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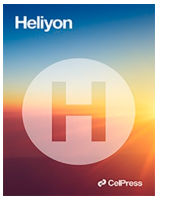

Review article

Siglec-15 as a potential molecule involved in osteoclast differentiation and bone metabolism

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ABSTRACT

Sialic acid-binding immunoglobulin-like lectin 15 (Siglec-15) is a well-conserved type I transmembrane protein of the Siglecs family, distributed in macrophages and dendritic cells in the human spleen and lymph nodes. As an immune receptor, Siglec-15 is expressed in almost all branches of the spinal cord. Siglec-15 participates in the metabolism of the skeleton by regulating osteoclast activity and differentiation and has an influential role in dynamic bone remodelling. The binding of DNAX activation protein of 12 kDa (DAP12), which contains the immunoreceptor tyrosine-based activation motif (ITAM) activation domain, to the Siglec-15 receptor provides a positive stimulatory signal for osteoclast growth, with the involvement of the receptor activator of nuclear factor-κB (RANK)/RANK ligand (RANKL) signalling. Recently, Siglec-15 antibodies have been shown to effectively prevent bone resorption in mouse models of osteoporosis and accelerate fracture healing to some extent. Therefore, exploring the molecular characteristics and functions of Siglec-15 may lead to new therapeutic strategies for common clinical skeletal diseases.

1. Introduction

Sialic acid-binding immunoglobulin (Ig)-like lectins (Siglecs) are crucial biological components of the immune system [1]. The transmembrane immunoreceptor family is expressed mainly in leukocytes and mediates intracellular signalling, intercellular adhesion, and peripheral immune responses by binding to cell-surface glycans [2,3]. Glycans act as signalling molecules for the immune system by binding terminal-specific sialic acid (Sia) to their receptors (e.g. Siglecs) to form complexes that initiate cellular transduction pathways and regulate immune system homeostasis [2,4,5]. Growing evidence suggests that Siglecs may act as potential therapeutic targets for diseases such as cancer, autoimmune, cardiovascular, and pulmonary diseases [6–8]. Multiple Siglecs subtypes, including 8 in mice and 15 in humans have been identified [9]. The human Siglec family is divided into classical Siglecs (Siglec-1,-2,-4, and-15) and Siglec subtypes related to cluster of differentiation (CD) 33 with similar sequence homology [10]. The classic type is highly conserved and has a low sequence homology in mammals, whereas other subtypes show high sequence similarity among different subspecies [11].

In 2007, Angata et al. identified a new member of the Siglec family, Siglec-15, present in myeloid cells [12,13]. Siglec-15 has recently been reported to be strongly associated with the risk of tumours, bone metabolic diseases, inflammation, and the development of infectious diseases [14]. Unlike other family members, Siglec-15 is highly conserved during vertebrate evolution and regulates osteoclast development to influence bone resorption and bone remodelling [12]. The effect of Siglec-15 on osteoclasts has been shown

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to be associated with DNAX activation protein of 12 kDa (DAP12), and the Siglec-DAP12 axis promotes cell differentiation and maturation by regulating downstream signalling molecules in osteoclasts [12,15]. Researchers have increasingly focused on Siglec-15; however, the mechanisms underlying its role in osteoclasts and the effects and treatments of bone-related diseases have not yet been comprehensively described. In summary, this review emphasizes the findings of previous studies and summarizes the effects of Siglec-15 on osteoclast development to provide potential molecular directions for immunotherapy in related bone metabolic diseases.

2. Molecular characteristics of Siglec-15

2.1. Structure and cellular location of Siglec-15

Siglec-15, previously known as CD33-related Siglecs [12], is a type I transmembrane protein with an activating signal located on human chromosome 18q12.3 [11]. Differences in the expression of Siglec-15 have been reported in human and mouse immune cell populations, with expression almost exclusively in osteoclasts in mice and widespread expression in haematopoietic and immune cells in humans [16] The DNA sequence of Siglec-15 protein has been detected in all vertebrates throughout biological evolution, and the cysteine residues in Siglec-15 are consistent with those of other members of the Ig family, which are evolutionarily similar [12,17]. In terms of molecular structure, Siglec-15 is similar to other proteins of the Siglec family members and consists of three classical domains: (1) a V-set Ig-like extracellular structural domain that mediates Sias binding at the N-terminus, as well as a constant C2 structural domain, (2) a transmembrane binding site, and (3) a short cytoplasmic tail (containing a YENL motif) [12,14,18,19] (Fig. 1).

