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REVIEW ARTICLE

Hippo-YAP/TAZ signaling in osteogenesis and macrophage polarization: Therapeutic implications in bone defect repair

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Abstract Bone defects caused by diseases or surgery are a common clinical problem. Researchers are devoted to finding biological mechanisms that accelerate bone defect repair, which is a complex and continuous process controlled by many factors. As members of transcriptional costimulatory molecules, Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ) play an important regulatory role in osteogenesis, and they affect cell function by regulating the expression of osteogenic genes in osteogenesis-related cells. Macrophages are an important group of cells whose function is regulated by YAP/TAZ. Currently, the relationship between YAP/TAZ and macrophage polarization has attracted increasing attention. In bone tissue, YAP/TAZ can realize diverse osteogenic regulation by mediating macrophage polarization. Macrophages polarize into M1 and M2 phenotypes under different stimuli. M1 macrophages dominate the inflammatory response by releasing a number of inflammatory mediators in the early phase of bone defect repair, while massive aggregation of M2 macrophages is beneficial for inflammation resolution and tissue repair, as they secrete many anti-inflammatory and osteogenesis-related cytokines. The mechanism of YAP/TAZ-mediated macrophage polarization during osteogenesis warrants further study and it is likely to be a promising strategy for bone defect repair. In this article, we review the effect of Hippo-YAP/TAZ signaling and macrophage polarization on bone defect repair, and highlight the regulation of macrophage polarization by YAP/TAZ.

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Introduction

There are situations in which bone regeneration is highly desirable in orthopedic surgery and oral and maxillofacial surgery, such as the skeletal reconstruction of bone defects caused by trauma, infection, tumor resection, skeletal abnormalities, and osteoporosis.[1] Current strategies for bone regeneration have certain positive results, but there are also some shortcomings and limitations, including conditions of use, efficiency, and cost-effectiveness.[1] How to accelerate bone defect repair and improve the effectiveness of bone healing is an urgent problem for researchers. One of the important ways is to explore new ideas from the biological mechanism of bone defect repair. Bone defect repair is a multistep and overlapping process of inflammation and osteogenesis.[2] It is initiated by inflammation via the release of various cytokines and growth factors.[2] The pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, and IL-6 are released in large quantities.[3] While the inflammatory process is crucial for tissue repair, dysregulation of inflammation, whether up-regulated or down-regulated, is detrimental to bone healing.[4,5] Subsequently, the inflammation rapidly subsides to stimulate the formation of a pro-regenerative environment rich in pro-osteogenic factors and associated cell populations to ensure normal tissue repair and optimal bone regeneration.[6] Pro-osteogenic molecules can regulate the phenotype and function of a range of osteogenesis-related cells, thus impacting bone healing.[6] During the phase of bone formation and remodeling, mesenchymal stem cells (MSCs) play an important role in osteogenesis,[7] and bone undergoes continuous remodeling by balancing bone-forming osteoblasts (OBs) and bone-resorbing osteoclasts (OCs).[8,9] In addition to the aforementioned cells, macrophages also play a crucial role in bone defect repair by mediating inflammatory responses and tissue repair.

Macrophages are a highly adaptive cell population that is present in almost all tissues of the body and participate in different biological processes such as infection, repair and regeneration.[10,11] The detrimental influence of macrophage depletion on osteogenesis confirms the significant contributions of macrophages to bone regeneration.[12–15] The role of macrophages in bone defect repair is increasingly revealed, and they modulate the inflammatory microenvironment by polarizing into different phenotypes (M1/M2).[16] M1 macrophages mainly release pro-inflammatory cytokines to mediate inflammatory responses, while M2 macrophages secrete factors involved in osteogenesis.[17–20] Activated macrophages can regulate the biological behavior of bone marrow stem cells (BMSCs), such as homing, proliferation, and osteogenic differentiation, by secreting various cytokines.[12,21] The polarization of macrophages is associated with diverse factors and its mechanism remains to be further explored.

