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Review

Dynamic Roles of Macrophage Autophagy in Tendon–Bone Junction Repair: A Review of the Mechanism

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Abstract

Tendon–bone healing has always been a difficult point in clinical orthopedics, tissue engineering, and sports medicine. The most important structure for stress transmission is the tendon–bone junction, which is the transition from soft tissue to hard tissue. Biological effects can be produced by a variety of cytokines in different cells. During the remodelling and repair of the tendon–bone junction, the key factor is the inflammatory microenvironment regulated by macrophages through various physiological processes such as autophagy, differentiation, and polarization, which mediate cytokine release and influence other cellular functions. This provides a theoretical basis for the development of new mechanisms for tendon–bone junction repair. This article aims to review the potential role of macrophage autophagy, differentiation, and polarization in the repair of tendon–bone injury. In addition, we propose that future research should integrate multidisciplinary approaches such as molecular biology and bioinformatics to conduct in-depth analyses of the dynamic networks of autophagy and polarization in macrophages, thereby guiding future research directions on the specific roles of macrophage autophagy in tendon–bone junction repair.

Keywords: macrophage; tendon-bone junction; autophagy; cytokines; mechanism; osteoclasts; review

1. Introduction

The tendon-bone junction—where tendon inserts into bone—is prone to rupture from stress concentration and represents a common sports medicine injury [1]. High retear rates and suboptimal outcomes after repair impose substantial socioeconomic burdens; 32 million US musculoskeletal injuries annually involve tendons/ligaments in 45% of cases [2]. Structurally categorized as direct/indirect attachments, repaired junctions exhibit significantly reduced biomechanical strength, increasing rerupture risk [3]. This weakness stems from early inflammatory cell influx causing excessive scar tissue that impedes fibrocartilage formation, or insufficient stem cell proliferation/chondrogenic differentiation to regenerate native structure [4].

During initial inflammation, peripheral blood/bone marrow/synovial-derived macrophages recruit to the junction, primarily polarizing to M1 phenotype. These macrophages phagocytose debris, eliminate pathogens, and secrete inflammatory factors to promote healing. M2 macrophages dominate subsequent remodeling, facilitating repair. Macrophages demonstrate activation states spanning pro-inflammatory to reparative, regulated by extracellular signals and metabolic programs. Macrophage autophagy, an immunologically regulated lysosomal pathway, clears damaged organelles/protein aggregates/pathogens. Activated macrophages initiate light chain 3 (LC3)-lipidated autophagosome formation, identify substrates, and phagocytose debris. Through secreted factors, they further regulate osteoblast/osteoclast differentiation to promote tendon-bone healing. This review examines macrophage autophagy, differentiation, and polarization in tendon-bone repair, exploring future therapeutic strategies.

2. Morphological Features of Tendon-Bone Junction

The human Achilles tendon-bone interface is narrow (≈200 μm to 1 mm) with a characteristic four-layer structure: tendon layer, non-calcified fibrocartilage layer, calcified fibrocartilage layer, and bone tissue [5,6]. The tendon layer contains orderly fibroblasts or elongated spindle-shaped tenocytes, exhibiting ligament-like biomechanical properties [7] and consisting of linearly aligned type I collagen fibers with minor glycosaminoglycan-modified proteins [8]. The non-calcified fibrocartilage layer houses rounded cells within extracellular matrix and collagen fibers, marking the soft-to-hard tissue transition (tendon: ~200 MPa; bone: ~20 GPa elastic modulus) [9]. Mineralization in this zone forms amorphous calcium phosphate bound to collagen residues. It contains collagen types II/III

interwoven with type X collagen, proteoglycans, dermatan sulfate, and sulfated cartilage core proteoglycans. The third layer features fibrochondrocytes with abundant aggrecan, type II and X collagen, transitioning clearly to bone. The bone tissue layer shows exponentially increasing mineral content within a lamellar type I collagen matrix containing osteoblasts, osteoclasts, and osteocytes [10,11]. Collectively, this graded structure mitigates stress concentration at the interface during mechanical loading [12].

3. Process of Tendon-Bone Junction Healing

Process of Tendon-Bone Healing

The healing process following tendon-bone junction injury comprises three distinct phases. The first phase is the inflammatory phase, characterized primarily by the infiltration of inflammatory cells and the phagocytosis and digestion of necrotic material by macrophages [13,14]. The second phase is the repair phase, during which macrophages release growth factors to recruit and activate tendon fibroblasts. These fibroblasts collaborate to synthesize fibronectin and type III collagen, forming an initial amorphous extracellular matrix. Subsequently, this type III collagen is replaced by the mechanically superior type I collagen [15]. The third phase is the remodeling phase, wherein elongated spindle-shaped tenocytes and collagen fibers become aligned along the direction of stress, thereby restoring tendon stiffness and tensile strength. Concurrently, type III collagen is progressively replaced by type I collagen, and tendon fibroblasts gradually differentiate into myofibroblasts [16].

4. Background to Autophagy

Autophagy is a conserved mechanism for protein degradation and organelle recycling in eukaryotic cells [17]. When induced by various cellular stress conditions, autophagy-associated proteins are recruited to cellular membranes to form double-membrane phagophores. These phagophores extend and encapsulate intracellular material, forming distinct autophagosomes [18,19]. Subsequently, autophagosomes fuse with lysosomes to form autolysosomes, wherein degradation occurs under the mediation of acidic hydrolases [20]. The degraded materials include damaged macromolecules, organelles, and misfolded cytoplasmic proteins, which are catabolized to provide energy and essential nutrients for intracellular activities [21]. Autophagy is classified microscopically into xenophagy, mitophagy, ER-phagy, pexophagy, ribophagy, and chaperone-mediated autophagy, and macroscopically into selective and non-selective autophagy [18]. Furthermore, hypoxia or nutrient deprivation serves as a key inducer of non-selective autophagy, while selective autophagy primarily facilitates the recycling of misfolded proteins and organelle degradation. Recent studies indicate that appropriate levels of autophagy also enable osteoblasts and osteoclasts to survive under extreme conditions [22].