Furthermore, Siglec-15 retains an arginine residue (R143) located in the IgV structural domain and, therefore, recognizes glycans in a similar manner as the other family members, and similarly retains aromatic amino acid residues known as a hallmark structure of Siglecs (one at the N-terminal end and the other near residue R143 [12]. Although Siglec-15 is structurally typical and conserved, it possesses specific properties with respect to general Siglec subtype genes. Siglec-15 contains two additional exons upstream of the first Ig-like structure; the transmembrane binding domain may be responsible for the exon encoding the second Ig-like structure, whereas the other subtypes require different exons encoding the structural domains of the second Ig and transmembrane domain [12]. Second, the amino acid sequence of the extracellular structural domain in Siglec-15 shares homology with members of the B7 family, and plays a role in the regulation of the tumour immune microenvironment, cell proliferation, and cytokine secretion. Therefore, the Siglec-15 and B7 genes have similar potential to regulate immunity [20]. Specifically, R143 within the Ig-v set region of Siglec-15 regulates cellular activation within the immune system by recognizing and binding to sialylated glycoproteins, thus participating in the formation of actin rings within multinucleated cells and maintaining the basic cellular skeleton [15]. Most Siglec family members contain an immunoreceptor tyrosine-based inhibitory motif (ITIM), a signalling molecule that inhibits cellular transduction within the cytoplasmic region [5]. Conversely, Siglec-15, which does not have an intracellular structural domain nor an ITIM, relies on positively charged residues as binding sites to interface with activating molecules containing active motifs based on immunoreceptor tyrosine-based active motif (ITAM) (e.g., DAP12 or DAP10), recruits spleen tyrosine kinase (Syk) to stimulate downstream signalling in response to immune activation, and plays an important role in the regulation of osteoclast development [2,15].

Thus, the above findings provide evidence indicating that Siglec-15 was highly conserved during the evolution of chordates and may represent an effective disease target. In conclusion, throughout the evolutionary process, Siglec-15 has been endowed with properties that play an important role in the immune response while retaining a typical structure.

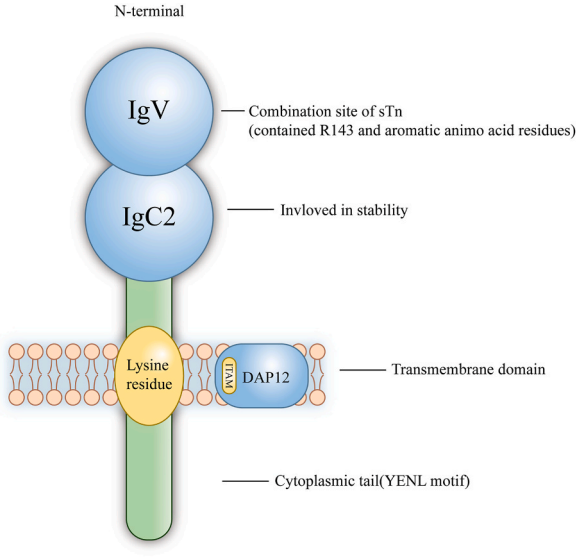

Fig. 1. Molecular structure of Siglec-15.

2.2. Ligands of Siglec-15

The mammalian spectrum is abundant in Sia and can be used as a recognition marker for its molecules by binding to Sia receptors. Sia is a nine-carbon monosaccharide present at the termini of cell surface glycans; however, only N-acetylneuraminic acid (Neu5Ac) is expressed in human cells [10,19,21]. Different Siglec proteins express corresponding dominant sialylated polysaccharides that induce cellular activation or inhibit signalling by preferentially binding to ligands. Previous studies reported that α2,6-glycoside-bonded sia is the preferred ligand for Siglec-15, for example, the structural domain of the N-terminal V set of human and murine Siglec-15 specifically recognizes the Neu5Acα2-6GalNAc (sTn) sialylated glycan chain structure [5,14]. However, murine Siglec-15 can also bind to Neu5Acα2-3Galβ1-4Glc to form a complex that positively regulates the cell differentiation process [12,18]. However, Mohammed et al. showed that Siglec-15 tends to show a weak affinity for α2,6-linked glycans, but a high glycan binding preference for α(2,3)-α(2,6) disialic acid glycosides. Sulfation can affect the binding affinity of Siglec-15 to sialoglycans, and this specific combination is driven by carbohydrate sulfotransferase 1(CHST1) [18]. In general, glycan ligands appear to play a critical role in Siglec-15-stimulated signalling, and different mammalian species may have different dominant Siglec-15 ligands. However, this unknown complex system remains to be revealed.

3. Siglec-15 in bone homeostasis

3.1. Property of osteoclasts

Bone resorption and new bone formation during dynamic bone remodelling depend on the cooperative and coordinated participation of osteoclasts and osteoblasts [22]. Therefore, normal osteoclast function is the key to maintaining bone homeostasis. Siglec-15 influences the bone resorption process during bone remodelling by regulating osteoclast differentiation and function.

