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Molecular Mechanisms of Osteoclast Precursor Fusion: From DC-STAMP to Novel Regulatory Proteins

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Abstract: Osteoclasts are multinucleated giant cells responsible for bone resorption in the skeletal system. Osteoclast precursors originate from monocytes and macrophages, as well as dendritic cells. Their multinucleation process is crucial for maintaining bone homeostasis. Studies indicate that efficient fusion of mononuclear precursor cells is a prerequisite for forming fully functional multinucleated osteoclasts. This unique cell fusion capability allows osteoclasts to form large, multinucleated cells with enhanced bone resorptive capacity. Under physiological conditions, a precisely regulated fusion process generates multinucleated osteoclasts of predictable size, with the number of nuclei positively correlating with bone resorption activity. Notably, each fusion event significantly increases the bone resorptive activity of osteoclasts, a characteristic vital for lifelong bone remodeling processes.

Keywords: Osteoclasts; DC-STAMP; Osteoclast precursor; Cell fusion

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1. Introduction

1.1. Cell fusion as a rate-limiting step in osteoclast maturation

The fusion of osteoclast precursors is a rate-limiting step in osteoclast maturation, subject to multi-layered, precise regulation. Fusion of osteoclast precursors (OCPs) is a complex process regulated by multiple factors. The fusion process can be divided into four steps: (1) cell attraction/migration; (2) cell recognition; (3) cell adhesion; and (4) cell fusion [1]. Various fusion-related genes are upregulated during osteoclast maturing, including CD9, MFR/SIRPα, ATP6V0d2, and DA-STAMP etc. Those proteins involved in the fusion procedure can be categorized into RANKL-independent or dependent proteins (**Figure 1**). RANKL-dependent proteins include CD47, CD9/CD81, ATP6V0d2, MFR/SIRPα, OC-STAMP, A2A, and DC-STAMP [2], whereas CD44 and TREM2 are classified as RANKL-independent proteins [3]. DC-STAMP and ATP6V0d2 are directly regulated by the transcription factor NFATc1; other fusion proteins, including MFR/SIRPα, CD9, CD44, E-cadherin, and meltrin-α, are not regulated by NFATc1 [4]. Otherwise, the number of nuclei in mature osteoclasts positively correlates with their bone resorption capacity; larger volume osteoclasts exhibit high resorption ability. Breaking the fusion or multinucleation process in osteoclasts can lead to decreased bone resorption, resulting in high bone mineral density (BMD) [5]. Research shows that RANKL-mediated caspase-8 activation is an early key event in osteoclast fusion [6,7], accompanied by the activation of

certain molecules related to apoptotic mechanisms [6]. This fusion process requires specific molecules, such as Dendritic Cell-Specific Transmembrane Protein (DC-STAMP) [8], and the expression of key transcription factors like c-Fos and NFATc1 etc. [9] Importantly, differentiating osteoclast precursors undergo mechanical changes, including reduced plasma membrane tension, which serves as a mechanical prerequisite for cell fusion [10]. Furthermore, the nuclear RNA chaperone La protein has been identified to possess a novel function in regulating osteoclast fusion [11], indicating that the regulatory network of fusion is far more complex than previously understood.

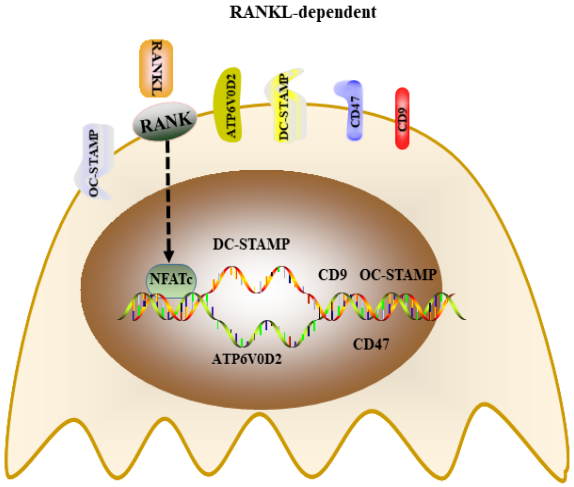

Figure1. RANKL-dependent proteins and the role they plays in the osteoclast differentiation stage.
Note: In response to RANKL stimulation, the expression of CD47, CD9/CD81, ATP6V0d2, MFR/SIRPα, OC-STAMP, A2A and DC-STAMP increases, ATP6V0d2 and DC-STAMP directly regulated by transcription factor NFATc1.