Macrophage autophagy comprises five distinct phases: initiation, nucleation, elongation, fusion/degradation, and termination [23]. This process requires multiple regulatory factors. Autophagy initiation commences with the activation of the Unc-51 like kinase 1 gene (*ULK1*) kinase complex, while autophagy related 5 gene (*ATG5*) plays a critical role in autophagic vesicle formation among autophagy-related genes [24]. During the elongation phase, ATG5 and ATG12 are activated by ATG7 and ATG10, forming a stable complex with ATG16L1 [25]. Microtubule-associated protein 1 LC3 is located on phagophores and autophagosome membranes. Upon autophagy induction, LC3 is activated by the complex formed by ATG5-ATG12-ATG16L1 [26]. LC3-I conjugates with phosphatidylethanolamine (PE) to form LC3-II, which both promotes continuous extension of the phagophore bilayer membrane structure and facilitates autophagosome maturation. Consequently, the LC3-II/LC3-I ratio serves as a key marker for monitoring autophagy occurrence [27]. Autophagy effector protein-1 (Arg1) is a crucial regulator of autophagosome formation. It binds Beclin-1, which dissociates during stress-induced autophagy to form a complex with class III phosphatidylinositol 3-kinase (PI3KC3). This complex regulates phagophore localization and upregulates transcription of cytokine genes, including *IL-1*β, *IL-6*, and Tumor Necrosis Factor (*TNF*)-α [28,29]. Upregulation of these cytokine genes results in extensive degradation or inhibition of the p65 subunit (RelA), a process that theoretically blocks the “inflammatory cascade”. Additionally, nuclear factor kappa-B (NF-κB) upregulation restricts inhibition of autophagy activators (e.g., BCL-2 interacting protein 3 (BNIP3), Reactive Oxygen Species (ROS), and mitogen-activated protein kinase (MAPK)8/C-Jun N-terminal kinase (JNK)1) and enhances inhibition of autophagy inhibitors (e.g., B-cell lymphoma-2 (BCL-2), BCL-2L1/BCL-XL) [30,31]. The autophagy receptor p62/ sequestosome 1 (SQSTM1), predominantly expressed in macrophages, functions in cellular homeostasis and antioxidative stress and can upregulate NF-κB activity [32]. Upon activation, p62 forms aggregates, delivers ubiquitinated substrates to the autophagy machinery, and is itself engulfed by autophagosomes via binding to LC3. The p62-LC3 complex then enters the lysosome [33]. Subsequent recruitment of phosphoinositide 3-kinase and Beclin-1 facilitates the assembly of autophagy-associated proteins and autophagic vesicle formation. Both the ATG12 conjugation system and the LC3 (ATG8) conjugation system cooperate in phagophore membrane elongation and double-membrane autophagosome formation [34]. Finally, the autophagosome outer membrane fuses with lysosomes. The resultant autolysosome degrades residual abnormal components, thereby concluding the autophagy process (Table 1, Ref. [24–29,32,34–43]).

Table 1. Key molecules, molecular functions and core molecular mechanisms of autophagy.

Process phase	Key molecules/complexes	Molecular functions	Regulatory mechanisms
Initiation signaling	NF-κB (p65/p50) NF-κB (p65/p50)	Transcriptional activation of autophagy-related genes	IκB phosphorylation and degradation $\rightarrow$ NF-κB entry into the nucleus $\rightarrow$ induction of IL-1β expression $\rightarrow$ activation of ULK1
Nucleation of phagocytic vesicles	VPS34-Beclin1-PI3KC3	Generates PI3P lipid membrane and Recruitment of ATG protein to phagosomes	Beclin1 dissociates from BCL-2 $\rightarrow$ binds PI3KC3 $\rightarrow$ recruits ATG14L/WIPI
Membrane extension	ATG5-ATG12-ATG16L1Complex	Mediates LC3 lipidation	ATG7/ATG10 activates ATG5-ATG12 $\rightarrow$ binds ATG16L1 $\rightarrow$ forms an E3 ligase complex
Autophagosome labeling	LC3-I $\rightarrow$ LC3-II	Autophagosome membrane localization marker	LC3-I cleaved by ATG4 $\rightarrow$ binds to PE $\rightarrow$ anchors to autophagosomal membrane (dependent on ATG5-ATG12-ATG16L1 complex)
Substrate recognition	p62/SQSTM1 + Parkin p62/SQSTM 1 + Parkin	Recruitment of ubiquitinated substrates	p62 aggregates ubiquitinated proteins $\rightarrow$ binds LC3-II via LIR domain $\rightarrow$ encapsulates into autophagosomes (Parkin mediates mitochondrial ubiquitination)
Lysosomal fusion	STX17-SNAP29-VAMP8	Mediates membrane fusion	Rab7-GTP hydrolysis $\rightarrow$ drives SNARE complex assembly $\rightarrow$ Autophagosome-lysosome fusion, cathepsin degradation of contents
References	[29,32,35–38,41]	[24–28,34,39,42]	[24–27,34,40,43]

NF-κB, nuclear factor kappa-B; ATG5, autophagy related 5 gene; LC3, light chain 3; SQSTM1, sequestosome 1; ULK1, Unc-51 like kinase 1 gene.

5. Different Modes and Mechanisms of Macrophage Death

5.1 Macrophage Apoptosis

Apoptosis serves as a critical host defense mechanism, activated through pathways including p38, NF-κB, Toll-like receptors-2 (TLR2), ERK, and Phosphatidylinositol 3-kinase (PI-3K) [44]. The BCL-2/Bax ratio within this protein family determines apoptotic fate [45]. In infected macrophages, TNF signaling initiates exogenous apoptosis, triggering TNF-α activation and osteoclast autophagy. This cascade simultaneously increases anti-apoptotic BCL-2 expression while decreasing pro-apoptotic Bax and Caspase-3 [46]. Phosphorylated Beclin-1—a key autophagy-apoptosis nexus—is upregulated during autophagy activation. This process liberates BCL-2 from Beclin-1 complexes, maintaining osteoblast anti-apoptotic function and cellular homeostasis [47].

5.2 Macrophage Necrosis

Macrophage necrosis comprises non-programmed and programmed forms. Non-programmed necrosis—typically induced by intense external stimuli—activates receptor-interacting protein kinase (RIPK) and provokes severe inflammation with irreversible damage [48,49]. Key inducers include high cytokine concentrations, oxidative/ER stress, and fas cell surface death receptor (FAS) ligand activation. Pathogen invasion (e.g., Staphylococcus aureus, Escherichia coli, Streptococcus pneumoniae) generates bacterial toxins causing membrane rupture, mitochondrial depolarization, Adenosinetriphosphate (ATP) depletion, ROS surge, and ultimately programmed necrosis (necroptosis) [50].

5.3 Macrophage Pyroptosis

Macrophage pyroptosis exhibits molecular and morphological features intermediate to apoptosis and necrosis. Pathogen exposure triggers caspase-1-mediated pyroptosis [51,52]. Under oxidative stress, Gasdermin D oxidation and NLRP3 inflammasome activation induce mitochondrial membrane potential collapse and ROS generation [53,54]. Caspase-1 cleaves Gasdermin D (GSDMD), releasing its N-terminal domain (NT-GSDMD). This forms membrane pores that facilitate IL-1β release and provoke lytic cell death [55].

5.4 Macrophage Ferroptosis

Ferroptosis, initially identified in cancer cells, is a novel mode of programmed death induced by iron-dependent depletion of polyunsaturated fatty acids and aggregation of toxic lipid reactive oxygen species [56]. Three main molecular pathways are involved: first, inhibition of small molecule peroxidation and glutathione peroxidase 4 degradation prevents lipids from being over-oxidised and further induces ferroptosis [57]. Second, inhibition of cystine glutamate transporter receptor reduces peroxidase activity and cellular antioxidant capacity, which eventually

Table 2. Different modes and mechanisms of macrophage death, include autophagy, necrotic apoptosis, necrosis, pyroptosis and ferroptosis.