Osteoclasts are multinucleated cells formed by differentiation of the monocyte/macrophage lineage and are transformed from mononuclear precursors to multinucleated tartrate-resistant acid phosphatase (TRAP)-positive cells after cellular and intercellular fusion [23]. Mature osteoclasts have a cytoskeletal structure that mediates bone resorption; when osteoclasts attach to the bone matrix, the annular sealing zone formed with actin (fixes bone resorption areas) and the ruffled border (secretes bone resorption factors) coordinate to control bone resorption [24,25]. As immune cells, the fusion and maturation of osteoclasts require the involvement of a large number of cytokines, growth factors, and intercellular co-stimulatory signals. Macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor-kappa B ligand (RANKL) signalling from the osteoblast lineage promote the differentiation of osteoclast precursors to mature osteoclasts, and co-stimulation with RANKL/receptor activator of nuclear factor-kappa B (RANK) regulates the formation of nuclear factor-activated T cells c1/2 (NFATc1/NFATc2) [26]. Similarly, ITAM signalling can also stimulate calcium flux to initiate activation of NFATc1, a major transcription factor essential for osteoclast differentiation and closely related to the multinucleated state of functional osteoclasts [15,27–29]. Osteoclast immunoreceptors with activating molecules containing ITAM signalling junctions such as the common chain of the DAP12 and Fc receptor common γ-chain (FcRγ) can mediate cell stimulation signalling, and DAP12 predominates in osteoclast differentiation [30]. DAP12-associated receptors (DARs) include the triggering receptor expressed on myeloid cells 2 (TREM-2), the signalling regulatory protein beta 1 (SIRPβ1) and Siglec-15 [31,32]. FcRγ binds to three other receptors, including osteoclast-associated receptor (OSCAR), paired immunoglobulin-like receptors A (PIR-A) and the Fc receptor, and the above specific receptors combine with ligands to form complexes and then regulate osteoclast maturation and differentiation [33,34].

3.2. Siglec-15 in osteoclast differentiation

Siglec-15 is specifically involved in osteoclast maturation triggered by RANKL and tumour necrosis factor alpha (TNF-α). Hiruma et al. first found that the expression of a new gene called Siglec-15 was significantly upregulated in giant cell tumours of the bone (GTC), which are characterized by osteolytic lesions, revealing new findings regarding the osteoclast genotype [35]. Subsequently, it was reported that Siglec-15 protein expression was significantly increased in osteoclast differentiation induced by both the mouse macrophage cell line RAW264.7, and primary human cell HOPs, and treated RAW264.7 cells exhibited intense immunofluorescent staining for Siglec-15 [36]. To verify whether this protein is an osteoclast-specific sialic acid-like lectin in vitro, the expression of only Siglec-15 mRNA was significantly up-regulated in mouse bone marrow macrophages (BMMs) in several Siglec members (Siglec-1, -3, -15, and -H) induced by RANKL [37]. Subsequently, BMMs derived from Siglec-15$^{-/-}$ mice treated with RANKL and M-CSF showed a significant downregulation of intracellular TRAP activity compared to wild-type (WT) BMMs; therefore, deletion of Siglec-15 blocked osteoclast differentiation [38]. Although the regulation of osteoclast differentiation is mainly associated with RANKL and M-CSF, TNF-α also plays an important role in pathological bone loss and joint destruction. During menopause, activated T cells increase the secretion of TNF-α, which binds to the TNF receptor P55 and indirectly induces osteoclast formation through the phosphoinositide 3kinase/serine-threonine kinase (PI3K/Akt) signalling pathway in synergy with M-CSF and RANKL [39,40]. Secondly, TNF-α can increase the level of sialylation of osteoclast precursor cells and stimulate osteoclast differentiation independently of the RANKL/-RANK system via the presence of M-CSF [41,42]. Siglec-15, in addition to its role in RANKL-induced osteoclast development is also required for TNF-α stimulation of osteoclast differentiation. Kameda et al. found that the induction of osteoclast marker genes such as TRAP and cathepsin K was suppressed in Siglece-15$^{-/-}$ BMMs compared to WT BMMs on the fifth day of TNF-α induction, suggesting that the development of functional osteoclasts in vitro induced by TNF-α requires the involvement of Siglec-15 [43]. Therefore, the above results suggest that Siglec-15 is closely related to osteoclast differentiation and is a specific receptor protein of sialic acid-binding

immunoglobulins that regulate the role of resorption in bone reconstruction.

