support the three-dimensional growth of cartilage cells, but the poly lactide-co-caprolactone (PLCL) block-PEG block-PLCL hydrogel has a wider gel temperature range and better hydrolytic stability. Furthermore, its controlled-release curve is closest to zero-order release kinetics. The PLCL-PEG-PLCL/NELL-1 compound can reverse osteochondral injury caused by TMJOA (56). Future research should further focus on developing NELL-1-PEG into a systemic treatment that can effectively prevent and treat osteoporosis, accelerate fracture healing and improve overall bone performance. Taken together, this method has excellent potential for practical application.

Enhancement of the osteogenic effect of NELL-1 through molecular-level changes in NELL-1570. NELL-1570 can significantly stimulate the proliferation of MSCs in multiple MSC-like cell groups, such as mouse C3H10T1/2 MSCs, mouse primary MSCs and PSCs, which are considered stem cells of perivascular origin. By contrast, NELL-1810 (normal molecular weight) only showed limited stimulation of MSC proliferation. *In vivo*, NELL-1570 can significantly induce the regeneration of skull defects given its effect of increasing cell proliferation (101). The proliferative effect of NELL-1570 is age-dependent and shows significant induction in adult mice but not in old mice (Table III) (102). In other words, NELL-1570 can potentially be used for bone regeneration therapy based on cells or hormones.

Table III. Changes at the molecular level.

Model	Changes at molecular level	Outcome	(Refs.)
Osteoporosis	PEGylated NELL-1	Improving thermostability, preserving bioactivity, increasing elimination half-life time, distributing >2-3 times the amount to bone tissues	(97)
Osteoporosis	PEGylated NELL-1	Increasing the half-life, improving pharmacokinetics upon systemic delivery	(98)
Osteoporosis	PEGylated NELL-1	Enhancing thermal stability, prolonging circulation time, retaining osteoblastic activity	(99)
Fracture	PEGylated NELL-1	Augmenting bone regeneration, increasing expression of bone turnover rate, bone formation rate, mineral apposition rate, accelerating fracture union, enhancing bone properties	(100)
TMJOA	PLCL-PEG-PLCL hydrogel	Wider gelation temperature range, better hydrolytic stability	(56)
Calvarial defect	NELL-1570	Stimulating MSC proliferation, functioning as a pro-osteogenic growth factor, potential for bone regeneration	(101)
Adult mice	NELL-1570	Proliferative effect of NELL-1570 is age-dependent	(102)

NELL-1, NEL-like molecule-1; PEG, polyethylene glycol; MSC, mesenchymal stem cell; PLCL, poly lactide-co-caprolactone.

Enhancement of the osteogenic effect of NELL-1 through bone tissue engineering. In this section, it is described how the osteogenic ability of NELL-1 can be enhanced through bone tissue engineering. A literature search revealed that when the osteogenic role of NELL-1 is examined, it is indicated that the osteogenic effect can be significantly enhanced via bone tissue engineering. Currently, relevant bone tissue engineering methods mainly include β-tricalcium phosphate (β-TCP), chitosan (Chi) nanoparticles (NNPs), polylactic-co-glycolic acid (PLGA) and demineralized bone matrix (DBM). Therefore, these methods were classified and summarized to better understand the related solutions.

Enhancement of the osteogenic effect of NELL-1 through bone tissue engineering of β-TCP. β-TCP is a bone conductive and biodegradable ceramic biomaterial that has been successfully used as a bone inducer for bone regeneration. It can be used as a carrier system for effectively delivering NELL-1 (103). The protein-carrying capacity of β-TCP particles can be enhanced and their initial rupture level can be improved by creating an apatite coating, surface etching with citric acid solution or immersing them in simulated body fluid. A study on the release kinetics of protein in modified β-TCP particles using the novel osteogenic protein NELL-1 as a model protein showed that the protein-carrying capacity of β-TCP can be regulated by surface modification, which allows the use of TCP as a controllable protein carrier (104). Autologous BMSCs modified by the NELL-1 gene and β-TCP particle scaffold can be used to lift the maxillary sinus floor in rabbits (86). TCP was modified through hydroxyapatite (HA) coating, after which a Chi coating was used to prepare Chi/HA-coated TCP particles. The NELL-1 protein showed a continuous release mode after being encapsulated in the modified Chi/HA-TCP particles. The NELL-1-integrated complex of Chi/HA-coated TCP particles demonstrates the advantages of these particles as a protein delivery carrier and highlights its potential as a modified bone matrix for bone regeneration research (105). The apatite-wrapped β-TCP vector promoted the sustained release of recombinant human NELL-1 protein over a prolonged period of time, resulting in the local inflow of Sca-1-positive MPCs, and induced complete bone fusion in all samples (100% spinal fusion rate) (59). Using tissue engineering technology, BMSCs after NELL-1 gene modification were combined with β-TCP at a concentration of $2x10^7$ cells/ml and implanted subcutaneously in the backs of nude mice. The percentage of new bone area in the NELL-1 group (18.1±5.0%) was significantly higher than that in the non-transfected (11.3±3.2%) and LacZ (β-galactosidase) groups (12.3±3.1%; $P<0.05$), suggesting that NELL-1 is a potential osteogenic gene for bone tissue engineering (82). One study that incorporated lyophilized recombinant human NELL-1 protein into a mixture of β-TCP and HA found that recombinant human NELL-1 vertebral body implantation significantly increased lumbar bone formation and successfully improved the regeneration of lumbar cortical and cancellous bones in osteoporotic sheep. This indicates that bone graft substitutes based on recombinant human NELL-1 have potential as a new local treatment method (106).

