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# Potential Role Of Netrin-1 In Rheumatoid Arthritis

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**Abstract**

Netrin-1, originally recognized for its role in guiding axonal growth, has recently been shown to interfere with monocyte movement by blocking chemokine-driven migration. Although it plays a crucial part in neuroimmune signalling, its specific function in osteoclast biology remains largely unclear. Notably, elevated levels of netrin-1 have been observed in the synovial fluid of individuals with rheumatoid arthritis. This protein is highly produced by osteoblasts and synovial fibroblasts, and its expression is markedly upregulated in response to interleukin-17 (IL-17). When netrin-1 interacts with its receptor UNC5b on osteoclasts, it triggers SHP1 activation, which in turn suppresses VAV3 phosphorylation and RAC1 activity. This chain of events disrupts actin filament organization and cell fusion processes essential for osteoclast function without affecting their overall differentiation. Remarkably, administering netrin-1 in experimental models of autoimmune arthritis and aging-related bone loss significantly reduced bone damage. These findings highlight the netrin-1/UNC5b signalling pathway as a promising target for treating diseases that involve bone degradation.

**Keywords**: Arthritis, Interleukin 17A,Osteoblast,Osteoclast,Osteoporosis, Netrin 1, fusion, Rheumatoid

## Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune condition that leads to progressive bone destruction. It is typically marked by the abnormal proliferation of synovial fibroblasts, intense inflammation within the joints, and significant bone erosion largely driven by overactive osteoclasts (1). Derived from the macrophage lineage, osteoclasts are sizable cells containing multiple nuclei (2). Both osteoblasts and synovial fibroblasts produce key cytokines such as RANK ligand (RANKL) and macrophage colony-stimulating factor (M-CSF), which stimulate myeloid precursor cells and initiate a cascade of transcriptional activity. This process involves the activation of several key transcription factors, including nuclear factor of activated T cells, cytoplasmic 1 (NFATc1) (3), Jun dimerization protein 2 (Jdp2) (4, 5), the proto-oncogene c-Fos (6), and nuclear factor kappa B (NF-κB) (7). In rheumatoid arthritis (RA), Th17 cells which are notable for their secretion of interleukin-17 (IL-17) play a direct role in promoting osteoclast formation through the release of RANKL, making them central contributors to excessive osteoclast activity (8).

Among the proteins upregulated by RANKL, ATP6v0d2, Sbno2, and DC-STAMP are crucial for the fusion of osteoclast precursors. Evidence from knockout mouse models indicates that the absence of these proteins prevents the formation of large, multinucleated osteoclasts and results in a mild osteoporotic phenotype. Notably, despite defective cell fusion, these mice still exhibit normal expression levels of typical osteoclast differentiation markers, highlighting the pivotal role of precursor fusion in effective bone resorption (9–11).

Denosumab, a monoclonal antibody that specifically binds to RANKL, inhibits osteoclast differentiation and is commonly employed in the treatment of both rheumatoid arthritis and osteoporosis (12, 13). However, concerns have arisen regarding its side effects, including increased risk of infections, low blood calcium levels, and jawbone necrosis (14). This has prompted interest in exploring strategies that selectively disrupt osteoclast multinucleation without broadly inhibiting osteoclast formation. While actin polymerization a process vital to the motility and fusion of osteoclast precursors is known to be a central component of multinucleation (15), the specific soluble mediator that governs this step remains to be identified.

Netrins are a class of proteins involved in axon guidance, named after the Sanskrit word "netr," meaning "one who guides." These proteins are conserved across species, with nematode worms, fruit flies, frogs, mice, and humans all sharing DNA encoding netrins. Structurally, netrins are similar to laminin, a protein found in the extracellular matrix. As chemotropic agents, netrins influence the direction of axonal growth, either attracting or repelling developing axons based on their concentration gradient. Axonal attraction to netrins is mediated by UNC-40/DCC (Deleted in Colorectal Cancer) cell surface receptors, while repulsion is facilitated by UNC-5 receptors.