Siglec-15 is involved in the formation of functional osteoclasts. Multinucleation is one of the characteristic manifestations of osteoclasts and a sign of cell maturation because multinucleated cells tend to possess greater bone resorption activity and can exert more powerful biological functions than mononucleated cells [44]. Although Siglec-15 knockdown cells were able to form TRAP-positive cells, most were mononuclear, and the number of TRAP-positive multinucleated cells were significantly lower in Siglec-15$^{-/-}$ cells than in WT cells [14,45,46]. Furthermore, Siglec-15$^{-/-}$ cells were smaller, contracted, and were unable to spread on the culture plate. Under conditions of abnormal cell morphology, reduced bone resorption by osteoclasts has been observed in dentin sections [15]. Importantly, TRAP-positive cells formed by Siglec-15$^{-/-}$ BMMs lack actin ring structures and even multinucleated cells, which may be related to the regulation of cytoskeletal remodelling by Siglec-15/DAP12 [16,43,45,47]. Therefore, the formation of functional osteoclasts requires the activation of Siglec-15, and blocking this protein inhibits bone resorption in the organism. Stuible et al. showed that activated RAW264.7, began to fuse into osteoclast-like cells after 3 days, and the expression of Siglec-15 mRNA was later than that of TRAP, NFATc1, and cathepsin K and showed increased expression on day 2 [36]. This suggests that Siglec-15 may be expressed before osteoclast precursor fusion and is induced in the later stages of RANKL stimulation, which may provide a potential clinical target for the treatment of Siglec-15-mediated bone-resorptive diseases.

To investigate the effects of this molecule on bone resorbing cells and bone physiology under in vivo conditions, several groups have established genetic mouse models harbouring Siglec-15 deletion to validate the effects of Siglec-15 on bone metabolic diseases in the organism [16,43,45–49]. Siglec-15$^{-/-}$ mice grew well with no obvious abnormalities in appearance, while c-fos and RANK knockout mice showed impaired development of long bones and impaired tooth eruption. Therefore, Siglec-15 may not have a greater negative impact on other tissues and organs throughout the body [38,45]. Further studies showed that Siglec-15$^{-/-}$ mice exhibit a mild osteosclerosis phenotype. In the lumbar spine and femur, bone markers, such as bone mineral density (BMD), bone volume fraction (BV/TV), and trabecular thickness (Tb. Th) levels were significantly higher than those of WT mice, accompanied by a decrease in bone resorption markers (urinary deoxyridinoline) [16,38,45]. This indicates that Siglec-15 participates in bone remodelling by affecting osteoclast activity. Additionally, to further clarify the role of other DARs in skeletal development, Kobayashi et al. constructed several mice deficient in DAR, showing that Siglec-15$^{-/-}$FcR γ$^{-/-}$ mice exhibited more severe osteosclerotic characteristics than Siglec-15$^{-/-}$ mice, while the bone mass of other DAR knockout mice (FcR γ$^{-/-}$, Trem-2$^{-/-}$FcR γ$^{-/-}$, and C-type lectin domain containing 5a (Clec5a)$^{-/-}$FcR γ$^{-/-}$) did not show any abnormal alterations in bone mass, which reinforces the fact that Siglec-15 dominates the DAR and is essential in osteoclasts to maintain bone mass [46]. In addition, DAP12 gene-deficient mice tend to exhibit osteosclerosis, and their clinical manifestations are similar to those of Siglec-15$^{-/-}$ [27], it is speculated that there may be a cascade relationship between Siglec-15-DAP12 mediating the biological effects of osteoclasts, or that each of them superimposes bone resorption to regulate bone metabolism. Kameda et al. found that Siglec-15$^{-/-}$ mice were resistant to bone loss induced after ovariectomy (OVX) compared to WT mice, and their osteoclasts could not spread on the bone surface in secondary sponges. Thus, blocking Siglec-15 attenuated the development of functional osteoclasts induced by acute oestrogen deficiency, which has some potential to combat osteoporosis [43]. Surprisingly, although osteoclasts were present in the primary spongiosa of Siglec-15$^{-/-}$ mice, there were fewer multinucleated osteoclasts in the secondary spongiosa, whereas Siglec-15$^{-/-}$ osteoclasts could restore the cytoskeleton and multinucleation in the presence of type II collagen and bone matrix [45]. This suggests that expression of the Siglec-15 gene is particularly critical for secondary spongiosa differentiation and that OSCAR/FcRγ signalling can act synergistically with signals activated by certain factors contained in the bone matrix to rescue the developmental defects of Siglec-15$^{-/-}$ osteoclasts in primary spongiosa [45]. This may also explain why Siglec-15-deficient mice exhibit mild osteosclerosis. Therefore, these findings suggest that osteoclast differentiation requires Siglec-15/DAP12 and OSCAR/FcRγ signalling, which together orchestrate the differentiation and maturation of functional osteoclasts.