1.2. Clinical translational value of molecular mechanism research

Investigating the molecular mechanisms of osteoclast fusion holds significant clinical translational value. DC-STAMP-deficient mice exhibit an osteopetrotic phenotype [12], suggesting that targeting fusion regulation could be a novel strategy for treating bone metabolic diseases. Current clinical drugs often completely inhibit osteoclast function, disrupting normal bone turnover [13], whereas specifically regulating precursor cell fusion might be more suitable for maintaining bone homeostasis [13]. Studies have attempted to use engineered bacteria for the targeted delivery of the osteoclast precursor fusion protein DC-STAMP [14], demonstrating good bone-targeting capability. Furthermore, regulating fusion-related molecules like NFATc1 and DC-STAMP can effectively inhibit pathological bone resorption [15,16], providing new therapeutic targets for osteolytic diseases such as osteoporosis. These findings highlight the importance of studying osteoclast fusion mechanisms for developing precise bone metabolism intervention strategies.

2. Research progress on the core osteoclast fusion molecule DC-STAMP

2.1. Molecular structure and functional characteristics of DC-STAMP

Dendritic Cell-Specific Transmembrane Protein (DC-STAMP) is a key molecule for forming functional multinucleated osteoclasts [17]. DC-STAMP-deficient mice exhibit an osteopetrotic phenotype under physiological conditions and develop systemic autoimmune symptoms with age [12]. This protein is highly expressed on the membrane of osteoclast precursors and mediates their phagocytosis of apoptotic bodies [16]. Structurally, DC-STAMP belongs to the seven-transmembrane protein family, with its extracellular domain involved in intercellular recognition and its intracellular domain participating in downstream signal transduction [18]. Functional studies show that DC-STAMP is not only a master regulator of osteoclast fusion [15] but also involved in regulating bone resorption function; its expression level increases synchronously with osteoclast markers (Nfatc1, Acp5, Ctsk, etc.) on stiffer matrix surfaces [15].

2.2. Molecular mechanism of DC-STAMP-mediated cell membrane fusion

DC-STAMP regulates the cell membrane fusion process through a unique molecular mechanism. During the fusion initiation stage, DC-STAMP promotes the formation of phagocytic cup-like structures between osteoclast precursors [19]. Experiments confirm that precursors with knocked-down DC-STAMP lose their ability to be phagocytosed [16]. In the membrane fusion stage, DC-STAMP activates the expression of cytoskeleton-related adhesion molecules (including fibronectin and integrin αvβ3), subsequently triggering biochemical signaling cascades involving paxillin, FAK, PKC, and RhoA [15]. Notably, SNX10 protein-deficient osteoclasts show persistent peripheral aggregation of DC-STAMP, which might be a key reason for their uncontrolled fusion [20]. Besides, immunoreceptor Tyrosine-based Inhibitory Motif (ITIM) in the cytoplasmic tail of DC-STAMP is a critical regulator of osteoclast differentiation through the Ca^{2+}/NFATc1 signaling axis [21]. Using optogenetic and mutational approaches shows that deletion of the ITIM disrupts intracellular Ca^{2+} flux, impairs NFATc1 nuclear translocation, and reduces osteoclast fusion, bone resorption, and cell motility [21].