	Autophagy	Necrotic apoptosis	Necrosis	Pyroptosis	Ferroptosis
Biochemical characteristics	Increased lysosomal activity	Caspase1 activation dependent or independent	ATP levels decreased; RIP1, RIP3 and MLK were activated	Activation of caspases oligosome DNA fragments	GSH and GPX4 inhibition; iron accumulation and lipid peroxidation
Morphological features	Formation of autophagic lysosomes with double-membrane structures	Plasma membrane blistering, reduced cell and nuclear volume, nuclear fragmentation	Plasma membrane rupture, organelles swelling, chromatin condensation	Nuclear retraction, cell swelling and cell membrane hole, cell collapse and rupture	Mitochondria become small and cristae of mitochondria decrease or disappear
Coregulated gene	LC3, ATG5, ATG-7, Beclin1, Other ATG family proteins	p53, Bax, Bak, BCL-2, BCL-XL, BCL-2 family proteins, Other anti-apoptotic	RIP1, RIP3, MLK L	CASP1, CASP11, GSDMD	VDAC2/3, Ras, NOX, TFR1, p53, CARS, GPX4, SLC7A11, HSPB1, NRF2
Regulatory path	PI3K-AKT-mTOR, MAPK-ERK1/2-mTOR	Csapase, P53, BCL-2	TNFα, TNFR1, TLR3, trail, FasL, ROS, PKC-MAPK-AP-1	Caspase-1, NLRP3	Gpx4, MVA, HSF1-HSPB1, p62-Keap1-Nrf2, LSH
Release of damage associated molecules	HMGB-1	HMGB1, ATP	DNA, IL-6	HMGB1, ATP, IL-1β, IL-18	HMGB1
Immunological characteristics	anti-inflammatory	anti-inflammatory	Proinflammatory	Proinflammatory	Proinflammatory
Detection method	Detection of changes in the levels of autophagy-related proteins such as Atg5, Atg7, BeclinI, LC3, P62, autophagosome fluorescence single/double labelling assay, lysosomal function assays	Mitochondria-free membrane potential assay, An-nexin V/PI, TU-NEL assay, apoptosis-related pathways, apoptosis-related proteins	Immunofluorescence or flowthrough methods by PI or 7-AAD staining	NLRP3, ASC, Procaspase-1, Cleavedcaspase-1, Pro-IL-1β, Cleaved-IL-1β, Pro-IL-18	Cellular activity assays: CCK-8, intracellular iron levels (PGSK probe), levels of reactive oxygen species, changes in death-associated factors such as COX-2, ACSL4, PTGS2, NOX1, GPX4, and FTH1
References	[15–21,63,64]	[46,50,56–59]	[19,27,46,47,50,66]	[20,31,61,62,65,66]	[35–38,52–54]

Table 3. The effects of autophagy on different types of cells in various biological processes.

Cell type	Autophagy function	Role in tendon-bone healing	Molecular mechanisms
Macrophages	Remove damaged mitochondria	Inhibition of NLRP3 inflammasome → reduces early inflammation(M1Macrophages secretes TNF-α → activates OC autophagy → enhances bone resorption)	PINK1/Parkin mediates mitochondrial autophagy → reduces ROS → blocks caspase-1 activation PINK1/Parkin
Osteoclasts	Promotes crease margin formation	Enhance bone resorption → accelerate bone remodelling	ATG5-dependent Rab7 localisation → drives integrin αvβ3 recombination → enhances cell migration (Table 1)
Osteoblasts	Maintains cell survival	Resist stress-induced apoptosis → promote bone matrix deposition	Autophagy degrades misfolded proteins → activates mTORC1 → upregulates RUNX2
Chondrocytes	Inhibits hypertrophic apoptosis	Protects fibrocartilage layer → promotes interface regeneration (M2Macrophages induces autophagy → secretes TGF-β3 → inhibits chon-drocyte apoptosis)	Autophagy removes damaged ER → inhibits CHOP pathway → reduces caspase-3 activation
Tendon stem cells	Maintain stemness	Promote differentiation to tendon cells → enhance collagen synthesis	Activation of p62/KEAP1-NRF2 axis → anti-oxidative stress → maintain stem cell pool
References	[15–21,63,64]	[46,50,56–59]	[19,27,46,47,50,66]

progresses to ferroptosis. Third, inhibition of the cystine glutamate transporter receptor system is mediated at the gene level through p53, which induces the activation of pro-apoptotic proteins and transcriptional repression of anti-apoptotic proteins by inhibiting the anti-apoptotic function of BCL-2 [58,59].

5.5 Macrophage Autophagy

Macrophage autophagy is modulated by NF-κB signaling—a nuclear transcription factor that integrates inflammatory and autophagic responses to prevent hyperinflammation and suppress inflammasome activation [60,61]. The NF-κB family comprises five eukaryotic transcription factors: c-Rel, p65/RelA, RelB, p50/NF-κB1, and p52/NF-κB2 [35]. These form homo-/heterodimers critical for inflammation control. Recent studies show autophagy-associated proteins complexed with IκB sequester inactive NF-κB in the cytoplasm [36]. Upon stimulation, IκB kinase activation triggers IκB phosphorylation, ubiquitination, and degradation. Liberated NF-κB translocates to the nucleus, binds nuclear localization sequences, and initiates downstream gene transcription. By inducing IL-1β expression, NF-κB emerges as a key activator of both inflammation and autophagy (Table 2, Ref. [15–21,27,31,35–38,46,47,50,52–54,56–59,61–66]).

6. Macrophages in the Healing Process of Tendon Bone

Stimulated by Receptor Activator of nuclear factor-κB ligand (RANKL) and macrophage colony-stimulating factor (M-CSF) [37], macrophages differentiate into multinucleated osteoclasts. Embryonically, yolk sac erythromyeloid progenitors and hematopoietic stem cells generate fetal osteoclasts that shape skeletal development [38]. Osteoclasts promote osteogenesis through: Direct Receptor Activator of nuclear factor-κB (RANK)-containing extracellular vesicle release binding osteoblast membrane RANKL, upregulating runt-related transcription factor (RUNX) transcription factors [62]; Indirect collagen triple helix repeat containing 1 (CTHRC1) and sphingosine-1-phosphate release recruiting MSCs for proliferation/differentiation [67]. Osteoblast-secreted RANKL drives osteoclast maturation via RANK binding, while osteoprotegerin inhibits this interaction [63]. RANKL (type II TNF receptor) forms homotrimers with receptor-binding clefts [63]; RANK (type I TNF receptor) trimerizes upon ligand binding, initiating signal transduction [64]. In osteoclastogenesis, TGF-β-activated kinase 1 (TAK1) activates IκB kinase (IKK) and MAPK cascades. As mitogen-activated protein kinase kinase kinase (MAPKKK), TAK1 phosphorylates mitogen-activated protein kinase kinase (MAPKK) which activates p38/JNK [68]. RANKL-activated JNK1 is essential—deficiency blocks osteoclast differentiation [69]. Osteoclast cytokines also suppress osteoblast activity. p62 degradation elevates LC3 and ATG5/7/12, accelerating actin ring formation that regulates macrophage autophagy and osteoclastogenesis [39]. Recent studies implicate G protein-coupled receptor kinase interacting protein 1 (GRKIP1), IL17A, CD147, TNF receptor-associated factor 6 (TRAF6), and kruppel-like fac-

tor 2 (KLF2) in autophagy-mediated osteolysis [70,71] (Table 3, Ref. [15–21,27,46,47,50,56–59,63,64,66]).