Enhancement of the osteogenic effect of NELL-1 through tissue engineering of Chi NNPs. Preloading NELL-1 into Chi NNP resulted in a significantly longer release time and greater released biological activity of NELL-1 compared to directly adding NELL-1 to the scaffold. As such, NELL-1 and dual-release scaffolds have potential applications in cartilage tissue engineering (70). A multifunctional polycaprolactone nano-HA Chi NNP composite fiber with long-term biological activity and bone induction was successfully prepared by electrospinning. Subsequent *in vivo* research found that this composite material effectively prolongs NELL-1 release and

shows good cell compatibility, indicating its superior ability to induce osteogenic differentiation. This makes it a promising scaffold for bone tissue engineering applications (107). Hyaluronic acid hydrogel is mixed with two types of particles (decalcified bone powder for bone conduction and biomimetic apatite-coated sodium alginate/Chi NNP for controlled NELL-1 delivery) to achieve the plasticity of biomaterials and improve the spinal fusion rate (108). Recombinant human NELL-1 has shown chondrogenic potential in a three-dimensional sodium alginate hydrogel microenvironment containing rabbit chondrocytes. Incorporating NELL-1 into Chi NNP can provide controlled delivery function and maximize its biological efficiency (109). NELL-1, incorporated into Chi NNP and embedded in alginate saline gel, can repair bone defects and promote obvious cartilage regeneration, closely mimicking the histological properties of natural cartilage. This makes it a promising candidate for treating various pathologies, such as cartilage defects and degeneration, using tissue engineering (71).

Enhancement of the osteogenic effect of NELL-1 through bone tissue engineering of PLGA. Transplanting the NELL-1 protein-coated PLGA scaffold into a rat skull defect showed that the osteogenic potential of NELL-1-induced bone regeneration was equivalent to that of BMP-2, revealing its potential therapeutic effects and establishing it as a currently recognized alternative to bone regeneration technology (40). After precoating a culture dish or PLGA scaffold with NELL-1, the degrees of cell adhesion and osteogenic differentiation increased significantly (67). The NELL-1-modified bone marrow MSC/PLGA group showed strong and rapid repair effects at 6 weeks, resulting in fibrocartilage regeneration and a completely repaired natural articular cartilage and subchondral bone at 24 weeks. Therefore, the NELL-1-modified bone marrow MSC/PLGA composite can rapidly repair large-area osteochondral defects of the mandibular condyle and promote the regeneration of the natural fibrocartilage and subchondral bone (110).

Enhancement of the osteogenic effect of NELL-1 through bone tissue engineering of DBM. In sheep, NELL-1, an independent and effective osteogenic molecule, is easy to use when combined with DBM (111). Micro-CT revealed that NELL-1 in DBM exerted a significant effect on spinal fusion, showing increased bone formation, endochondral ossification and vascularization (112). β-TCP/DBM, which is a carrier system for the efficient delivery of biologically active NELL-1, can improve the biochemical stability and biological efficiency of NELL-1 (103). In rats, NELL-1 in DBM carriers can significantly and dose-dependently promote bone regeneration in critical-size femoral segmental defects. NELL-1 is an effective bone-specific growth factor, which can be used as a substitute for bone transplantation in various clinical scenarios, including repairing severe bone loss when autologous bone is limited or unavailable (Table IV) (113).

4. Tumor expression

NELL-1 is also expressed, to a certain extent, in bone tissue tumors and plays an important role. Sarcoma, as the most common malignant tumor of bone tissue, should be examined. Studies have revealed that NELL-1 is stably and reliably expressed in chondrogenic bone tumors (114) and exhibits extensive and reliable expression in benign, nonmalignant osteogenic tumors (115). Recombinant human NELL-1 mainly increases the activation of the JNK pathway, which is necessary for mediating the final osteogenic differentiation of Saos-2 osteosarcoma cells (116). Upregulation of NELL-1 has been positively associated with the metastasis of rhabdomyosarcoma and negatively associated with prognosis (117).