Currently, Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ) have been shown to be involved in osteogenesis,[22–25] and their effect can be partially achieved by regulating macrophage polarization. YAP and TAZ are downstream effectors of the Hippo pathway (Fig. 1), a kinase cascade capable of inhibiting YAP/TAZ activity.[26,27] In addition to YAP/TAZ, the core of the Hippo pathway also includes two kinases, mammalian STE-like 1 and 2 (MST1/2) and large tumor suppressor (LATS) 1/2.[28,29] As transcriptional coactivators, active YAP/TAZ accumulate in the nucleus and induce gene expression primarily by interacting with TEA domain (TEAD) transcription factors.[30–32] In the Hippo pathway, the activated LATS1/2 directly phosphorylate YAP/TAZ, preventing them from entering the nucleus.[33–35] The phosphorylation and activation of LATS1/2 are up-regulated by upstream MST1/2.[36–38]

In this review article, we summarize the intricate effects of Hippo-YAP/TAZ signaling and macrophage polarization on osteogenesis, as well as the regulation of macrophage polarization by Hippo-YAP/TAZ signaling. Although the mechanism of macrophage polarization during osteogenesis needs to be further studied, YAP/TAZ-mediated macrophage polarization is a novel and instructive idea, which is expected to become one of the therapeutic strategies for bone defect repair.

The role of Hippo-YAP/TAZ signaling in osteogenesis

Hippo-YAP/TAZ signaling affects osteogenesis by regulating the osteogenic function of MSCs

YAP/TAZ are crucial factors regulating the osteogenic differentiation of MSCs.[39,40] The positive regulation of YAP/TAZ on osteogenic differentiation of MSCs has been demonstrated by many studies. For example, the up-regulation of receptor activity-modifying protein 1 (RAMP1) expression promoted YAP-calcitonin gene-related peptide (CGRP)-mediated osteogenic differentiation of BMSCs.[41] Dupont et al[42] found that an active YAP mutation (YAP5SA) in BMSCs promoted osteogenic differentiation. Consistently, TAZ drives osteogenic differentiation of MSCs by activating Runt-related transcription factor 2 (Runx2).[43] Correspondingly, the lack of YAP/TAZ is not conducive to osteogenesis. Heterozygous deletion of YAP/TAZ inhibited the osteogenic differentiation of BMSCs, manifested by decreased osteogenic gene expression.[44] Furthermore, RNAi-mediated deletion of YAP/TAZ in BMSCs inhibited alkaline phosphatase (ALP) activity and mineral deposition even in an osteogenic environment.[24,42] In addition to the deletion of YAP/TAZ, G-protein alpha-subunit (GNAS), a gene encoding multiple transcripts, played an inhibitory role in the osteogenic

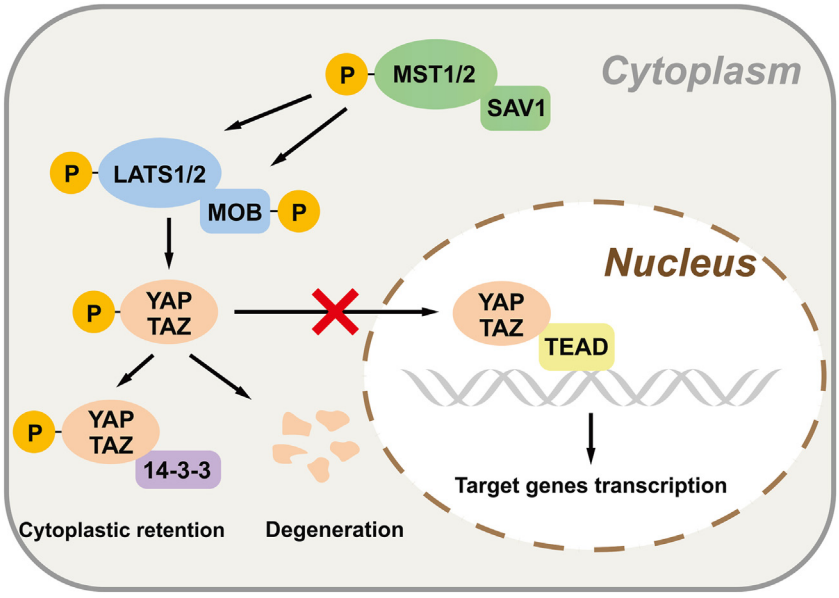

Figure 1 Hippo-YAP/TAZ signaling pathway. When the Hippo pathway is activated, MST1/2 bind to the regulatory protein SAV1 to form an active enzymatic complex. The complex phosphorylates and activates LATS1/2, as well as MOB, which is the cofactor for LATS1/2. Phosphorylated LATS1/2 directly phosphorylate YAP/TAZ and prevent them from entering the nucleus to promote transcription mainly by binding to TEAD, thereby inhibiting target genes expression. Phosphorylated YAP/TAZ is retained in the cytoplasm by 14-3-3 proteins or degraded by ubiquitination. When the Hippo pathway is inhibited, a series of kinases are inactive, and dephosphorylated YAP/TAZ enter the nucleus to promote target genes expression. YAP/TAZ are usually partly in the cytoplasm and partly in the nucleus, depending on the regulation of their upstream molecules and phosphorylation level. Deficiency or dephosphorylation of MST1/2 and LATS1/2 can increase the level and activity of YAP/TAZ.