2.3. Transcriptional network regulating DC-STAMP expression

DC-STAMP expression is subject to multi-level transcriptional regulation. The RANKL-NFATc1 signaling pathway upregulates DC-STAMP expression by inducing key transcription factors like c-Fos and NFATc1 [22,23]. Research indicates that RANKL-activated JNK, p38, and ERK MAPK signaling pathways, as well as the IκBα degradation pathway, are upstream events regulating DC-STAMP expression [22]. At the epigenetic level, the histone modification enzyme Ctsk might affect the chromatin accessibility of the NFATc1 promoter by regulating H3K27me3 cleavage status, indirectly modulating DC-STAMP expression [24]. Additionally, Chi3l1 significantly promotes RANKL-induced upregulation of DC-STAMP expression by activating the MAPK (ERK/P38/JNK) and AKT pathways [25].

2.4. Synergistic effects of DC-STAMP with other fusion-related proteins

DC-STAMP forms a functional synergistic network with various fusion-related proteins. Studies found correlations between the expression levels of DC-STAMP and the co-receptor CCR5, as well as tetraspanins CD9 and CD81 [26]. At the signaling level, DC-STAMP, together with transcription factors like NFATc1 and c-Fos, constitutes the core regulatory module for osteoclast fusion [27]. Experimental evidence shows that inhibiting DC-STAMP simultaneously downregulates the expression of osteoclast-specific marker genes such as ATP6V0D2 and CtsK [22,28]. In inflammatory arthritis models, DC-STAMP interacts with the TNF-α signaling pathway, jointly regulating synovitis and bone erosion progression [12,29]. Furthermore, recent studies reveal that SARS-CoV-2 variants can upregulate osteoclastogenesis-related genes like DC-STAMP, promoting RANKL-independent osteoclast formation [30].

3. Discovery and functional validation of novel regulatory proteins

3.1. Role of la protein in precursor recognition

La is generally recognised as an abundant and ubiquitous RNA-binding protein, also known as LARP3 and La autoantigen [31]. The most extensively studied function of nuclear La is its role in protecting precursor tRNAs from exonuclease digestion. This is achieved through specific interactions between La's highly conserved N-terminal La domain and the 3′ ends of tRNA. La protein exhibits unique regulatory functions during osteoclast precursor fusion. In the initial stage of monocyte differentiation into osteoclasts, La protein levels decrease significantly [8,15,19]. As the fusion process initiates, a low molecular weight form of La protein reappears on the osteoclast surface [11]. This non-classical form of La protein anchors to transiently exposed phosphatidylserine on the fusing cell surface by directly interacting with membrane-anchored Annexin A5 [8,11]. Notably, the function of La protein in promoting osteoclast fusion is independent of its classical RNA-binding activity [8,11]. Reactive Oxygen Species (ROS) signaling regulates the subcellular localization and functional switch of La protein by oxidizing cysteine residues at its C-terminus, leading to the formation of this fusion-promoting atypical La protein form [8,11].

La was present in primary human monocytes but virtually absent in m-CSF-derived osteoclast precursor cells. However, following rankl-induced osteoclastogenesis, La protein reappeared on the surface of fused osteoclasts. As osteoclast fusion entered the stable phase, LMW La was observed to gradually disappear, and higher molecular weight phosphorylated full-length La protein (FL-La) was observed in the nuclei of mature multinucleated osteoclasts. Inhibition of La expression, cleavage, or surface function impedes osteoclast fusion, whereas the addition of exogenous La facilitates osteoclast fusion. In fact, the carboxy-terminal portion of La promotes human osteoclast fusion, unlike the classical function of La [11]. Osteoclasts secrete factors that regulate osteoblast activity [32]. The targeted blockade of the RANKL pathway may impede osteoclast-osteoblast signalling, whereas the targeting of La to inhibit the fusion phase of osteoclast formation may simultaneously preserve osteoclast differentiation capacity and maintain this osteoclast-osteoblast crosstalk within bone remodelling lesions.