Considering the limited research on osteosarcoma, to enrich the content of the present review, a bioinformatics analysis was further conducted. The differential expression of NELL-1 in sarcomatous tissues was analyzed using several databases and bioinformatics methods.

DNA methylation is closely related to tumors and changes in the methylation level of NELL-1 may be a key factor in osteosarcoma development (118). NELL-1 methylation levels in sarcoma tissues vary. Analysis on the SMART platform (http://www.bioinfo-zs.com/smartapp/) revealed the chromosomal distribution of the methylation probes associated with NELL-1 (Fig. 3A) and provided detailed genomic information on NELL-1 (Fig. 3B). The results showed that the CpG-aggregated methylation value (β-value) of NELL-1 in sarcomatous tissues was significantly lower than that in normal tissues (Fig. 3C), indicating that a decrease in NELL-1 methylation levels can cause osteosarcoma. Continuing this in-depth research, the probe of cg10964385 was selected for closer examination; however, no significant difference between tumor and normal tissue was found for the methylation value of cg10964385 (Fig. 3D). The specific significant methylation probe will also become the main focus of research in the future.

The most common cause of tumor occurrence is genetic mutations. Mutation in the NELL-1 gene is closely linked to sarcoma occurrence (119). Using the cBioPortal platform (https://www.cbioportal.org), the alteration frequency of NELL-1 in sarcomatous tissues was assessed. A certain level of mutation, structural variation, amplification, deep deletion and multiple alterations in sarcomas were found (Fig. 4A); hence, the frequency of mutation sites was further analyzed (Fig. 4B). Varying degrees of mutation sites can be observed throughout the NELL-1 sequence. Among them, the 468th amino acid showed the highest frequency. Subsequently, data on the fraction genome altered, mutation count and mRNA expression RNA-sequencing by Expectation-Maximization (RSEM) for NELL-1 across various tumors were obtained (Fig. 4C-E). For the fraction genome altered of NELL-1, a shallow deletion of NELL-1 was most commonly observed in sarcoma (Fig. 4C). For the mutation count of NELL-1, a shallow deletion of NELL-1 was most commonly observed in sarcoma (Fig. 4D). For mRNA expression (RSEM) of NELL-1, a shallow deletion of NELL-1 was most commonly observed in sarcoma (Fig. 4E). Finally, the following relationships were analyzed using Spearman and Pearson correlation: Fraction genome altered and mutation count (statistically significant; Spearman: 0.34, $P=2.51\times10^{-263}$; Pearson: 0.21, $P=9.24\times10^{-96}$) (Fig. 4F); metrology and NELL-1 mRNA expression (statistically significant; Spearman: -0.25, $P=7.92\times10^{-145}$; Pearson: -0.27, $P=3.80\times10^{-162}$) (Fig. 4G); NELL1 mutations and NELL1 mRNA expression (RSEM) [among mutations of NELL-1 (missense, truncation, splice, multiple, no mutation and not

Table IV. Bone tissue engineering.

Model	Bone tissue engineering	Outcome	(Refs.)
Rat spinal fusion model	β-TCP	Delivering osteoinducers for bone regeneration	(103)
-	Apatite-coated TCP	Enhancing carrying capacity, reducing initial burst	(104)
Rabbit maxillary sinus floor elevation model	β-TCP	Elevating the maxillary sinus floor	(86)
Bone regeneration	Chi/HA-TCP	Sustained release pattern, improving bone matrix for use in bone regeneration	(105)
Nonhuman primate lumbar spinal fusion model	Apatite-coated TCP	Prolonging release, completing osseous fusion	(59)
Nude mouse model	β-TCP	Inducing osteogenic differentiation of bMSCs, enhancing bone formation	(82)
Osteoporotic sheep model	β-TCP mixed with HA	Increasing bone formation, improving cortical and cancellous bone regeneration	(106)
hBMSCs	Chi nanoparticles	Extending release time, increasing released bioactivity	(70)
Bone defect	PCL/nHA/Chi nanoparticles composite fiber	Prolonging the release time, good cytocompatibility, inducing osteogenic differentiation. Used as scaffolds in bone tissue engineering	(107)
Rat spinal fusion model	Demineralized bone powder and biomimetic apatite-coated alginate/Chi microparticles	Moldability achieved, enhancing spinal fusion rates	(108)
Rabbit chondrocytes	Chi microparticles	Providing controlled delivery, maximizing biological efficiency	(109)
Circular osteochondral defects	Chi nanoparticles and embedded into alginate hydrogels	Improving cartilage regeneration, producing functional cartilage	(71)
Calvarial defects	PLGA scaffolds	Stimulating bone regeneration	(40)
-	PLGA scaffold	Increasing cell attachment and osteogenic differentiation	(67)
Osteochondral defect	BMMSCs/PLGA composite	Regeneration of native fibrocartilage and subchondral bone	(110)
Sheep spinal fusion model	DBM	Promoting bone formation	(111)
Athymic rats with posterolateral spine fusion model	DBM	Increasing bone formation, endochondral ossification and vascularization	(112)
Rat spinal fusion model	β-TCP/DBM	Increasing biochemical stability and biological efficiency	(103)
Femoral segmental defect model	DBM	Greater bone formation, improving bone regeneration	(113)