7. Typing and Characterisation of Macrophages

During early inflammation, macrophages (MΦ) recruited from peripheral blood, bone marrow, and synovial tissues to the tendon-bone interface predominantly polarize into M1 phenotype. These phagocytose debris, clear pathogens, and secrete inflammatory factors (including CXCL8/IL-8, CCL2, SDF-1) to promote healing [72]. In subsequent repair phases, M2 macrophages dominate and facilitate healing. Polarized into four subtypes (M2a/b/c/d), M2 exhibit limited pathogen phagocytosis and pro-inflammatory factor production but enhanced anti-inflammatory factor release, creating a regenerative microenvironment [73]. Notably, M2 demonstrate greater osteoclastogenic potential than M1 [74]. While M1 promote osteoclast formation via pro-inflammatory factors, M2 inhibit it through anti-inflammatory mediators. Osteoclasts express M2-specific markers (CD163, CD206, YM1, IL-10), indicating possible M2-derived origin [75,76].

8. The Role of Macrophages on Chondrocyte Apoptosis

M1 macrophage polarization induces chondrocyte apoptosis and hypertrophy, extracellular matrix (ECM) degradation, and ultimately exacerbates cartilage damage and OA progression [77]. Selective macrophage depletion significantly reduces the M1/M2 ratio, downregulates IL-1β and matrix metalloproteinase (MMP)-13 levels, and attenuates cartilage damage. Within the OA synovium, M1 macrophages secrete inflammatory cytokines and MMPs, accelerating ECM degradation and chondrocyte apoptosis. IL-1β activates key NF-κB and MAPK signaling pathway enzymes (p38, p50, p52, Rel, JNK, ERK), regulating chondrocyte proliferation, differentiation, and apoptosis [78]. Type II collagen activates M2 macrophages, inhibits chondrocyte hypertrophy and apoptosis, and promotes slow repair of degenerative OA cartilage. Interleukin-1 receptor antagonist, chemokine ligand 18, and insulin-like growth factor enhance chondrogenic mediators [79].

9. The Role of Macrophages in Osteoclast Formation

M1 macrophages secrete cytokines to induce osteoclastogenesis from osteoclast precursor cells (OCPs). Studies demonstrate that M1 macrophages enhance osteoclast precursor proliferation or directly induce their differentiation into osteoclasts via cytokine secretion, while increased pro-inflammatory factor release indirectly upregulates RANKL secretion by osteoblasts and stromal cells, thereby augmenting osteoclast formation [80,81]. In contrast, M2 macrophages polarize into the M2a subtype upon IL-4/IL-13 stimulation, secreting insulin-like growth factor-1 (IGF-1) and arginase-1, releasing chemokines such as CCL13 and CCL17, and recruiting basophils, eosinophils, and Th2 cells [82]. Notably, high surface expression of CD86 and CD163 facilitates neovascularization and collagen deposition. Glucocorticoids, IL-10, and TGF-β induce M2c polarization, promoting matrix metalloproteinase (MMP) secretion; meanwhile, moderate CCL16 release recruits resting T cells and eosinophils, regulating extracellular matrix (ECM) remodeling and fibrosis [83].

Osteoclasts, regulated by autophagy or pathway-associated upstream/downstream cytokines, also feedback by releasing bioactive signals that inversely modulate macrophages and autophagic complexes, further regulating tendon-bone junction healing (relevant cytokines are detailed below) (Table 4, Ref. [30,63,64,72–76,84–88]).

10. The Effect of Correlating Factors on Tendon Bone Healing

10.1 TNF-α

TNF-α, a key factor secreted by M1 macrophages promoting osteoclast formation, induces apoptosis of tendon stem cells, increases matrix metalloproteinase synthesis, and decreases type I collagen synthesis, leading to extracellular matrix degradation at the tendon-bone junction [84]. TNF-α also activates the NF-κB signalling pathway, triggering substantial pro-inflammatory factor secretion and thereby exacerbating the inflammatory response [89]. *In vitro* experiments demonstrated that TNF-α addition following RANKL co-stimulation significantly increased osteoclast numbers compared to RANKL alone [85]. This effect may stem from TNF-α's inhibitory role in macrophage differentiation into osteoclast precursor cells.

10.2 IL-1β

IL-1β, an inflammatory cytokine secreted by M1 macrophages, is minimally expressed in the normal tendon-bone junction but significantly upregulated in post-injury scar tissue and peripheral joint cavities [90]. This pro-inflammatory mediator directly promotes osteoclast differentiation by enhancing stromal cell-derived RANKL secretion, or indirectly induces osteoclast formation via TNF-α secretion—particularly in the presence of RANKL—through the p38 mitogen-activated protein kinase (MAPK) pathway [40]. IL-1β also potently enhances prostaglandin synthesis in bone tissues. In both human and murine models, it upregulates RANKL expression in regulatory T cells, thereby facilitating osteoclast formation from precursor cells [91]. Notably, in these systems, IL-1β-mediated RANKL upregulation in Tregs accelerates osteoclastogenesis, with early IL-1β blockade exerting a more pronounced inhibitory effect on arthritis and osteoclast formation compared to late intervention [92]. Additionally, IL-1β modulates tendon-bone healing via the NF-κB signaling pathway: it inactivates NF-κB dimer inhibitors through the

Table 4. Different pathways and functional components of macrophage polarisation and autophagy interaction.

Regulatory module	Key components	Biological significance	References
M1 polarisation pathway	TLR4/NF-κB $\rightarrow$ TNF-α, IL-1$\beta\uparrow$	Pro-inflammatory signals inhibit autophagy initiation, but ROS can activate stress-induced autophagy	[72–76,84,85]
M2 Polarisation Pathway	IL-4/STAT6 $\rightarrow$ Arg1, CD206$\uparrow$	Inhibits autophagy by activating Akt-mTOR, promoting a reparative phenotype	[72–76,86,87]
Autophagy regulates polarisation	TG5 degradation STAT1 $\rightarrow$ inhibits M1 p62 activation NRF2 $\rightarrow$ promotes M2	Resist stress-induced apoptosis $\rightarrow$ promote bone matrix deposition	[63,64,73,76]
Metabolic switch	Hypoxia $\rightarrow$ HIF-1$\alpha\uparrow$ $\rightarrow$ BNIP3-mediated mitochondrial autophagy	Removes damaged mitochondria, reducing M1-related inflammation	[30,88]

$\uparrow$: be promoted.