differentiation of BMSCs by activating the Hippo signaling pathway to inhibit YAP.[45] Additionally, YAP/TAZ also act as key mechanotransducers in MSCs to promote osteogenesis. Culturing MSCs on microfluidic chips that mimic interstitial shear stress enhanced the activity of YAP/TAZ, which was associated with RAS homology family member A (RhoA)/Rho-associated coiled-coil containing protein kinase (ROCK), thereby promoting osteogenic differentiation.[46] Moreover, mechanical stimulation of MSCs with acoustic tweezing cytometry (ATC) enhanced YAP activity and promotes osteogenesis, which was mediated by F-actin (an actin filament), myosin II, and RhoA/ROCK signaling.[22] Furthermore, when MSCs were cultured under microgravity, TAZ accumulation in the nucleus was reduced, resulting in decreased osteogenesis.[47] This mechanotransduction capability can be utilized to fabricate biomaterials that promote osteogenesis.

After reviewing numerous studies, we postulate that TAZ primarily plays an active role in the osteogenic differentiation of MSCs, while YAP has a dual role.[48] A few studies have proposed that YAP also mediates detrimental effects on osteogenesis by regulating downstream molecules such as Runx2, Wnt, and β-catenin, showing the controversial role of YAP in osteogenesis. For instance, activator protein 2a (AP2a) can compete with Runx2 to recruit YAP to form a YAP-AP2a complex, releasing the inhibition of Runx2 activity from YAP and promoting the osteogenic differentiation of MSCs.[49] In addition, as a downstream effector of alternative Wnt signaling, overexpressed YAP inhibits Wnt/β-catenin signaling and osteogenesis.[24] One of its specific mechanisms was identified by Seo et al,[50] who found that YAP1 was required for the inhibition of osteogenesis by SRY-box transcription factor 2 (SOX2). Under the treatment of SOX2, YAP1 bound to β-catenin and induced Dkk1-mediated repression of the pro-osteogenic signaling Wnt in BMSCs.[50] Overall, the regulation of YAP/TAZ on MSC osteogenic differentiation is complex and its molecular mechanism remains to be further studied.

Hippo-YAP/TAZ signaling affects osteogenesis by regulating OBs and OCs

YAP/TAZ also play an important role in regulating the formation and biological behavior of OBs and OCs. There is evidence that YAP/TAZ promote osteogenesis by facilitating the differentiation and function of OBs and inhibiting those of OCs. Piezo1 is a non-selective Ca^{2+} channel located in OBs that senses the mechanical load and promotes translocation of YAP into the nucleus.[51] The nuclear YAP up-regulates the expression of target genes such as type II and IV collagen, and the deposition of collagen can inhibit bone resorption.[51] Furthermore, β-catenin is the target of YAP as Pan et al[25] found that YAP deletion impaired the proliferation and differentiation of OBs induced by the interaction between YAP and β-catenin. Likewise, differentiation into OCs is inhibited in bone marrow-derived macrophages (BMDMs) that were conditionally knocked out of YAP or treated with the YAP inhibitor verteporfin (VP), as demonstrated by significantly reduced expression of nuclear factor of activated T-cells cytoplasmic 1 (NFATc1), tartrate-resistant acid phosphatase (TRAP), and cathepsin K (CTSK), which are markers of OCs,

compared with the control group.[9] The attenuated tendency of BMDMs to differentiate into OCs reduces bone resorption and has a positive effect on new bone formation. Cysteine-rich protein 61 (CYR61), another target gene of YAP/TAZ, was reported to significantly suppress the formation of TRAP-positive multinucleated cells and reduce the expression of the osteoclast phenotypic markers.[52]