TCP, tricalcium phosphate; HA, hydroxyapatite; Chi, chitosan; bMSCs, bone marrow stromal cells; PLGA, polylactic-co-glycolic acid; BMMSCs, bone marrow mesenchymal stromal cells; DBM, demineralized bone matrix.

profiled), a shallow deletion of no mutation was most closely related to NELL1 mRNA expression (RSEM)] (Fig. 4H); and putative copy number alterations from Genomic Identification of Significant Targets in Cancer (GISTIC) and NELL-1 mRNA expression (RSEM) [among putative copy number alterations from GISTIC of NELL-1 [deep deletion, shallow deletion, diploid, gain and amplification], shallow deletion, diploid and gain were similar and most closely related to NELL1 mRNA expression (RSEM)] (Fig. 4I).

Recently, immune infiltration, a biological process, has been reported to be closely related to tumor occurrence and development (120). NELL-1 expression in sarcomatous tissues correlates with immune infiltration. First, the The Cancer Genome Atlas (TCGA) database (https://www.cancer.gov/ccg/research/genome-sequencing/tcga) was used to analyze the correlation between NELL-1 expression and immune cell infiltration into sarcomatous tissues (Fig. 5A). It was found that the levels of natural killer (NK) cells and NELL-1 had the highest positive correlation, whereas those of dendritic cells (DCs) and NELL-1 had the highest negative correlation. Next, the relationship between the enrichment fraction of NK cells and DCs and the expression level of NELL-1 was studied, which was subsequently determined to be statistically significant ($P<0.05$ in NK cells, $P<0.001$ in DC; Fig. 5B). Finally, the relationship between the purity and infiltration levels of NK cells and DCs and the expression