3.2. Spatial regulatory role of the CD47-SIRPα signaling axis

The CD47-SIRPα signaling axis plays an important spatial regulatory role in osteoclast fusion. CD47, a transmembrane protein, interacts with the SIRPα receptor expressed on osteoclast precursor surfaces [33,34], transmitting intercellular recognition signals [35].

In the immune system, CD47-SIRPα interaction generates a “don’t eat me” signal [36,37]; this mechanism might participate in regulating the recognition and contact of precursor cells during osteoclast fusion. The SIRPα receptor belongs to the immunoreceptor tyrosine-based inhibitory motif (ITIM)-bearing receptor family; its N-terminal Ig-like domain triggers downstream signaling upon binding to CD47 [38,39]. Studies indicate that CD47-SIRPα interaction can influence cell metabolism by regulating JAK/STAT and ERK/MAPK signaling pathways [40]. Additionally, this signaling axis can activate the Hedgehog/SMAD pathway and regulate NF-κB activity [41], potentially forming a multi-layered regulatory network in osteoclast fusion. CD47 could facilitate leukocyte chemotaxis and migration by binding receptor MFR. Furthermore, blocking CD47/SIRPα recognition with monoclonal antibodies significantly inhibits osteoclast maturation [42]. In RANKL-treated BMMs, $CD47^{-/-}$ cells generate significantly fewer $TRAP^{+}$ osteoclasts than wild-type controls [43,44]. Similarly, $CD47^{-/-}$ mice exhibit a clear reduction in $TRAP^{+}$ osteoclasts in vivo [45]. CD47 inhibition decreases fusion events between mononuclear OCPs and between binuclear osteoclasts, without affecting fusion involving osteoclasts with three or more nuclei [46,47].

3.3. Other identified new regulatory factors

Besides the aforementioned proteins, various other novel regulatory factors are involved in osteoclast fusion. ROS signaling has been confirmed to promote osteoclast fusion and bone resorption activity [8,11]. During differentiation, ROS signaling regulates the subcellular localization and functional switch of La protein via redox-sensitive cysteine residues [8]. Furthermore, Annexin A5, as an anchoring protein for La protein, forms functional complexes on transiently exposed phosphatidylserine on the fusing cell surface [11]. Recent studies also found that caspase-8 activation is an early key event in osteoclast fusion [6], suggesting that proteins associated with programmed cell death might also regulate the fusion process. These newly discovered regulatory factors collectively constitute a complex regulatory network for osteoclast precursor fusion.

4. Cascade signaling network in osteoclast fusion

4.1. Central role of the RANKL-NFATc1 signaling pathway

The RANKL/RANK signaling pathway is the core cascade regulating osteoclast differentiation, with NFATc1 acting as a pivotal transcription factor [48]. Studies show that NFATc1 activation is regulated through three aspects: direct activation by the upstream RANKL/RANK signaling pathway, amplification by Ca^{2+}-related co-stimulatory signals, and positive feedback regulation at the NFATc1 transcriptional level itself [48]. Mechanistically, RANKL stimulation promotes the non-apoptotic cleavage of downstream effector molecules via caspase-8 activation, an early event critical for osteoclast fusion [49,50]. Single-cell RNA sequencing analysis confirms that NFATc1-associated super-enhancers (SEs) and their resulting enhancer RNAs

(eRNAs) play key regulatory roles in osteoclast differentiation; interfering with these elements significantly reduces NFATc1 expression and osteoclast differentiation capacity [40]. Moreover, various inhibitors like PLM and mussaendoside O block osteoclast formation by inhibiting the RANKL-induced p38/JNK-cFos/NFATc1 signaling cascade [51,52].