IKK complex, promoting nuclear translocation of NF-κB dimers and subsequent upregulation of pro-inflammatory factor gene expression [41,93].

10.3 IL-4

IL-4 is an anti-inflammatory factor secreted by M2 and inhibits osteoclast formation by suppressing the expression of RANKL and TNF-α [86]. IL-4-induced reduction of RANKL expression is mainly caused by inhibiting the activation of NF-κB in a STAT6-dependent manner and inhibiting the MAPK signalling pathway, and the lack of IL-4 leads to the reduction of bone resorption capacity [87].

10.4 IL-6

IL-6, a downstream product of the NF-κB signaling pathway, is abundantly expressed in torn tendon tissue. M1 macrophages also secrete copious amounts of IL-6 during inflammatory responses [94]. Characterized by dual pro- and anti-inflammatory properties, IL-6 exerts anti-inflammatory effects via its membrane-bound receptor (IL-6R) and pro-inflammatory effects through the soluble form of IL-6R [95]. While IL-6 recruits monocytes and promotes their differentiation into osteoclasts, it simultaneously enhances the release of IL-1 receptor antagonists and IL-10 to suppress excessive inflammation [65]. STAT3, activated via the JAK-IL-6 signaling axis, drives osteoclastogenesis; notably, IL-6 inhibition markedly diminishes osteoclast formation both *in vitro* and *in vivo* [96].

10.5 IL-10

IL-10 is negatively associated with the development of osteoporosis and inhibits osteoclast formation by decreasing the secretion of pro-inflammatory factors and inhibiting the expression of RANKL. IL-10 levels are significantly lower in osteoporotic patients compared to healthy individuals [97].

10.6 Chemokines

M1 macrophages release chemokines (e.g., CCL2, CXCL8, SDF-1, etc.) to recruit MSCs to the injury site to participate in tendon bone healing [98]. In the early stage of tendon-bone junction healing, a large number of M1 macrophages infiltrate to phagocytose cellular debris and foreign pathogens, and at the same time recruit MSCs to the tendon-bone junction by secreting chemokines [99].

10.7 TGF-β

TGF-β, a member of a superfamily regulating cell growth and differentiation, is implicated in tissue healing. Persistently elevated TGF-β1 levels during the healing process activate signaling pathways such as mitogen-activated protein kinase (MAPK) and Smad2/3, inducing fibroblasts to overproduce extracellular matrix (ECM) and driving scar tissue formation [100]. Chronic inflammation sustains high TGF-β1 expression within the TGF-β/Smad3 pathway, further inducing M2-type macrophages to undergo macrophage-to-myofibroblast transition [101]. In a rat model of acute rotator cuff injury, exogenous TGF-β1 administration enhances tendon-bone healing, improves biomechanical strength, upregulates type III collagen transcription, and promotes fibrous scar tissue formation [102–104].

10.8 VEGF and Bone Morphogenetic Protein-2

M2 macrophage polarization enhances endogenous vascular endothelial growth factor (VEGF) expression, promoting vascular neovascularization and fat infiltration in ischemic conditions. This creates a favorable microenvironment for tendon-bone healing [88]. BMSC-derived exosomes induce M2 polarization, elevate VEGF expression, and stimulate peripheral vascular neovascularization around injured tendon-bone interfaces. This improves ligament healing strength without increasing adhesion [105]. Bone morphogenetic proteins (BMPs), abundant in bone matrix, promote chondrogenic and osteogenic differentiation of bone marrow stromal stem cells. BMP-2—among the most active isoforms—drives trilineage differentiation (adipogenesis, chondrogenesis, osteogenesis), enhances vascular density, and upregulates alkaline phosphatase and osteocalcin [106,107] (Table 5, Ref. [32,40,41,65,84–87,89–95,100–104]).

Table 5. The bidirectional regulatory effect of autophagy on different types of cytokines (mainly divided into positive and negative effects).

Cytokine	Regulatory role of autophagy	Effects on tendon-bone healing	Molecular pathways	References
TNF-α	⊙ Autophagy degrades TNF-α receptors	Negative: Induces tendon stem cell apoptosis + inhibits type I collagen synthesis	NF-κB/p38 MAPK activation → Caspase-3 cleavage	[32,65,84,85,87,89,94,95]
	⊙ TNF-α inhibits autophagy initiation	Positive: Low concentrations promote M1 differentiation into OC precursors	Inhibits AMPK → activates mTORC1	
IL-1β	⊙ Autophagy clears inflammasomes	Negative: Promotes MMP expression → ECM degradation	NLRP3-Caspase-1/GSDMD pathway activation	[40,41,85,89–93]
	⊙ IL-1β induces stress-induced autophagy	Positive: Enhances cellular stress survival	PI3K-AKT-FOXO3a feedback loop	
IL-4	⊙ Autophagy promotes IL-4 secretion	Positive: Inhibits RANKL expression → Reduces bone resorption	STAT6 phosphorylation → Inhibits NF-κB nuclear translocation	[86,87]
	⊙ IL-4 inhibits excessive autophagy	Negative: Delays damage clearance	Activates Akt → Inhibits FOXO1	
TGF-β3	⊙ Autophagy activates TGF-β3 release	Positive: Promotes fibrocartilage regeneration + inhibits scar formation	Smad2/3 phosphorylation → Upregulates SOX9/type II collagen	[41,100–104]
	⊙ TGF-β3 enhances autophagy flux	Positive: Clears damaged ECM → creates space for new tissue formation	Inhibits PI3K-Akt → relieves mTOR inhibition of ULK1	
IL-10	⊙ Autophagy maintains IL-10 levels	Positive: Inhibits pro-inflammatory cytokine storm + promotes M2 polarisation	Activates JAK1-STAT3 → Upregulates BCL-2	[89,93]
	⊙ IL-10 promotes mitochondrial autophagy	Positive: Protects macrophage anti-inflammatory phenotype	PINK1/Parkin ubiquitinates damaged mitochondria	

11. Crucial Factor of Tendon-Bone Healing

11.1 Growth Factors

These growth factors, when highly expressed, modulate both the biological and mechanical microenvironments of the tendon-bone junction through the following mechanisms: First, distinct growth factor types are upregulated at different stages—those recruiting inflammatory cells and promoting neoangiogenesis are predominant during the inflammatory phase, while those driving stem cell differentiation into specific lineages and collagen synthesis dominate during the reparative and remodeling phases [108]. Second, acting as signaling molecules, these growth factors regulate cellular behaviors via paracrine or autocrine mechanisms to facilitate tendon-bone healing [109]. Third, growth factors exhibit interactive effects [103]. However, due to their short half-lives, sustained local delivery via appropriate carriers is necessary to maintain their temporal efficacy.