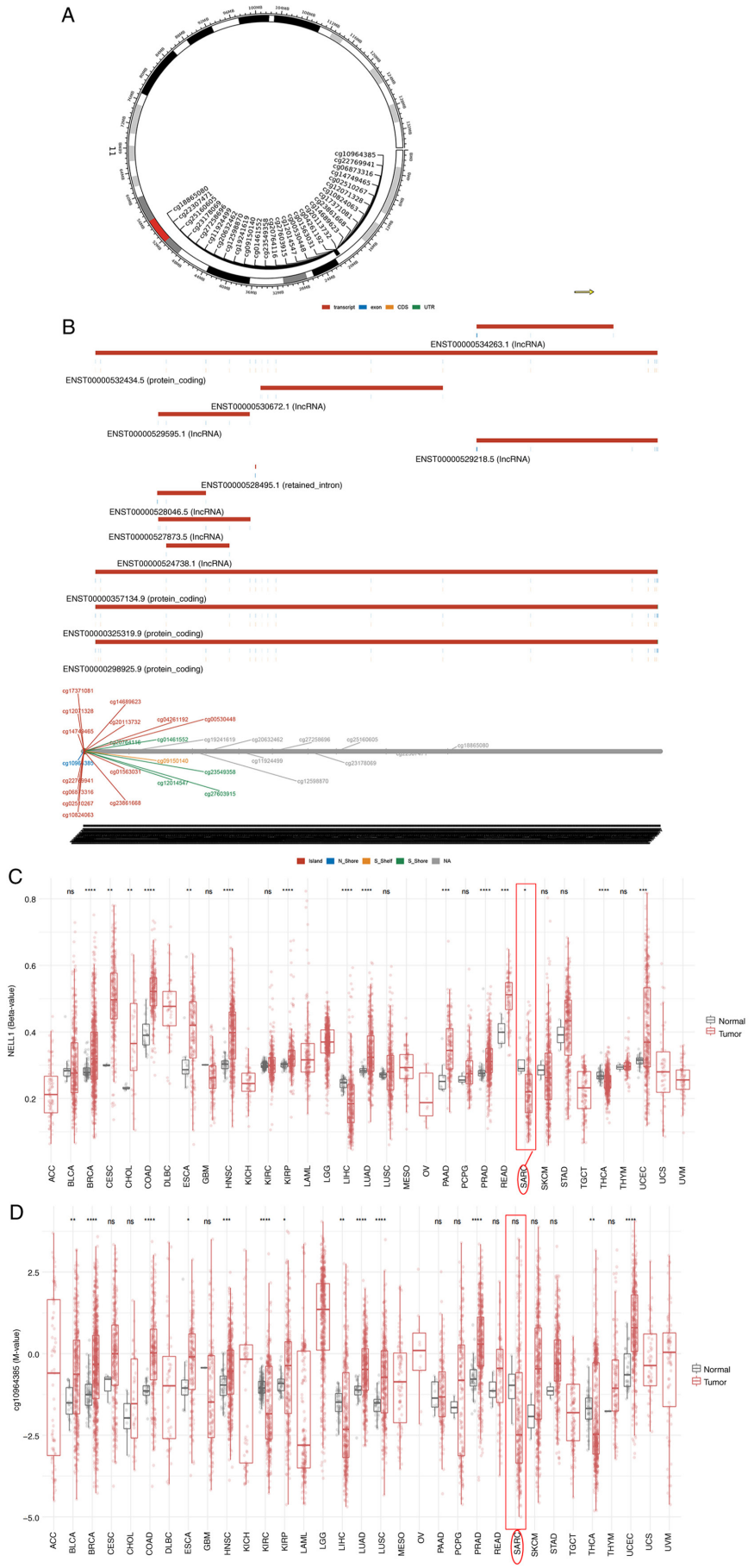

Figure 3. Methylation levels of NELL-1 in sarcoma tissues. (A) Chromosomal distribution of the methylation probes associated with NELL-1. (B) Detailed genomic information on NELL-1. (C) CpG-aggregated methylation values across all samples. (D) Methylation values across all samples. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001; ns, no significance, tumor vs. normal. NELL-1, NEL-like molecule-1.

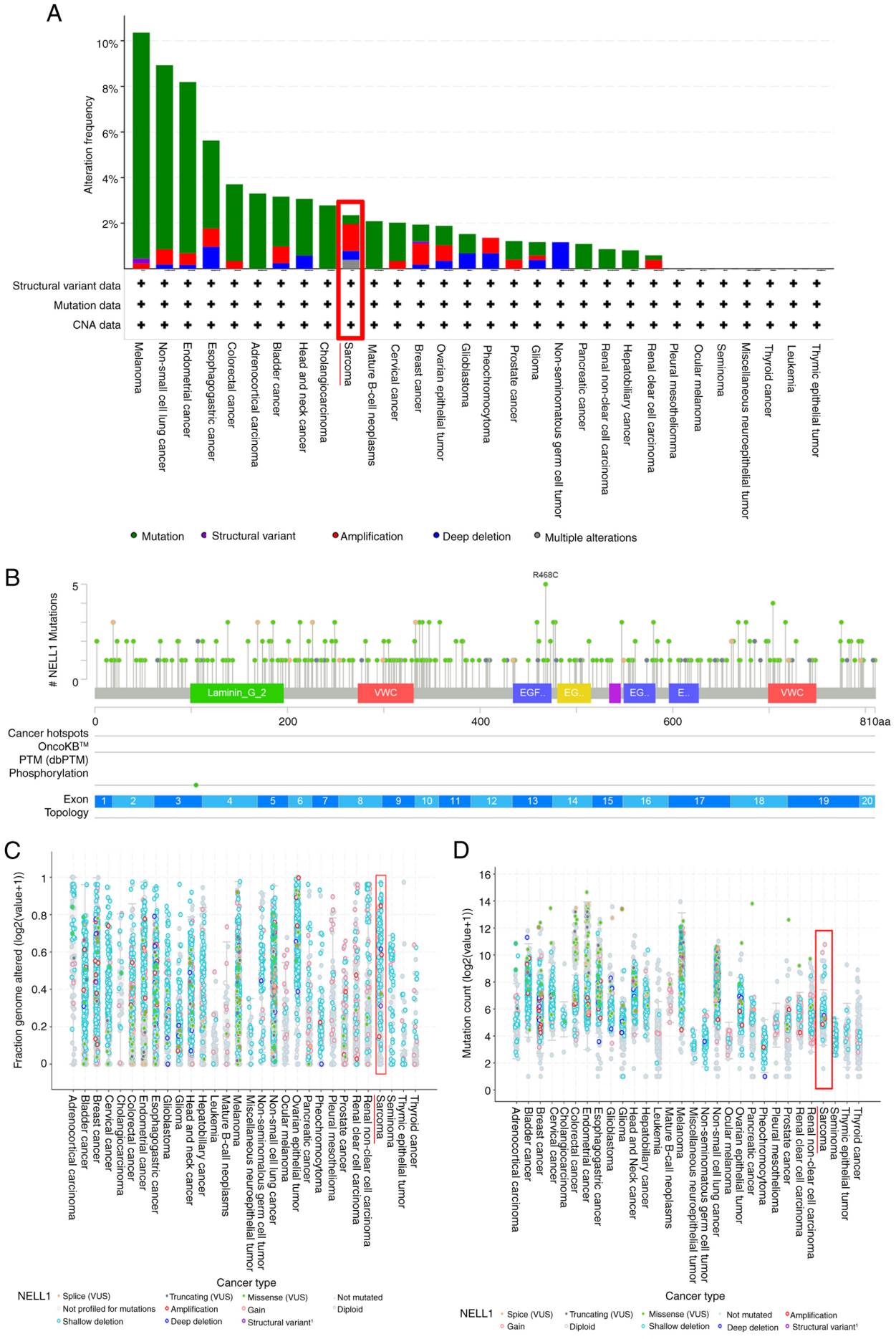

Figure 4. Continued.