4.2. Enhanced fusion via extracellular signals by collagenase inhibition

Extracellular matrix remodeling plays a significant role in osteoclast fusion. Research suggested that the collagenase inhibitor CA significantly inhibits RANKL-induced actin ring formation, osteoclastogenesis, and bone resorption function [37]. Its mechanism involves two aspects: On the one hand, it suppresses RANKL-induced ROS production by elevating antioxidant enzyme levels like catalase and NQO1; on the other hand, it interferes with the fusion process by modulating the expression of key proteins such as integrin αvβ3, NFATc1, and Cathepsin K (CTSK) [37]. Notably, CA has no significant effect on cortical bone, suggesting its potential as a candidate drug for targeted therapy of bone metabolic diseases due to this selective action [37].

4.3. Molecular basis of stage-specific regulation by IFNγR

Interferon-γ receptor (IFNγR) regulation of osteoclast fusion exhibits a unique stage dependency [53]. In early osteoclast precursor stages, IFNγR activation completely inhibits multinucleated osteoclast formation; whereas, in immature osteoclast stages, the same activation further enhances cell fusion [53]. The molecular basis for this biphasic regulation lies in the differential downstream activation of MAPK pathways: IFNγR activation in early precursor cells induces FcγR expression, a co-regulatory phenomenon dependent on MAPK signaling. Phosphokinase array analyses reveal significant differences in IFNγR signal transduction between precursor cells and immature osteoclasts, providing new insights into the dynamic regulation of bone resorption under inflammatory conditions [53].

4.4. Association between Metabolic Reprogramming (PKM2) and fusion capacity

Pyruvate Kinase M2 (PKM2), a key molecule in metabolic reprogramming, has been identified as a novel switch regulating osteoclast differentiation [54]. Immunoglobulin superfamily member 11 (IgSF11) regulates osteoclast differentiation in a PKM2-dependent manner by interacting with the postsynaptic scaffolding protein PSD-95. Experiments confirm that inhibiting PKM2 activity with the specific inhibitor Shikonin rescues the osteoclast differentiation defect in IgSF11-deficient cells, while the activator TEPP46 inhibits osteoclast differentiation in wild-type cells [54]. More importantly, PKM2 activation inhibits osteoclast-mediated bone loss without affecting bone formation, making it a potential target for treating pathological bone loss due to this selective regulation [38]. The link between metabolic reprogramming and cell fusion capacity is also reflected in PKM2's influence on the expression of fusion-related proteins like DC-STAMP via regulating NFATc1 activity [52,54].

5. Dynamic regulatory mechanisms of the fusion process

5.1. Molecular events in precursor chemotaxis and initial contact

The chemotaxis and initial contact of osteoclast precursors are critical initiating steps of the fusion process. Studies indicate that RANKL-mediated caspase-8 activation is a core molecular event at this stage [6]. Single-cell RNA sequencing analysis shows that partial activation of apoptotic mechanisms accompanies the differentiation of osteoclast precursors into mature multinucleated osteoclasts [53]. Caspase-8 activation promotes the non-apoptotic cleavage of downstream effector molecules, which subsequently translocate to the plasma membrane, triggering the activation of phospholipid scramblase Xkr8 [6]. Xkr8-mediated phosphatidylserine exposure provides the molecular basis for mutual recognition and initial contact between precursor cells [6]. Additionally, the binding of Siglec15 to TLR2 has been confirmed as an indispensable cell recognition mechanism in RANKL-mediated osteoclast formation [55].

5.2. Molecular switches in the membrane fusion execution stage

The membrane fusion execution stage is precisely regulated by various molecular switches. Research finds that decreased plasma membrane (PM) tension is a mechanical prerequisite for osteoclast fusion [10]. During RANKL-induced differentiation, reduced ezrin expression in fusion-committed progenitors leads to Membrane-Cortex Attachment (MCA)-dependent PM tension reduction [10,11]. Artificially increasing PM tension inhibits cell fusion. Furthermore, La protein plays a key role at this stage, reappearing on the osteoclast surface in a low molecular weight form and anchoring to transiently exposed phosphatidylserine on fusing cell surfaces via direct interaction with Annexin A5, thereby promoting fusion [11,56]. Notably, La protein's fusion-promoting role is independent of its classical RNA-binding function.