Netrin-1 is well known for its role in guiding axons during neural development, where it influences axonal movement by modulating the cell's cytoskeleton, leading to either attraction or repulsion (16–19). This duality in function largely depends on the type of receptor expressed on the surface of neural cells. The presence of the UNC5b receptor typically causes axonal repulsion, while the DCC receptor is associated with axonal attraction (20). Beyond its neurological role, netrin-1 has also been shown to inhibit monocyte migration by altering cytoskeletal dynamics in cells of the myeloid lineage (21, 22). Previous studies have also demonstrated that human osteoblasts express netrin-1 (23). Together, these findings raised the possibility that netrin-1 might also influence osteoclast multinucleation, prompting further investigation into its role in bone biology.

**Structure of netrin-1 and its receptors:**

Netrin-1 is a protein made up of 604 amino acids and is organized into three main structural domains. At its N-terminal region lies the highly conserved laminin domain, also called domain VI (LN). This is followed by domain V, which contains three cysteine-rich laminin-type epidermal growth factor (EGF)-like repeats—designated as LE1, LE2, and LE3. The C-terminal region features a small, positively charged segment known as the netrin-like (NTR) module. Domains VI and V are crucial for the interaction of netrin-1 with its receptors, particularly those in the UNC5 and DCC families. The DCC receptor itself is structured with four immunoglobulin-like domains and six fibronectin type III domains, enabling its binding to netrin-1 and mediating downstream signalling.

**Figure 1: Structure of netrin-1 and its receptors (24)**



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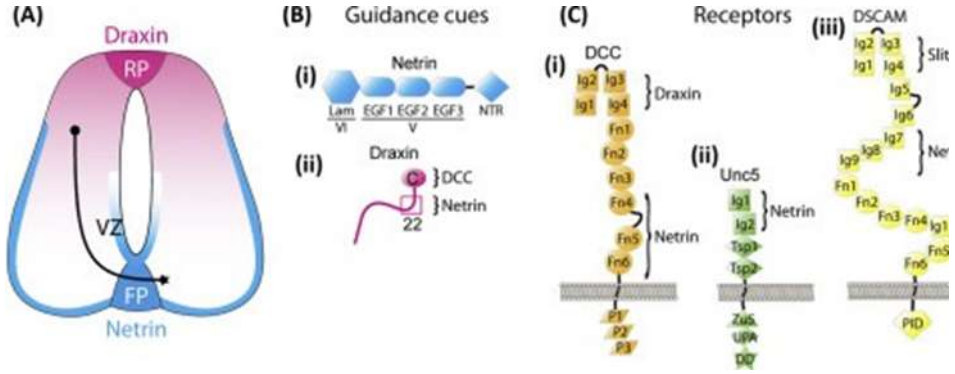


Netrin-1 plays a complex role in the regulation of inflammation, acting as both an anti-inflammatory and pro-inflammatory factor depending on the surrounding physiological or pathological conditions. This functional duality reflects the nuanced ways in which netrin-1 can influence immune activity, positioning it as a promising therapeutic target for inflammatory disorders. The term "anti-inflammatory" refers to any process or mechanism that works to reduce or control inflammation in the body. Inflammation itself is a protective response triggered by infection, injury, or harmful stimuli, involving the coordinated action of immune cells, blood vessels, and various signalling molecules. While this response is essential for healing and defence, unchecked or chronic inflammation can lead to tissue damage and is a contributing factor in many long-term illnesses (24).

**Netrin-1 Inhibits Osteoclast Multinucleation**

In patients with rheumatoid arthritis (RA) who exhibit elevated levels of netrin-1, the bone resorption marker known as human type I collagen cross-linked C-terminal telopeptide (CTXI) is found at relatively lower concentrations. Furthermore, in individuals with osteoarthritis (OA), synovial fluid analysis revealed an inverse relationship between netrin-1 and CTXI levels. Nonetheless, no association was found between CTXI and IL-17 levels in the synovial fluid of patients with either OA or RA. Histological analysis of the tibial metaphysis in mice deficient in netrin-1 expression showed a notable decrease in trabecular bone, along with a significant rise in both osteoclast numbers and nuclei per cell. On a molecular level, netrin-1 inhibits the VAV3-RAC1 signalling cascade through its binding to UNC5b, a known receptor on osteoclasts (25). These results indicate that netrin-1 specifically restricts the multinucleation of osteoclasts while leaving their general differentiation process unaffected.