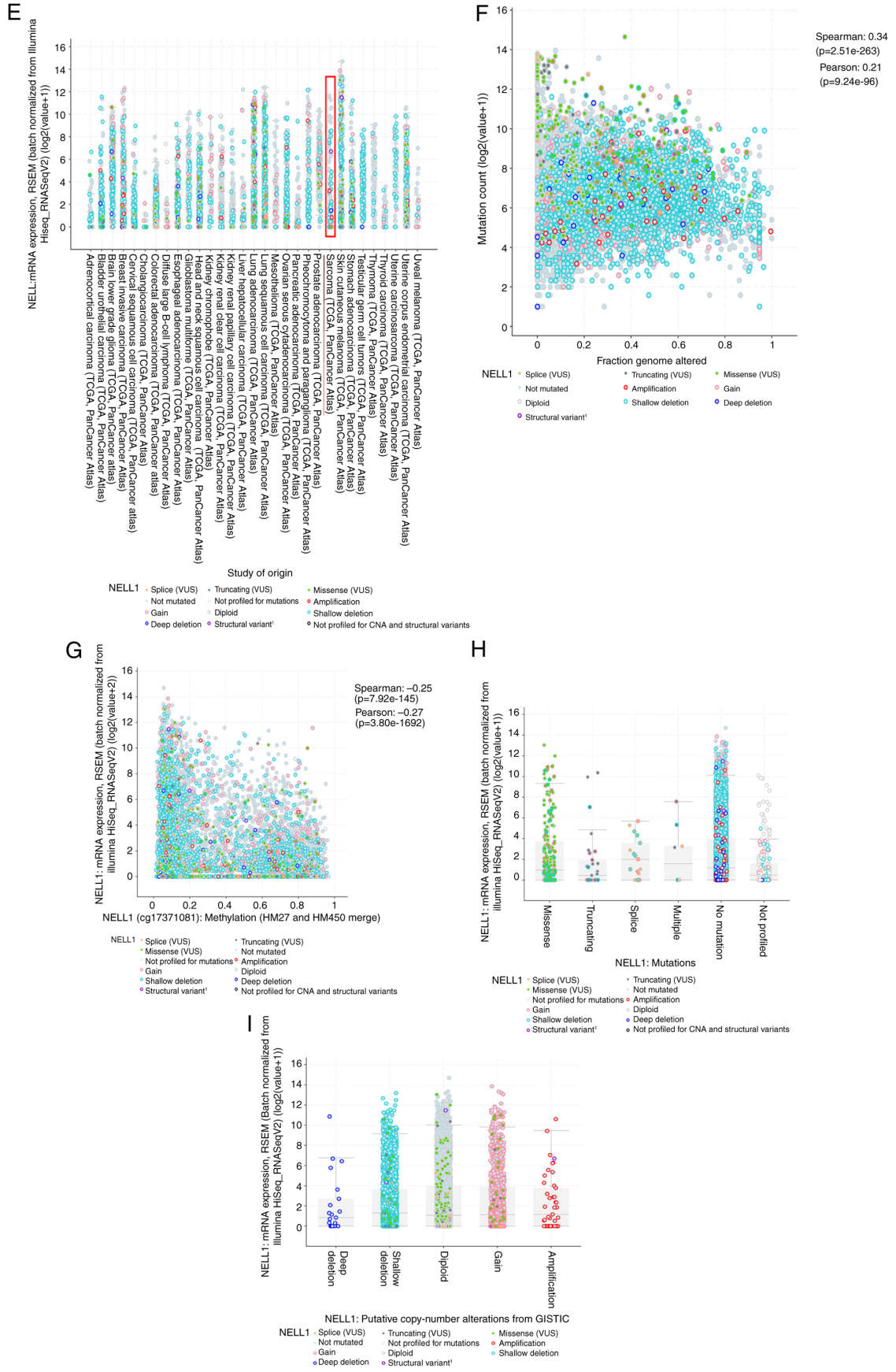

Figure 4. Mutation levels of NELL-1 in sarcoma tissues. (A) Alteration frequency of NELL-1. (B) Lollipop diagram of NELL-1 mutations. (C) Fraction Genome Altered of NELL-1 in different cancer types. (D) Mutation Count of NELL-1 in different cancer types. (E) mRNA expression (RSEM) of NELL-1 in different cancer types. (F) Relationships of NELL-1 between Fraction Genome Altered and Mutation Count. (G) Relationships of NELL-1 between metrology and NELL-1 mRNA expression. (H) Relationships between NELL1 mutations and NELL1 mRNA expression (RSEM). (I) Relationships of NELL-1 between NELL-1: Putative copy number alterations from GISTIC and NELL-1 mRNA expression (RSEM). NELL-1, NEL-like molecule-1; RSEM, RNA-sequencing by Expectation-Maximization; CNA, copy number alterations.