11.2 Stress Stimulation

Shear stress, distraction stress, mechanical stimulation, and noose stress can affect collagen and extracellular matrix synthesis, and under limited conditions can promote the differentiation of cells at the tendon-bone junction into cartilage [110]. Existing studies have found higher expression of type I collagen and glycosaminoglycans in the cartilage matrix of the loaded stress group, with a higher resistance to distraction and healing closer to normal tissue morphology [111].

11.3 Other Factors

The Other tendon-bone junction repair method include biomechanical materials, cellular applications (e.g., osteoinductive materials, biodegradable scaffolds, bionic patch gene therapy and cellular therapy, etc.), and postoperative rehabilitation strategies [105,109].

11.4 Low-Intensity Pulsed Ultrasound (LIPUS)

LIPUS delivers non-invasive mechanical stimuli that activate the PI3K-Akt pathway via mechanotransduction, polarizing macrophages toward the M2 phenotype [112]. This polarization upregulates IL-10 while downregulating pro-inflammatory cytokines (TNF-α, IL-1β) [113]. M2 macrophages promote collagen matrix degradation through autophagy-dependent lysosomal pathways, facilitating TGF-β3-mediated fibrocartilage regeneration and tendon-bone junction healing [114]. Although inflammatory cues (TGF-β/IL-1β) induce autophagy via the ATG7-Beclin1 complex [25–27]. Coman *et al.* (2011) [115] demonstrated that mechanical stimulation activates PI3K-Akt signalling in myeloid cells, laying the foundation for LIPUS-mediated immune regulation. As a non-invasive physical stimulus, LIPUS mediates its effects through dual mechanisms: direct mechanotransduction via macrophage surface receptors and indirect immunomodulation through microcirculatory enhancement. Furthermore, LIPUS induces macrophage upregulation of BMP-2 and TGF-β1 expression, activates autophagy flux, enhances lysosomal permeabilization, and accelerates LC3-II conversion—collectively promoting osteoblast differentiation and collagen realignment at the tendon-bone junction (TBJ) interface [116].

12. Regulation of Osteoclast Differentiation by Autophagy

Osteoclast (OC) differentiation and maturation are regulated by multiple signaling pathways, including mTORC1 which mediates autophagy and influences OC differentiation [97]. mTORC1 is a serine/threonine kinase regulating protein synthesis. Under nutrient-rich conditions, mTORC1 binds and inhibits the ULK1 complex, suppressing autophagy; during malnutrition, their dissociation activates ULK1 through dephosphorylation, initiating autophagy [66,117]. The mTOR pathway is modulated by upstream regulators including AMPK, Akt, and PI3K pathways [118,119]. mTORC1-mediated autophagy may bidirectionally regulate OC. AMPK maintains cellular homeostasis by activating during energy depletion. High glucose decreases p-AMPK and p-ULK1 while increasing p-mTOR [120]. Concomitantly, reduced LC3-II and Beclin-1 with elevated P62 correlate with decreased OC production and bone resorption, indicating mTORC1-mediated autophagy promotes osteoclastogenesis [121].

13. Regulation of Osteoclast Migration by Autophagy

Osteoclasts (OCs) migrate to and adhere to bone surfaces during differentiation and maturation to execute bone resorption. Their migratory capacity depends on adaptive morphological changes and the continuous formation and degradation of pseudopodia. Integrin αvβ3 is localized in the basement membrane of pseudopodia—for example, on the outer side of the basement membrane and at the edges of folds—not only reorganizing OC cytoskeleton to polarize these cells but also facilitating migration via continuous adhesion and detachment to/from the extracellular matrix [122,123]. The autophagy-associated protein LC3B plays a critical role in pseudopod degradation and OC migration by regulating integrin αvβ3 and kindlin-3 [24]. Thus, integrin αvβ3 and its downstream effectors such as focal adhesion kinase (FAK) are likely key autophagy targets governing OC migration.

14. Regulation of Osteoclast Bone Resorption Function by Autophagy

Osteoclasts (OCs) attach to bone surfaces forming ruffled borders, secreting acid and proteolytic enzymes to resorb bone. ATG4B, ATG5, ATG7, and LC3 critically regulate ruffled border formation and lysosomal secretion [24,39]. ATG5/ATG7 knockout impairs ruffled border for-

mation and reduces bone resorption without affecting OC differentiation [42]. Autophagy and resorption functions interconnect via lysosomes: Fusion of lysosomal vesicles forms ruffled borders, while vesicular transport of resorptive factors relies on GTP hydrolases. Rab7 localizes to ruffled borders in an ATG5-dependent manner; ATG5 deficiency disrupts Rab7/LC3 expression and reduces cathepsin K secretion [43,124]. ATG7 and ATG4B/LC3 further recruit Rab7 to enhance resorption.

15. Macrophage Autophagy/Polarization Mechanisms and Corresponding Therapeutic Strategies

The dynamic interplay between macrophage autophagy and polarization offers actionable targets for refining therapeutic strategies in tendon-bone junction (TBJ) repair. For graft integration, targeted modulation of macrophage phenotypes can optimize the foreign body response. Biomimetic scaffolds (e.g., gradient-mineralized hydrogels [7,109]) engineered to sustainably release autophagy inducers or M2-polarizing cytokines could promote early M2 dominance, suppress chronic inflammation, and enhance fibrocartilage regeneration at the graft-host interface. Crucially, scaffold surface topography and biochemical cues can direct macrophage polarization toward regenerative phenotypes, reducing fibrotic encapsulation and improving biomechanical integration [105]. In scaffold design, incorporating "smart" nanocarriers (e.g., exosomes) enables spatiotemporally controlled delivery of autophagy modulators (e.g., ATG5/7 agonists) or anti-inflammatory miRNAs specifically to TBJ-resident macrophages [105, 125]. For postoperative recovery, LIPUS exemplifies a non-invasive mechanobiological intervention. By activating the PI3K-Akt pathway [112,126], LIPUS polarizes macrophages toward M2 phenotypes, enhances autophagy flux, and upregulates BMP-2/TGF-β1 [114,115]. Integrating LIPUS with early controlled rehabilitation protocols could synergistically optimize ECM remodeling while preventing ectopic ossification—addressing a key limitation in current physical therapy regimens.