**Netrin-1 as a Protective Agent against Bone Damage in Arthritis**

Given its function in inhibiting osteoclast fusion, the potential of recombinant netrin-1 as a therapeutic agent was assessed using a collagen-induced arthritis (CIA) mouse model. At six weeks of age, mice were initially immunized with type II collagen, followed by a secondary booster dose three weeks later. Following immunization, the animals received intravenous injections of either netrin-1 or a phosphate-buffered saline (PBS) control. Although netrin-1 did not significantly alter clinical scores, paw swelling, or circulating pro-inflammatory cytokines, it notably reduced bone erosion. Histological examination showed that inflammation scores were comparable between netrin-1– and PBS-treated mice; however, the number of osteoclasts was substantially lower in the netrin-1 group.

Further evidence came from a collagen antibody-induced arthritis (CAIA) model using adult mice heterozygous for netrin-1. While these mice showed normal clinical and inflammation scores, the extent of bone erosion was significantly greater compared to controls, and osteoclast numbers in the calcaneus were elevated. Collectively, these results support the conclusion that netrin-1 plays a protective role in autoimmune arthritis by limiting bone destruction through the inhibition of osteoclast fusion and function.

**Netrin-1 Enhances Bone Mass**

To investigate the impact of netrin-1 on skeletal metabolism, researchers examined how its expression varies with age. Older mice exhibited lower serum levels of netrin-1, along with decreased expression in bone marrow cells and osteoblasts derived from mesenchymal stem cells (MSCs), compared to younger counterparts. The transcription factor p53



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previously identified as an activator of netrin-1 expression (26, 28) was explored to uncover the underlying molecular mechanism. In osteoblasts from aged MSCs, both p53 expression and its binding affinity to the netrin-1 promoter were notably reduced. Furthermore, silencing p53 in osteoblasts led to diminished netrin-1 expression, suggesting that impaired p53 activity plays a key role in the age-related decline of netrin-1.

To assess the biological effects of netrin-1 supplementation, mice at two developmental stages 4 weeks (young) and 20 weeks (adult) received intravenous injections of either netrin-1 or phosphate-buffered saline (PBS). Micro-computed tomography (μCT) and histological analysis revealed that netrin-1 treatment resulted in increased bone volume. Histomorphometric evaluation showed a marked reduction in osteoclast function alongside a rise in bone formation markers. Notably, levels of tartrate-resistant acid phosphatase (TRAP) in bone marrow cells remained within normal ranges, and expression of bone morphogenetic protein-2 (BMP-2) was elevated in the netrin-1–treated group. These findings suggest that netrin-1 enhances bone formation through upregulation of BMP-2, indicating its potential role in preserving bone mass and mitigating age-related bone loss.

**Discussion**

Growing evidence highlights the role of pro-inflammatory cytokines, including IL-17 and TNF-α, as strong inducers of osteoclastogenesis. Although the involvement of osteoblasts and synovial fibroblasts in mediating inflammatory bone degradation is well established, their potential to secrete protective factors during inflammation has been comparatively underexplored. Recent studies have identified netrin-1 as a soluble factor released by both osteoblasts and synovial fibroblasts that inhibits osteoclast multinucleation (25). Of all netrin family members, netrin-1 emerges as the predominant isoform expressed in these cells, with its expression notably enhanced following IL-17 stimulation.

These findings suggest that, in response to acute inflammation, osteoblasts and synovial fibroblasts may promptly release soluble mediators that help limit excessive bone loss. Interestingly, in osteoarthritis (OA) patients, levels of netrin-1 in the synovial fluid exhibit a negative correlation with CTXI, a biomarker for bone resorption. This implies that netrin-1 may act as a suppressor of bone degradation even in conditions where inflammation is not the primary driver. Additionally, studies using arthritis models have shown that treatment with netrin-1 significantly reduces bone erosion, indicating its potential utility as a preventative strategy in Th17-related bone disorders in humans.