5.3. Regulation of multinucleation maintenance and functional polarization

The maintenance of the multinucleated state and functional polarization involves complex regulatory networks. Research finds that dynamic changes in O-GlcNAcylation are crucial for osteoclast maturation: increased O-GlcNAcylation in early stages promotes differentiation, while its downregulation later favors maturation [57]. In inflammatory arthritis, TNFα promotes osteoclastogenesis by regulating the dynamic changes of O-GlcNAcylation [57]. Additionally, ROS signaling and the atypical low molecular weight form of La protein jointly promote osteoclast fusion and bone resorption function [11]. Abnormal expression of SELENOW protein also affects cell fusion; its overexpression stimulates cell fusion, crucial for osteoclast maturation, while its deficiency inhibits osteoclast formation [58]. These findings collectively reveal a multi-layered regulatory mechanism for maintaining multinucleation and functional polarization.

6. Research models and technological methodological breakthroughs

6.1. Optimization and standardization of in vitro fusion models

Significant progress has been made in recent years in optimizing in vitro osteoclast fusion models. Researchers have established standardized culture systems based on bone marrow-derived macrophages (BMMs) and RAW264.7 monocytic cells by optimizing culture conditions [15]. Particularly noteworthy is the finding that modulating the stiffness of polydimethylsiloxane (PDMS) substrates significantly influences osteoclast differentiation progression; stiffer substrates accelerate differentiation, manifested by morphological changes and enhanced fusion/division activity [15]. Regarding model standardization, studies confirm that RANKL-mediated caspase-8 activation is an early key event in osteoclast fusion [59], providing an important basis for selecting molecular markers in vitro. Furthermore, interference experiments confirm that reduced plasma membrane (PM) tension is a mechanical prerequisite for osteoclast fusion [10], a discovery offering new standards for setting mechanical parameters in vitro models.

6.2. Application advances in high-resolution imaging technologies

High-resolution imaging technologies have played a pivotal role in osteoclast fusion research. Scanning electron microscopy has successfully revealed the fine morphological characteristics of tunneling nanotubes (TNTs) during osteoclastogenic fusion [60]. These actin-based membrane structures are confirmed to participate in the cell-cell fusion process between osteoclast precursors and multinucleated osteoclast-like cells, allowing observation of membrane vesicle and nuclear movement [61]. Two-photon microscopy live imaging enables real-time visualization of immune cell dynamics within the synovial microenvironment [62], providing a powerful tool for studying osteoclast fusion behavior in physiological contexts. Notably, these imaging techniques have also revealed the spatial localization characteristics of La protein reappearing on the osteoclast surface as a low molecular weight species promoting fusion [26].

6.3. Application of single-cell omics in mechanism studies

Single-cell RNA sequencing (scRNA-seq) technology has greatly advanced the understanding of osteoclast fusion mechanisms. This technology successfully identified specific macrophage subpopulations in joints that differentiate into

pathological mature osteoclasts [62]. In a rheumatoid arthritis (RA) mouse model, scRNA-seq library analysis revealed a novel RANK+TLR2+ monocyte population [63]. Integrated multi-omics analysis combined with single-cell technology has uncovered the transcriptional and epigenetic mechanisms guiding the continuous fusion process of osteoclasts from monocytes in adulthood [64]. These technologies also confirmed that partial activation of apoptotic mechanisms accompanies the differentiation of osteoclast precursors into mature multinucleated osteoclasts [8], providing new perspectives for understanding the molecular basis of fusion.