16. Different Outcomes of Macrophages in Tendon-Bone and Skin/Muscle Healing

Macrophage polarization is a key regulatory factor in tissue repair processes, but its regulatory mechanisms exhibit significant context-dependent discrepancies across different parts of the musculoskeletal system [127]. This difference is particularly evident in tendon-bone interface (TBJ) versus skin/muscle healing, where M2 macrophage polarization may even exert diametrically opposite effects. The divergence in fibrotic outcomes is a prominent manifestation of this discrepancy. In skin wound healing, excessive M2 polarization drives pathological fibrosis formation by overactivating the TGF-β1/Smad3 signaling pathway [128]. However, during tendon-bone interface (TBJ) healing, the situation is different. Here, M2 macrophages primarily secrete TGF-β3; this isoform promotes type III collagen deposition without causing scar formation, strongly suggesting tissue-specific isoform selectivity exists in the fibrogenic signaling pathway [129]. The variability in macrophage polarization behavior also differs depending on the tissue environment. Single-cell transcriptomic analysis reveals that macrophages at the tendon-bone interface tend to undergo terminal differentiation, with limited phenotypic plasticity making reversal difficult [130]. In contrast, macrophages in the skin dermis exhibit dynamic M1-M2 phenotypic switching capability during the wound resolution phase. This dynamism is considered constrained by the local calcified extracellular matrix (ECM), an environment that hinders the physical space required for cytoskeletal remodeling [130]. The cytokine-mediated mechanisms similarly exhibit tissue specificity. In skin repair, M2 macrophages, by secreting TGF-β1 and PDGF, activate the profibrotic DDR2 receptors on fibroblasts, leading to disorganized collagen deposition [131]. Whereas at the tendon-bone interface, the same cytokines derived from M2 cells instead stimulate tenocyte proliferation via integrin $\alpha 10\beta 1$-mediated FAK/Src signaling, or by activating the Akt/mTOR-dependent pathway, without inducing significant fibrosis [7,110]. Furthermore, the risk of ectopic ossification is a challenge specific to tendon-bone interface healing. Early excessive dominance of M2 macrophages at this site may aberrantly activate the BMP-2/Runx2 pathway within mesenchymal stem cells, thereby triggering chondro-osseous metaplasia and ectopic mineralization [132,133]—a pathology extremely rare in skin or muscle healing.

17. Macrophage-Driven Ectopic Ossification in Tendon Healing

Recent studies reveal that dysregulation of macrophage polarization disrupts the intrinsic healing program of tendons, thereby promoting the occurrence of ectopic ossification [134]. This phenomenon stands in sharp contrast to the healing process of bone defects: in bone repair, polarization of M2 macrophages typically favors osteogenesis; whereas in tendon injury, persistent activation of M1 macrophages drives pathological chondro-osteogenic differentiation through unique mechanisms. The shift in the cytokine microenvironment is a key mechanism therein. Activated M1 macrophages significantly increase the secretion of oncostatin M; this factor synergizes with BMP2 signaling to potently activate the Runx2 transcription factor in tendon-derived stem cells [125,135]. This signaling axis overrides the canonical TGF-β3/Smad2/3 pathway in tendon repair, leading to a fundamental shift in collagen synthesis—from tendon-characteristic type I and III collagen toward markers associated with hypertrophic cartilage [133]. External stimuli factors also profoundly influence this pathologi-

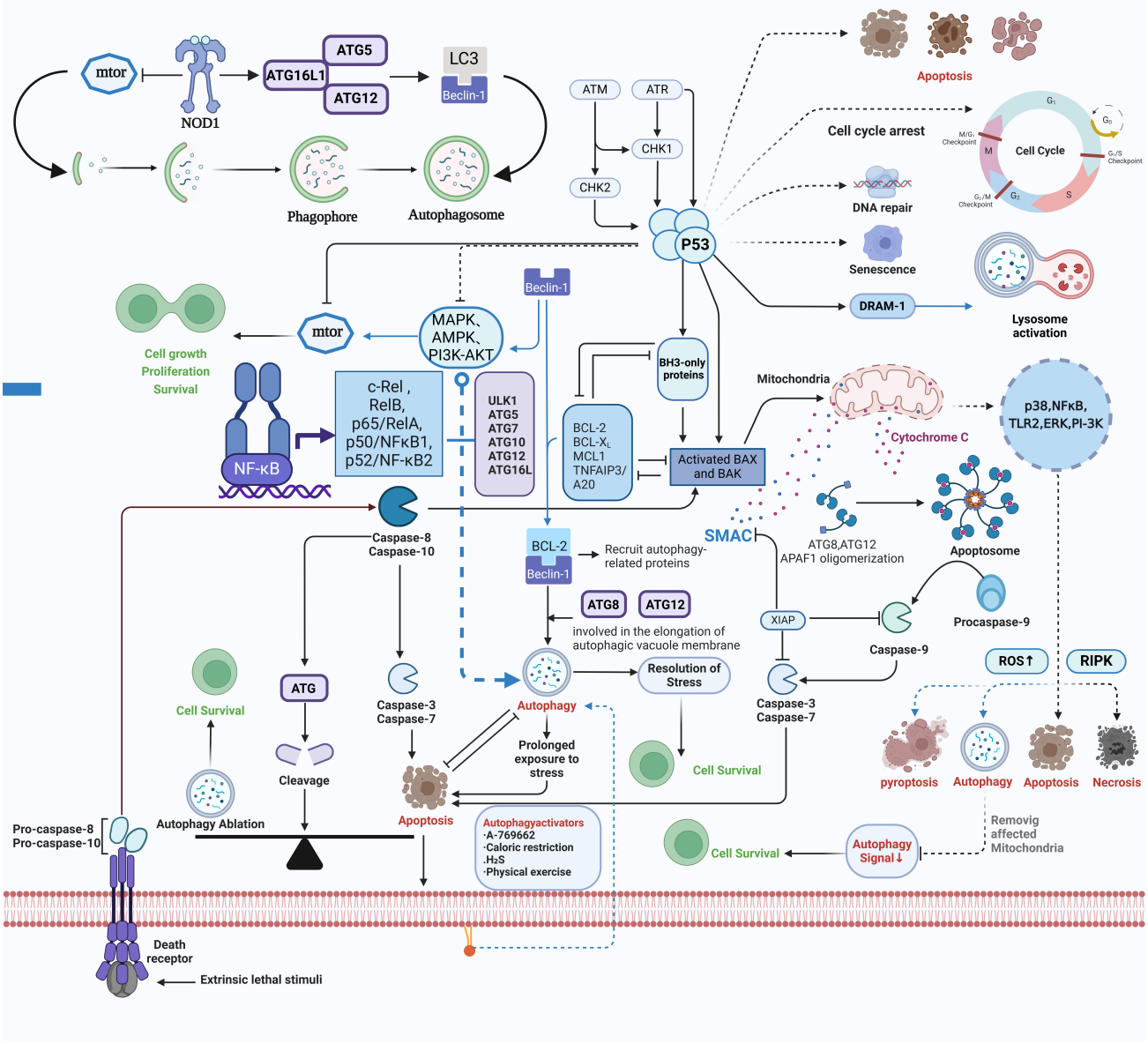

Fig. 1. Signaling pathways and the sequential process of autophagy. This figure illustrates: (1) the roles of cytokines and various molecules in autophagy through different pathways; (2) the morphological differences between autophagy and other forms of apoptosis; (3) the complete process of autophagy, from initiation, vesicle formation, membrane extension, substrate recognition, to lysosomal fusion.

cal process. Hypoxic conditions induce overexpression of BMP-2 in M2 macrophages, thereby promoting the transition of tenocytes to osteoblasts [125,136]. Notably, mechanical stimulation exerts a dual regulatory effect on BMP-2 secretion: on one hand, it enhances BMP-2 secretion from macrophages; on the other hand, within tendon tissue, this stimulation precisely maintains BMP-2 at pro-regenerative levels while effectively inhibiting ectopic bone formation [137] (Fig. 1).