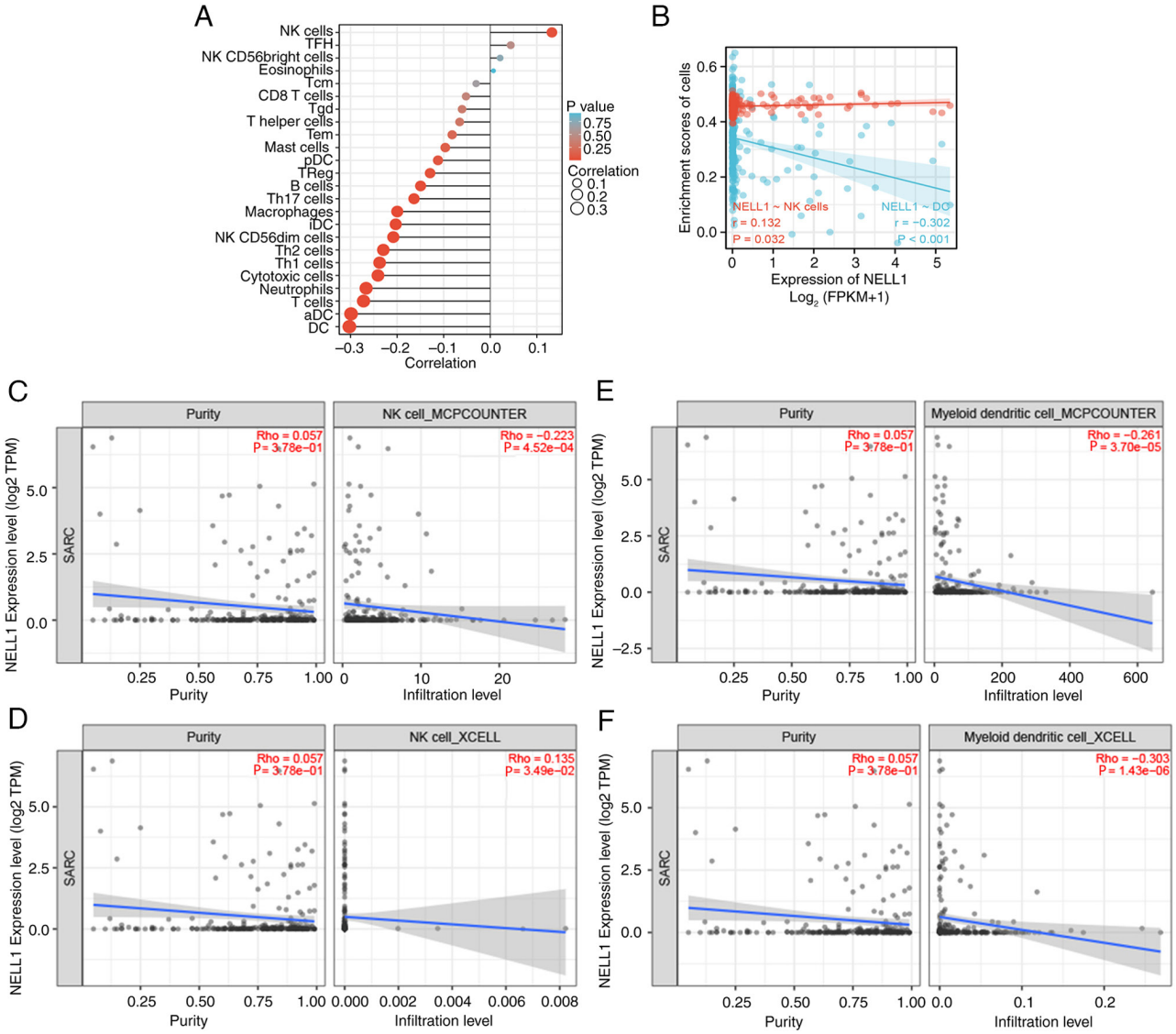

Figure 5. Immune infiltration level of NELL-1 in sarcoma tissues. (A) Association between immune cell infiltration and NELL-1 expression. (B) Correlation between the enrichment fraction of NK cells and DCs and NELL-1 expression. (C) Correlation between NK cell purity and infiltration and NELL-1 expression by MCPcounter. (D) Correlation between NK cell purity and infiltration and NELL-1 expression by XCELL. (E) Correlation between DC purity and infiltration and NELL-1 expression by MCPcounter. (F) Correlation between DC purity and infiltration and NELL-1 expression by XCELL. NELL-1, NEL-like molecule-1; NK, natural killer; TFH, T follicular helper; Tcm, natural killer; DC, dendritic cell; pDC, plasmacytoid DC; Treg, regulatory T; iDC, immature DC; aDC, activated DC.