Siglec-15 may also be involved in the development of pathological arthritis, in addition to affecting the long bones of the body. To date, 2 groups have successfully constructed antigen-induced and serum-induced arthritic Siglec-15$^{-/-}$ model mice, respectively [16,47]. In an antigen-induced arthritis (AIA) model, Siglec-15 knockout mice lost significantly less bone around the joints than that in the WT group, but there was no significant difference in bone erosion area or extent of inflammation [47]. This is in contrast to the findings of Dam et al., who noted that compared to the WT group, Siglec-15$^{-/-}$ mice had significantly less pathological bone erosion area and osteoclast counts in a serum transfer-induced arthritis model and no significant differences in the extent of inflammation [16]. We believe that this difference is probably related to the inconsistency of the conditions used to induce rheumatoid arthritis (RA) models, as the above models were only selected to induce a specific factor and did not reflect all the properties of RA. Therefore, further experiments are still needed. In summary, Siglec-15 expressed on osteoclasts can notably affect bone metabolism in multiple parts of the body, and its deficiency can interfere with the multinucleation of osteoclasts, leading to the development of osteosclerosis and providing protection for the bone.

3.3. Mechanism of Siglec-15 that affects osteoclasts

The Siglec-15 and DAP12 adaptor proteins regulate osteoclast development through ITAM signalling. Osteoclast maturation cannot be achieved without the involvement of DAP12, which carries an ITAM motif in the transmembrane region that transmits stimulatory signals to the intracellular compartment. Lys-272, presented in the Siglec-15 transmembrane region, and the Asp-52 site within DAP12 can bind to each other and transmit extracellular signals to the intracellular compartment to regulate osteoclast [15]. Therefore, the Siglec-15-ITAM-DAP12 signalling pathway plays a key role in osteoclast differentiation. Syk is a kinase related to osteoclast and bone resorption, and its phosphorylation is influenced by ITAM [50]. It has been shown that binding of the ITAM structural domain to the corresponding receptor can trigger sarcoma oncogene tyrosine kinase (Src) to phosphorylate tyrosine residues, which subsequently form a docking site with the C-terminal SH2 motif of Syk and activate the phosphorylation process of downstream proteins, thus

regulating cell growth and development [27,50]. Meanwhile, Syk and DAP12 deficiency has been correlated with a disordered cytoskeleton, which is involved in the normal development of functional structures in osteoclasts [50,51]. Based on these findings, it has been proposed that Siglec-15 interferes with DAP12-Syk and that Siglec-15 located on the plasma membrane of osteoclasts and the Syk kinase downstream of DAP12 are recruited to influence the terminal differentiation of osteoclasts [15,36]. Thus, the generation of functional osteoclasts is regulated by the Siglec-15-DAP12-Syk signalling cascade.

In addition to classical DAP12-Syk regulation, Siglec-15 may also regulate osteoclasts through the RANK-RANKL signalling pathway. Activation of signalling molecules downstream of RANK-TNF receptor-associated factor 6 (TRAF6) may require the co-stimulatory effect of Siglec-15. *In vitro*, Siglec-15 deficient cells inhibited RANKL-induced phosphorylation of downstream signalling molecules such as PIK3/Akt and extracellular signal-regulated kinase (ERK) [45]. However, with the regulation of this signalling pathway, the target molecule does not receive a single RANKL signal, but it still requires DAP12 and Siglec-15 to act in combination [36]. Researchers have found that K273A mutants (lacking the ability to combine with DAP12) cannot rescue impaired RANKL signalling in Siglec-15$^{-/-}$ osteoclast precursors, whereas WT-Siglec-15 can play a rescue role [45]. Therefore, when Siglec-15 and DAP12 accumulate on the surface of osteoclast precursor cells, they can activate Syk kinase through the ITAM motif and, together with the RANK-RANKL-TRAF6 pathway, stimulate downstream Syk transducers, such as Akt and ERK, to play a biological role in the regulation of the cytoskeletal structure of osteoclasts and inducing the transcription of NFATc1. NFATc1 is a key transcription factor in osteoclast formation, provided by the co-stimulation signals of FcRγ and DAP12, and triggers the circulation of Ca^{2+} after activating phospholipase cγ2 (PLCγ2) [27]. Subsequently, NFATc1 completes the transcription and then induces the expression of a lysosomal cysteine proteinase (Cathepsin K), TRAP, calcitonin receptor (CTR), OSCAR, and β3 integrin, to promote the maturation and multinucleation of osteoclast precursors [29,51–53] (Fig. 2).