6.4. Value of gene editing technologies in mechanism elucidation

Gene editing technologies have become essential tools for studying osteoclast fusion mechanisms. Specific knockout of the chromatin remodeler Arid1a in bone marrow-derived macrophages (BMDMs) inhibits cell-cell fusion and maturation of osteoclast precursors [65]. RNA interference confirmed that reducing plasma membrane tension promotes osteoclast fusion, whereas forcibly increasing PM tension by enhancing membrane-cortex attachment (MCA) inhibits cell-cell fusion [10]. Genetic manipulation also revealed the critical regulatory role of Selenoprotein W (SELENOW) in cell-cell fusion during osteoclast maturation [58]. Furthermore, DLEU1 silencing was found to hinder the fusion process, leading to the disappearance of the phagocytic cup fusion pattern and reduced fusion events between mononuclear precursors and multinucleated osteoclasts [55]. These findings highlight the unique value of gene editing technologies in mechanism elucidation.

7. Current controversies and challenges in research

7.1. Controversy regarding the hierarchy of different regulatory factors

There is significant controversy regarding the hierarchical relationship among different regulatory factors in osteoclast fusion. Although DC-STAMP is widely considered a core molecule [55], recent studies identify RANKL-mediated caspase-8 activation as a key upstream event [57], suggesting a more complex regulatory hierarchy. Furthermore, multiple studies show NFATc1's regulatory effect on DC-STAMP expression [63,66], while HIV infection experiments indicate the virus simultaneously affects the transcription levels of multiple key factors like RANK, NFATc1, and DC-STAMP [26], complicating the determination of causal relationships. Particularly noteworthy is that SARS-CoV-2 variants can even promote RANKL-independent osteoclast formation [30], further challenging the completeness of the existing regulatory network.

7.2. Limitations of species differences on mechanism studies

Species differences pose significant limitations to osteoclast fusion mechanism research. Mouse DC-STAMP deficiency models exhibit clear osteopetrotic phenotypes [55], but the spectrum of manifestations associated with DC-STAMP mutations in human diseases remains incompletely elucidated. In HIV infection studies, the extent of the virus's impact on osteoclast precursors was found to be closely related to the viral inoculum size [26], and this dose-dependent response may vary between species. Additionally, studies on TNF-transgenic mouse arthritis models found that the impact of DC-STAMP deletion on the arthritic phenotype requires accurate evaluation through bone marrow chimera experiments [29], indicating complex species-specificity in the immune-bone axis regulation.

7.3. Issues with consistency validation between in vivo and in vitro models

Current research exposes significant issues in consistency validation between in vivo and in vitro models. In vitro experiments show that the IGF-PI3K-AKT pathway influences osteoclast differentiation by regulating marker genes like DC-STAMP [67], but the spatiotemporal specificity of this pathway's regulation in vivo remains unclear. While scRNA-seq can reveal the heterogeneity of osteoclast precursor differentiation [9,57], in vitro culture systems struggle to fully simulate the cell interactions within the bone marrow microenvironment. Notably, experiments using engineered E. coli to deliver DC-STAMP protein show that targeting strategies validated in vitro may produce unexpected bone-targeting effects in vivo, highlighting the complexity of translational research between models [14]. Furthermore, the role of membrane

mechanical properties in fusion revealed by new technologies like optical tweezers requires further validation of their physiological relevance across different model systems [10,15].

8. Future research directions and technological pathways

8.1. Single-cell resolution strategies for spatiotemporal dynamic regulation

Single-cell RNA sequencing has revealed the activation characteristics of apoptosis-related genes during osteoclast precursor differentiation [6], but the spatiotemporal dynamics of the fusion process require deeper analysis. Future research needs to integrate single-cell transcriptomics with live-cell imaging to precisely capture molecular events at key fusion nodes (e.g., initial contact, membrane fusion execution) [9]. Particular attention should be paid to how RANKL-induced caspase-8 activation spatiotemporally regulates membrane fusion [6], and the changes in La protein's subcellular localization at different fusion stages [68]. Developing novel time-resolved single-cell multi-omics technologies holds promise for constructing a continuous differentiation map from mononuclear precursors to mature multinucleated osteoclasts [69].