However, YAP/TAZ also have a controversial role in regulating OBs and OCs. Dual deletion of MST1/2 inhibits endochondral bone formation and accumulation mainly through YAP during early osteoblast differentiation.[53] Zhao et al[9] reported that inhibition of YAP1 and its association with the main transcription factor TEAD attenuated the formation and resorption functions of OCs, as well as nuclear factor-kappa B (NF-κB) signaling induced by receptor activator of NF-κB ligand (RANKL), which was the major signaling pathway regulating OC differentiation in previous research. Moreover, Hossain et al[54] found unusual results regarding TAZ, in which the skeletal development of mice with germline deletion of TAZ was apparently normal. Previous studies have not reached a consensus and the mechanism is still unclear. Xiong et al[55] demonstrated that deletion of YAP/TAZ in OB progenitors increased OB formation, which was associated with increased Wnt signaling and Runx2 activity. In contrast, deletion of YAP/TAZ in mature OBs and osteocytes decreases bone mass, indicating distinct functions of YAP/TAZ at several stages of osteoblast differentiation.[55] This may partly explain the mechanism of YAP/TAZ regulating OBs, but the mechanism of their regulation of OBs and OCs still needs to be further studied. Taken together, YAP/TAZ exhibits diverse regulation of OBs and OCs, which has a non-negligible impact on osteogenesis.

In summary, the complex effect of YAP/TAZ on osteogenesis is achieved through the regulation of MSCs, OBs, and OCs, but is not limited to these. YAP/TAZ modulate the expression of downstream target genes through various signaling pathways in these cells to promote or inhibit their osteogenesis-related biological functions. However, the regulation of target genes by YAP/TAZ has intricate biological mechanisms, which remains to be further explored.

Therapeutic potential of macrophage polarization in bone defect repair

A glimpse of macrophage polarization

In addition to MSCs, OBs, and OCs, macrophages are also an important group that affects osteogenesis. Macrophages can be classified into many phenotypes according to their activation pathways and functions. There are two main phenotypes of macrophages, the classically activated M1 phenotype and the alternatively activated M2 phenotype[56,57] (Fig. 2). The two phenotypes of macrophages are morphologically different, showing that M1 macrophages have a more elongated and irregular shape, while M2 macrophages are round in appearance.[58,59] M0 macrophages (primary macrophages) polarize towards the M1 phenotype when stimulated by inflammatory molecules such as interferon (IFN)-γ, TNF-α, lipopolysaccharide (LPS), granulocyte-macrophage colony-stimulating factor (GM-CSF), and opsonins.[60–62] IL-4, IL-10, IL-13, and macrophage colony-stimulating factor (M-CSF) can induce macrophage polarization to M2 phenotype.[63,64] Studies have shown that enhancement of mitochondrial oxidative phosphorylation correlates with M2 macrophage polarization.[65,66] M2 phenotype macrophages can be further subdivided into multiple phenotypes, namely M2a, M2b, M2c, M2d, and M2f.[67] The polarization of M2a is stimulated by IL-4, IL-13, Jumonji domain containing-3 (Jmjd3), and interferon regulatory factor (IRF) 4.[68,69] M2b polarization requires immune complexes and toll-like receptor (TLR) ligands.[21,69] M2c macrophages are induced by transforming growth factor (TGF)-β, IL-10, and glucocorticoids.[69] Polarization of M2d is induced by TLR ligands and adenosine.[70] M2f macrophages are stimulated by phagocytosis of apoptotic cells.[71] The molecular mechanisms of macrophage polarization are intricate and have been summarized in detail by previous studies.[61,72–74]

Although the current classification of macrophage phenotypes is complicated, the classic M1/2 phenotype remains important in tissue repair. In the early phase of wound formation, a large number of M1 macrophages aggregate at the wound site, releasing a large number of antiviral proteins, reactive oxygen species (ROS), inducible nitric oxide synthase (iNOS), and pro-inflammatory cytokines such as IL-1, TNF-α, IL-6, IL-12, and IL-23, of which iNOS is the most reliable marker of M1 phenotype macrophages.[21,72,75,76] The subsequently formed M2 macrophages with anti-inflammatory and repair functions produce IL-10, TGF-β, polyamines, and arginase 1 (Arg1).[21,77,78] If M1 macrophages exist in large numbers for a long time and fail to transform into M2 macrophages more, it is likely to lead to long-term inflammation and poor tissue repair.[79] In general, M1 macrophages clear tissue foreign bodies and damaged cells relying on their potent bactericidal and phagocytic abilities, while M2 macrophage activation reduces inflammation and promotes tissue repair and angiogenesis.[61]

M1/M2 macrophages exert distinct functions in bone defect repair

M1/2 macrophages are part of osteoimmunology,[80] which largely influences bone formation.[81] Horwood et al[82] found that in the early phase of osteonecrosis, M1 macrophages infiltrated the necrotic tissue and secreted a large amount of TNF. Subsequent histology findings revealed increased new bone formation, accompanied by a progressive increase in M2 macrophages.[82] Bone defect repair is similar to the healing of other tissues, with M1 macrophages playing a pro-inflammatory role in the early phase, while M2 macrophages promote bone regeneration in the later phase (Fig. 3).