However, downstream signals of TNF receptor 1, such as PI3K, Akt, Erk, and c-Jun N-terminal kinase (JNK), are not involved in the TNF-α-induced osteoclast differentiation of Siglec-15, in contrast to RANKL-induced phosphorylation of PIK3/Akt and ERK [43]. Thus, the exact mechanism by which Siglec-15 regulates TNF-α-induced osteoclastogenesis is unknown, suggesting a possible future research direction. In general, the mechanism by which Siglec-15 regulates osteoclast differentiation is quite complex and may be related to the existence of multiple signalling cascades that interact at different molecular levels. There are still many unexplained pathways that warrant exploration in the future.

4. Application of Siglec-15-targeted therapy

Osteoporosis is a systemic metabolic disease that is difficult to treat definitively and can be accompanied by structural bone abnormalities, osteopenia, and an increased risk of fractures [54]. This disease affects a wide range of people and can occur in children, young adults, middle-aged people, and older adults [55]. There are many causes of osteoporosis, such as physiological bone loss caused

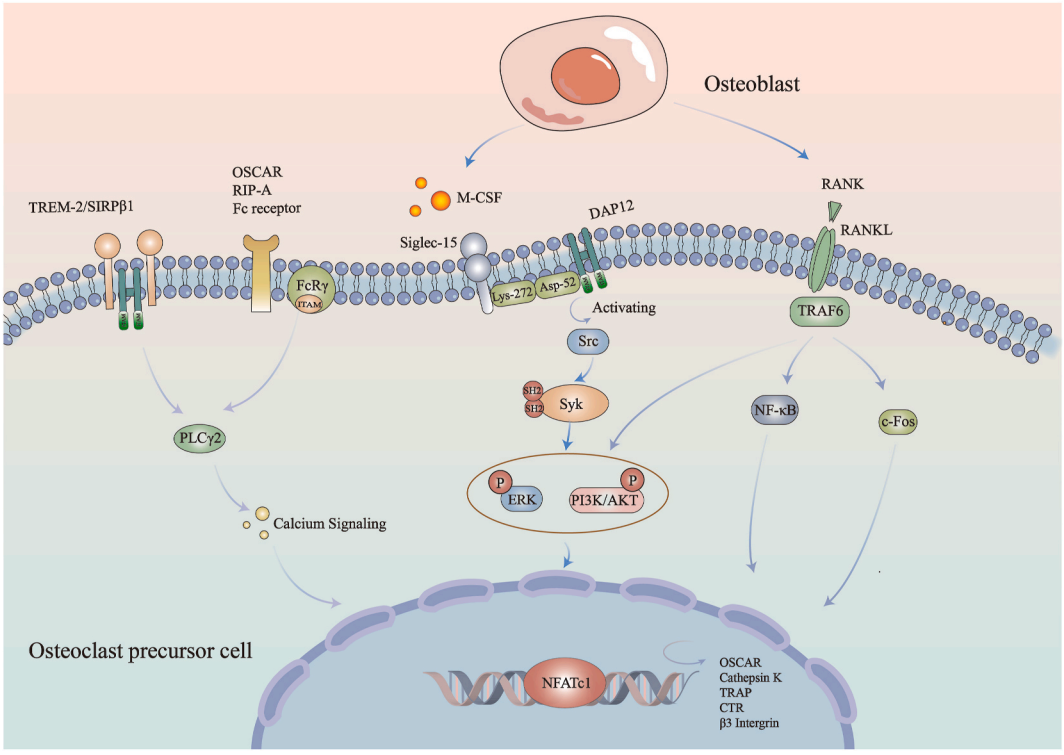

Fig. 2. The Siglec-15/DAP12 signal axis and RANK/RANKL/TRAF6 regulate osteoclasts differentiation together.

by ageing, oestrogen deficiency, or pathological osteoporosis caused by hormone and drug treatment [50]. However, these first-line drugs also tend to inevitably cause varying degrees of side effects in patients, such as bisphosphonate-associated osteonecrosis of the jaw (BRONJ) and the risk of fracture triggered by long-term high-dose administration [56–58].

Recent studies have revealed that Siglec-15 antibodies may be an effective targeted therapy for bone resorption diseases. Several teams have successfully constructed Siglec-15 neutralising antibodies derived from rats or humans and found that they may be safe modulators for ameliorating multiple aetiologies of osteoporosis [3,43,48–60]. A sudden drop in oestrogen levels after menopause is strongly predictive of bone loss in middle-aged and older women and has become a worldwide health concern. By constructing OVX models, researchers observed a significant increase in BV/TV of vertebrae and femurs in mice treated with Siglec-15 antibodies without significant functional impairment [61]. Therefore, antibody therapy can effectively alleviate osteoporosis caused by ovariectomies and promote bone formation to some extent.