18. Discussion

Emerging evidence confirms cytokine-mediated autophagy as pivotal for macrophage function at tendon-bone interfaces. However, physical stimuli represent an underutilized therapeutic lever. Coman *et al.* [115] demonstrated that mechanical strain activates PI3K-Akt via integrin β1 clustering in myeloid cells—providing the mechanistic foundation for LIPUS-mediated immunomodulation. Inflammatory microenvironments activate pathway cascades that yield potent cytokines (TGF-β, IL-1β, TNF-α) [40,84,85,90,91], autophagy-related proteins (ATG7, Beclin-1) [25–27], and autophagosome complexes. These components reciprocally stimulate macrophage autophagy, suppressing osteoclastogenesis and chondrocyte apoptosis.

19. Future Directions

19.1 Precise Regulation of Autophagy and Polarization Signaling Pathways in Macrophages

Interaction mechanisms between autophagy and polarization: Future studies should clarify whether autophagy-related proteins (e.g., ATG5, Beclin-1, LC3) modulate macrophage polarization direction (M1/M2) by degrading key polarization proteins or activating specific transcription factors [79]. Spatiotemporal-specific regulation of autophagy and polarization: Autophagy levels may differ between the inflammatory phase (M1-dominated) and the reparative phase (M2-dominated) [73–76].

19.2 Clinical Translational Strategies Targeting Autophagy

Optimization of drug delivery systems: Current autophagy modulators (inducers/inhibitors) lack tissue specificity and elicit systemic side effects [18,21–24]. Future investigations should explore nanocarrier-based systems (e.g., exosomes, liposomes) encapsulating autophagy regulators to achieve spatiotargeted modulation of local TBJ macrophages. Synergistic effects of biomaterials and autophagy: Biomimetic scaffolds (e.g., gradient mineralized hydrogels) can replicate the four-layer architecture of the tendon-bone junction (TBJ) while co-loading bioactive factors (e.g., IL-4, TGF-β3) to induce M2 macrophage polarization, thereby enhancing their therapeutic efficacy for TBJ repair [100,105].

19.3 Application of Multi-Omics Technologies in Macrophage Autophagy

Bioinformatics and high-throughput sequencing: transcriptome characterization of macrophage subsets during TBJ repair by single-cell RNA sequencing, combining bioinformatics to predict key targets [42].

19.4 Clinical Validation

Large animal experiments with clinical trials: testing intervention strategies targeting autophagy in large animal models of rotator cuff injury or anterior cruciate ligament reconstruction, and a gradual transition to clinical trials [31, 56].

19.5 Precision Mechanotherapy Integrating Coman's Viewpoint

Conformal LIPUS transducers such as 3D-printed devices to match TBJ stiffness gradients to optimize integrin β1 activation pathway. And further validate the upstream and downstream pathways through rigorous animal experiments or human trials.

19.6 LIPUS

Combining autophagy-modulating nanoparticles with LIPUS stimulation could provide a synergistic approach to resolving chronic inflammation at tendon-bone junctions and enhancing matrix regeneration at the repair site [126]. Combine LIPUS with thermosensitive liposomes co-loaded with autophagy inducer. Nanoscale LIPUS materials could be developed in future to achieve this goal.

20. Conclusions

Despite advances in understanding macrophage autophagy in tendon-bone junction repair, critical limitations persist: Current models (e.g., rodent rotator cuff injuries) fail to fully recapitulate human TBJ complexity, particularly regarding mechanotransduction and immune cell crosstalk. Biomaterial scaffolds show promise *in vitro* but lack clinical validation for sustained immunomodulation. We also put forward some testable hypotheses. Hypothesis 1: Precision timing of autophagy induction will accelerate M1-to-M2 transition, reducing scar formation (Testable via ATG7-knockout murine models). Hypothesis 2: Scaffold-driven autophagy (e.g., activated integrin β1/PI3K) synergizes with LIPUS to enhance collagen realignment (Testable using 3D-bioprinted TBJ constructs under mechanical loading). Based on the inflammatory microenvironment, precise regulatory strategies should be developed to ensure that the autophagy process in macrophages occurs within a predictable range, enabling them to fully utilise their role in promoting the remodelling or repair of tendon-bone junctions while minimizing heterotopic ossification. Furthermore, elucidating the mechanisms of autophagy provides a solid theoretical foundation for developing new mechanisms for repairing tendon-bone junctions, which is a crucial component in advancing the fields of sports medicine and tissue engineering.

Abbreviations

M1, Macrophage-1; M2, Macrophage-2; OC, Osteoclasts; OB, Osteoblasts; BMP, Bone Morphogenetic Protein-2; BMSCs, Bone marrow mesenchymal stem cells; MMPs, Matrix MetalloProteinases; mTOR, Mammalian target of rapamycin; IL-, Interleukin-; AMPK, AMP-activated protein kinase; GSH, Glutathione; GSSG, Oxidized Glutathione; GPX4, Glutathione PeroXidase4; ATG, Autophagy-related gene; NF-κB, Nuclear factor kappa-B; TRPV1, Transient receptor potential vanilloid-1; LC3, Microtubule-associated protein light chain 3; BCL-2, B-cell lymphoma-2; TLR2, Toll-like receptors-2; PI-3K, Phosphatidylinositol 3-kinase; ULK1, Unc-51 like Kinase 1 Gene; ATP, Adenosinetriphosphate; ROS, Reactive Oxygen Species; BINP3, BCL-2 interacting protein 3; TNF, Tumor Necrosis Factor; MAPK, Mitogen-Activated Protein Kinase; RANKL, Receptor Activator of Nuclear Factor-κB Ligand; JNK, C-Jun N-terminal kinase.

Author Contributions

DJY: Conceptualization, Methodology, Writing—Original Draft Preparation, Supervision. GWG, JFS: Col-

lecting References. JJC: Collecting References, Resources, Writing—Review & Editing. GYW, JCL: Collecting References, Project Administration, Funding Acquisition, Supervision. All authors contributed to editorial changes in the manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

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Conflict of Interest

The authors declare no conflict of interest.

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