level of NELL-1 in sarcomatous tissues were studied using the TIMER2.0 tool (http://timer.cistrome.org/), and the results are summarized in Fig. 5C-F. Specifically, the first focus was on NK cells. Using the MCPCOUNTER algorithm, no significant correlation between the purity of NK cells and NELL-1 expression levels was found ($P=3.78x10^{-1}$); however, a negative correlation was found between the infiltration level of NK cells and NELL-1 expression levels ($P=4.52x10^{-4}$) (Fig. 5C). Using the XCELL algorithm, no significant correlation was found between the purity of NK cells and NELL-1 expression levels ($P=3.78x10^{-1}$); however, a negative correlation was detected between the infiltration level of NK cells and NELL-1 expression levels ($P=3.49x10^{-2}$) (Fig. 5D). The subsequent focus was on DCs. Using the MCPCOUNTER algorithm, no significant correlation was found between the purity of DCs and NELL-1 expression levels ($P=3.78x10^{-1}$); however, a negative correlation was noted between the infiltration level of DCs and NELL-1 expression levels ($P=3.70x10^{-5}$) (Fig. 5E). Using the XCELL algorithm, no significant correlation between the purity of DCs and NELL-1 expression levels was found ($P=3.78x10^{-1}$); however, a negative correlation was noted between the infiltration level of DCs and NELL-1 expression levels ($P=1.43x10^{-6}$) (Fig. 5F).

Future research is needed to further clarify the basic biological, diagnostic and prognostic significance of NELL-1 in bone tumors.

5. Conclusion

Research on NELL-1 and bone tissue diseases is gaining increasing attention. To date, >80 relevant studies, including 10 reviews on NELL-1 and bone tissue, have been published; however, the current review was mainly conducted from a partial perspective and the findings (NELL-1 can induce

osteogenesis) are not comprehensive. For instance, certain studies reviewed the therapeutic effects of NELL-1 in orthopedic surgeries such as spinal fusion; however, NELL-1 only makes up a small part of an array of other growth factors (21,23,24,26,30). Another study reviewed the efficacy of NELL-1 in osteoporosis; however, osteoporosis is only a small aspect of metabolic diseases (27). A study reviewed the relationship between NELL-1 and osteogenesis, but it was only limited to stem cells (29). Another study explored the relationship between NELL-1 and RUNX2 in dental diseases (22). Other reviews were conducted on the osteogenic effects of NELL-1, similar to the current review, but from a different perspective, such as different functions of NELL-1, different sites of NELL-1 and different applied models of NELL-1 (25,28,38). Therefore, the present review provides a more systematic and comprehensive overview of all relevant studies in the literature.

This review had certain limitations. Although it was attempted to collect all relevant research data, the results may not be complete because of limitations in search platforms and language. Furthermore, the present review mainly focused on categorizing and summarizing the results and the scope for discussion was slightly insufficient.

Bone remodeling is a complex process (121). As a newly discovered protein, NELL-1 has been found to induce osteogenesis, which can promote the differentiation of stem cells into osteoblasts and inhibit the differentiation of stem cells into adipocytes. First, NELL-1 binds to cells through integrin β1 adhesion (67) and then acts as a ligand to specifically bind to Cntnap4 or Robo2 on the cell surface (63,64) or combines with APR3 (68), exerting biological effects. The subsequent effect on stem cell differentiation is mediated by the transduction of a series of signaling pathways, such as BMP, Hedgehog, RUNX2 and Nfatc, thereby affecting the differentiation of stem cells into osteoblasts. Despite its osteogenic capabilities, it is not biologically stable, which markedly limits its development. Through a series of pharmacokinetic changes, modification with organic polymers can increase its biological stability without weakening its ability to induce osteogenesis. Furthermore, when combined with other drugs, such as BMP, its osteogenic effects can be markedly improved. To date, NELL-1 has been used in several *in vivo* bone tissue engineering studies and has achieved some notable results. In the future, the related pathways and pharmacokinetics of NELL-1 need to be further studied before it can be considered a new target for promoting osteogenesis.

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Authors' contributions

YT contributed to the study's conception and design, performed the literature selection/review and wrote and edited the manuscript. ZL performed language editing, tabulation and drawing, and bioinformatics predictions. All authors have read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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