In addition to their application to physiological bone loss in middle-aged and older people, Siglec-15 antibodies can also improve bone loss associated with adolescence. In vivo experiments have shown that anti-Siglec-15 antibody treatment can increase bone mass and skeletal mechanical properties in a growth-age rat model without affecting skeletal growth and development and improve glucocorticoid (GC)-induced osteoporosis in juvenile rats [3,60]. Therefore, Siglec-15 antibody therapy is an important preventive and therapeutic tool to treat bone loss in adolescents. Teriparatide (Parathyroid Hormone [PTH]-1-34) is an osteosynthesis drug used in severe osteoporosis or high risk of fracture. There are certain dosing deadlines for this therapy considering the risk of cardiovascular and other diseases after long-term use [62–64]. As a result, the application of antiresorptive drugs, such as the most commonly used bisphosphonates and denosumab, is crucial after discontinuing bone anabolic therapy [65]. Alendronate (ALN) is a common bisphosphonate drug whose powerful inhibition of bone resorption has been universally proven effective in increasing bone mass in osteoporotic populations. However, long-term use of ALN can increase the risk of fractures of the vertebral and hip bones and retard bone growth [66]. It has been found that mature rats with osteoporosis showed stagnant bone formation in the late stage of PTH treatment. After oral administration of ALN and subcutaneous injection of rat anti-Siglec-15 monoclonal antibody (32A1), compared with ALN therapy that increased bone density only after 8 weeks, the use of 32A1 as a follow-up treatment effectively inhibited bone resorption and actively promoted bone turnover in a short period of time, with effects observed after 4 weeks of treatment [59].

Anti-Siglec-15 antibodies have efficient anti-bone resorption properties, which may be attributed to Siglec-15 inhibiting the activity and differentiation of multinucleated osteoclasts. In this regard, anti-Siglec-15 antibody is highly effective against bone resorption, and compared to ALN, which is detrimental to bone homeostasis, treatment with Siglec-15 antibody does not have significant negative modulation of bone growth and protects growth plate development more efficiently [60], a property that may be due to the absence of effects on osteoclast numbers and differentiation in primary cancellous bone regions [3]. In contrast, after ALN treatment, the number of osteoclasts in both primary and secondary cancellous bone regions tended to decrease, interfering with the initial and terminal differentiation of bone-resorbing cells. This may be one of the reasons for the abnormal development of the metaphysis and growth plates of long bones [60]. Denosumab, another anti-bone-resorption agent, blocks RANK activation by binding to RANKL, thus functioning as a highly effective anti-bone-resorption agent and is used in the treatment of osteoporosis with a high risk of fracture [67]. However, because RANKL is also expressed by T and B cells, inhibition of RANKL by denosumab may affect the immune system, leading to cellulitis [68,69]. Second, discontinuation of denosumab triggers a rapid rebound in bone conversion and rapid bone loss and exacerbates the risk of multiple cone fractures [70]. This side effect may be associated with inhibition of osteoclasts and osteoblasts in cancellous bone [71]. A recent study reported that an anti-Siglec-15 antibody (NP159) almost completely prevented

Table 1
Application of the Siglec-15 targeted therapy in vivo models.

Author	Year	Animal model	Antibody type	Result
Stuible M [36]	2014	Growing mice	Siglec-15 monoclonal antibody (B02)	Significantly increased trabecular bone volume (BV/TV) (over 55 %) compared to controls
Sato D [3]	2018	Growing rats	Anti-Siglec-15 antibody (32A1)	Antibody treatment increased bone mass and bone mechanical properties in growth-phase rats without concomitant bone growth retardation.
Sato D [60]	2020	GC-induced juvenile osteoporosis in rats	Anti-Siglec-15 antibody (32A1)	Anti-Siglec-15 antibody had no negative effect on the growth plate, but maintained normal osteoclast and chondrocyte functions in the primary cavernous body and protected the growth plate.
Zhen G [48]	2021	1 OVX mice 2 Bone-healing mice	Anti-Siglec-15 antibody (NP-159, NP-158, or NP-149)	1 Increased the number of osteoclasts on the bone surface to prevent bone loss induced by oestrogen deficiency in adult female mice 2 Increased the number of mononuclear preosteoclasts in the damaged area to promote cortical bone healing and intramembranous and intrachondral ossification.
Tsukazaki H [61]	2021	OVX mice	Rat Anti-Siglec-15 Monoclonal antibody(32A1)	Inhibited multinucleate osteoclastogenesis to protect against bone loss and also maintained bone formation more efficiently than berezophosphonate.
Tsuda E [59]	2022	1 OVX rats 2 Osteoporosis rats after PTH treatment	Rat anti-Siglec-15 monoclonal antibody (32A1) Humanised anti-Siglec-15 monoclonal antibody (DS-1501a)	1 Inhibited the reduction of BMD of the lumbar vertebra of OVX rats. 2 Reduced bone resorption parameters of osteoporosis rats after PTH treatment (lower than that of the ALN treatment group).
Peng [49]	2023	1 Acute spinal cord rats	Anti-Siglec-15 antibody (NP159)	1 Prevented sublesional loss of BMD and metaphysis trabecular bone volume