Among the various axon guidance molecules secreted by osteoblasts, netrin-1 exhibits the strongest inhibitory effect on osteoclast formation. Comparative analysis have shown that osteoclast differentiation is reduced by roughly 50% with 2 μg/ml of clustered EphB4 (27) and 1 μg/ml of Sema3A; however, Sema3A loses its suppressive effect when applied after RANKL activation (26). Structurally, netrin-1 belongs to the laminin-related protein family and includes domain VI, three EGF-like repeats, and a heparin-binding region features suggest a high affinity for binding within the extracellular matrix. This binding capability likely influences its local availability and stability in tissues. Since osteoblasts and synovial cells are known to produce extracellular matrix components, netrin-1 may be concentrated in their surrounding microenvironments. Still, additional in vivo studies are essential to better understand how netrin-1's distribution and function are regulated within bone tissue.

Netrin-1 functions as a strong inhibitor of osteoclast fusion. Its receptor, UNC5b, is highly expressed on osteoclasts, and upon binding netrin-1, it initiates activation of the phosphatase SHP1. This activation disrupts the signalling pathways involving VAV3 and RAC1 two critical regulators of cytoskeletal reorganization and cell fusion thereby hindering the formation of multinucleated osteoclasts. Notably, diminished SHP1 activity has been associated with increased osteoclast fusion (29). In osteoclasts exposed to netrin-1, there is also elevated expression of ITIM-containing inhibitory receptors such as PIR-B and SIRPα, both of which are known to activate SHP1. This suggests these receptors may contribute to the downstream signalling network initiated by UNC5b. Nevertheless, further investigation is needed to clarify the exact molecular mechanisms through which netrin-1 disrupts osteoclast fusion.

Interestingly, despite netrin-1's established role in inhibiting osteoclast fusion, emerging research



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indicates that it is also expressed in murine osteoclasts and may enhance their differentiation through autocrine signalling mechanisms (30). This is supported by observations that osteoclasts with reduced netrin-1 expression showed increased osteoclastogenesis in vitro (30), suggesting that the loss of netrin-1 may impair internal regulatory pathways critical for balanced osteoclast development. Additionally, treatment with 250 ng/ml of recombinant netrin-1 (R&D Systems) was found to modestly elevate RANKL-induced osteoclast numbers by approximately 1.2-fold (30). Despite these in vitro findings, systemic administration of netrin-1 in vivo has consistently demonstrated a protective effect against bone loss. This discrepancy implies that the experimental conditions in ref. 30 may not fully capture the physiological environment necessary for netrin-1's bone-preserving activity. Further complicating its interpretation, a modest increase in bone mass was also observed in wild-type mice transplanted with fetal liver cells from netrin-1-deficient embryos (30), underscoring the complexity of netrin-1's function and the need for more comprehensive in vivo investigations.

Studies have demonstrated that circulating netrin-1 levels decrease with age, and administration of netrin-1 to healthy mice can inhibit osteoclast fusion, resulting in increased bone mass. Although netrin-1 does not seem to have a direct effect on osteoblast differentiation in vitro, it promotes bone formation in vivo, indicating that its bone-conserving effects may be mediated through indirect mechanisms. This idea is reinforced by findings from knockout models such as DC-STAMP (10) and ATP6v0d2 (9), where reduced osteoclast activity is accompanied by enhanced bone formation. In vitro assays using cells from these knockout mice revealed impaired osteoclast fusion with no observable changes in osteoblast differentiation, suggesting that the fusion process itself may generate signals that contribute to bone formation in physiological contexts.

## Conclusion

Taken together, these findings highlight netrin-1 as a promising prophylactic candidate for the prevention and management of osteoporosis and other bone-degenerative conditions. While most research to date has focused on its role in neurobiology, growing evidence underscores the importance of exploring netrin-1's function in bone metabolism. Future investigations particularly those involving the netrin-1–UNC5b signalling pathway in osteoclasts and studies using conditional netrin-1 knockout models will be crucial to unravelling the full therapeutic potential of this molecule in skeletal health.

## Author's Contribution

All author have contributed equally for bringing this review article effectively.

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