8.2. AI-assisted construction of molecular interaction networks

Existing research indicates that DC-STAMP forms dynamic interaction networks with proteins like Annexin A5 [11], but the hierarchical relationships among various regulatory factors (e.g., Dyrk2 kinase, CD47-SIRPα axis) remain controversial. Artificial intelligence (AI) can integrate vast omics data to predict the weight of key molecular switches (e.g., Cited2) in the fusion cascade [69]. Deep learning models can help analyze the synergistic regulation patterns between mechanical signals (matrix stiffness) and biochemical signals (RANKL pathway) [15] and predict the regulatory role of membrane trafficking proteins like Rab11A in later fusion stages [70]. Furthermore, based on the hypothesis that ROS signaling regulates La protein trafficking [10], machine learning can be used to build redox-sensitive molecular interaction prediction models.

8.3. Translational medical research pathways targeting fusion regulation

Engineered bacterial vectors (BEV-DCS) have proven capable of targeted delivery of DC-STAMP fusion protein to precursor cells [67], providing proof-of-concept for developing therapies based on membrane fusion inhibitors. Future translational research should focus on three directions: (1)Developing specific antibodies targeting the low molecular weight form of La protein [50] to block its binding to phosphatidylserine [11]; (2) Utilizing gene editing to construct DC-STAMP conditional knockout models [16] to evaluate the therapeutic differences of intervention at various differentiation stages; (3) Optimizing biomaterials based on matrix stiffness regulation [15] to physically intervene in the fusion tendency of precursor cells. These strategies have clear application prospects for osteoporosis and other osteolytic diseases [71].

9. Summary and core biological insights

Current research has established a molecular regulatory framework for osteoclast fusion centered on DC-STAMP. This framework comprises three key layers: the upstream signal input layer (RANKL-NFATc1 pathway), the core execution layer (DC-STAMP-mediated membrane fusion), and the auxiliary regulatory layer (including novel regulators like La protein and Dyrk2 kinase) [12,16]. It is particularly noteworthy that RANKL signaling promotes the fusion readiness of precursor cells in a non-apoptotic manner by activating caspase-8 and other apoptosis-related molecules [15,72]. Simultaneously, NF-κB and MAPK signaling pathways influence the transcription levels of fusion marker genes like DC-STAMP by regulating the expression of c-Fos and NFATc1 [70,73]. These findings collectively depict a multi-layered, dynamically regulated molecular network, providing a systematic theoretical framework for understanding osteoclast multinucleation.

Based on fusion mechanism research, DC-STAMP has emerged as the most promising therapeutic target for translation. Animal experiments show that DC-STAMP deficiency significantly alleviates arthritis symptoms and bone

erosion in TNF-transgenic mice [12]. In osteoporosis models, engineered bacterial vectors (BEV-DCS) targeting DC-STAMP demonstrate excellent bone-targeting capability and therapeutic potential [14]. Furthermore, small molecule compounds targeting fusion-related signaling pathways (e.g., NF-κB, JNK/p38/ERK MAPKs) can effectively block pathological bone resorption by inhibiting the NFATc1-DC-STAMP axis [22,55]. These discoveries provide a molecular basis for developing therapeutic strategies that specifically intervene in osteoclast fusion, holding significant application promise, particularly in inflammatory bone diseases and postmenopausal osteoporosis [9,12].

The mechanisms revealed by osteoclast fusion research have broad biological implications. First, the caspase-8-mediated non-apoptotic cell fusion mechanism might be widely present in multinucleated giant cell formation processes. Second, the membrane reorganization mechanisms mediated by transmembrane proteins like DC-STAMP provide important references for understanding other cell fusion phenomena, such as myotube formation and placental syncytiotrophoblast development. Particularly noteworthy are the metabolic reprogramming features and mechanical signal sensing capabilities (via the integrin αvβ3-FAK-RhoA pathway) exhibited during osteoclast fusion, revealing a deep-level connection between cell fusion and microenvironmental adaptation. These findings expand the understanding of cell multinucleation phenomena.

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The authors declare no conflict of interest.

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