the loss of BMD and metaphyseal trabecular bone volume and greatly preserved bone strength in a rat model of acute spinal cord injury (SCI) [49]. Although this novel Siglec-15 antibody is different from purely anti-bone resorption drugs, NP159 inhibits osteoclast maturation and generation while promoting the formation of osteoblast mineralised nodules and upregulates the expression of mRNAs, such as runt-related transcription factor 2 (Runx2) and osteocalcin (OCN), preserving the coupling of bone resorption and formation [49]. Furthermore, treatment with NP159 did not cause adverse effects such as death or weight loss in animals, and no histological toxicity was detected in tissues or organs [49]. Therefore, we hypothesised that Siglec-15 antibodies could provide better efficacy than bisphosphonates and denosumab in the treatment of pathological bone loss, and that in the future, it may be a novel, effective, and possibly safer drug. (Table 1).

How does Siglec-15 retain its anti-bone-resorption effects without affecting bone growth? Stuible et al. found that osteoclast activity and differentiation were inhibited, the structure of the actin ring was altered and the spreading area of multinucleated cells was reduced after using the Siglec-15 monoclonal antibody; This inhibition of bone resorption was similar to the results of in vivo experiments [15,36]. Conversely, the Siglec-15 antibody could prevent the binding of Siglec-15 and Neu5Acα2-6GalNAc to induce dimerization. This leads to rapid endocytosis and degradation of this molecule [36]. The Siglec-15 antibody inhibits multinuclear osteoclast maturation and differentiation, but does not affect mononuclear osteoblast and osteoclast differentiation, which can be achieved by increasing platelet-derived growth factor-BB (PDGF-BB) production by TRAP positive monocytes to promote H-type angiogenesis [36,48]. Specialised H-type capillaries play a vital role in angiogenesis because new bone formation is inseparable from blood vessels [72]. Osteoclasts and osteoblasts are inseparable systems that regulate each other through intercellular contacts, cytokines, and various pathways that affect intercellular communication [73]. Excessive inhibition of osteoclast function and activity when excessive antiresorptive therapies are used to treat skeletal disorders can also affect osteoblast differentiation and thus may be detrimental to bone formation. Therefore, the Siglec-15 neutralising antibody achieves anti-osteoporosis and restores fractures by increasing the number of TRAP positive mononuclear cells and preventing the maturation of multinucleated osteoclasts without inhibiting osteoclast function [48]. Siglec-15 antibody therapy preserves the coupled relationship between osteoblasts and mononuclear osteoclasts by significantly improving bone quality and promoting a balanced switch in bone metabolism.

In conclusion, the Siglec-15 antibody is a safe and highly effective drug for inhibiting bone resorption, showing great superiority, and can be widely used in patients with osteoporosis of different ages. This suggests that the Siglec-15 antibody may not only be a potential novel agent for the follow-up of PTH treatment, but may also offer good therapeutic prospects for patients experiencing osteoporosis, especially postmenopausal women and those in growth spurts.

5. Conclusions

Several studies have suggested that Siglec-15 is a classical and interesting immune receptor that regulates osteoclast differentiation through several molecular signalling pathways. In addition to effectively treating osteoporosis of various aetiologies, targeting Siglec-15 may be a safe therapy for fracture repair and acute bone loss. Although it is now clear that Siglec-15 plays a regulatory role in the formation of functional osteoclasts and that Siglec-15 antibody therapies have therapeutic promise, Siglec-15 still has many unknown biological functions. The signalling pathway through which Siglec-15 mediates the role of other osteoclast-stimulating factors in the regulation of osteoclasts is unclear, and its mechanism of action in physiological bone reconstruction deserves to be investigated. Additionally, there is still a paucity of studies related to how anti-Siglec-15 antibodies crosstalk between bone resorption and formation, as well as a lack of side-by-side comparisons between this therapy and other commonly used osteoporosis drugs. Therefore, further detailed understanding of the molecular properties of Siglec-15 and its functions is necessary to contribute to the development of future targeted drugs.

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Data availability statement

No data was used for the research described in the article.

CRediT authorship contribution statement

Jiaqi Lu: Conceptualization. **Yinyin Zhang:** Writing – review & editing. **Huiyu Wen:** Visualization. **Junlin Li:** Supervision. **Chen Chen:** Investigation. **Liwei Xiao:** Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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