During the early inflammatory phase, M1 macrophages are present in the bone defect area, phagocytosing cell debris, recruiting MSCs, and infiltrating T cells by releasing TNF-α, IFN-γ, and IL-6.[83] Activated M1 macrophages not only inhibit the osteogenic differentiation of BMSCs but also the osteogenic function of OBs by releasing a variety of pro-inflammatory factors such as IL-1, IL-6, and TNF-α.[84,85] M1 macrophages also induce the expression of RANKL in OBs and activated T cells to promote osteoclastogenesis,

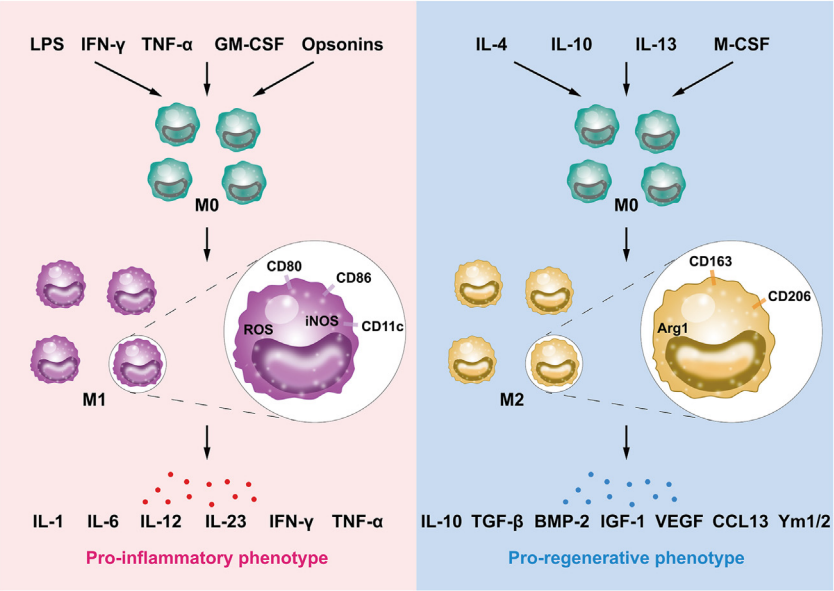

Figure 2 Polarization and function of macrophages. Macrophages can polarize to the pro-inflammatory M1 phenotype and pro-regenerative M2 phenotype. M1 macrophages are stimulated by LPS, IFN-γ, TNF-α, GM-CSF, and opsonins. They are identified by intracellular markers, such as ROS and iNOS. They also express high levels of cell surface markers, such as CD80, CD86, and CD11c. M1 macrophages secrete a large number of pro-inflammatory cytokines such as IL-1, IL-6, IL-12, IL-23, and IFN-γ, as well as TNF-α, which in turn triggers M1 polarization. M2 macrophages are polarized by IL-4, IL-10, IL-13, and M-CSF. Pro-regenerative M2 macrophages express higher CD206, CD163, and Arg1, which are recognized as markers of M2 phenotype. They also release massive secretions such as IL-10, TGF-β, BMP-2, IGF-1, VEGF, CCL13, and Ym1/2. IL-10 and TGF-β play a significant role in attenuating inflammation, while BMP-2 and VEGF are favorable to form a pro-regenerative microenvironment.

resulting in the acceleration of bone resorption.[83] Wang et al[86] found that advanced glycation end product (AGE)-induced M1 polarization inhibited the osteogenic function of BMSCs and impaired bone regeneration in type 1 diabetes, which can be reversed by adrenomedullin 2 (ADM2). This confirms the idea that excess M1 macrophages are detrimental to osteogenesis. On the other hand, depletion of M1 macrophages altered the expression of inflammatory cytokines and severely impaired bone healing.[6] From the above results, it can be seen that M1 macrophage polarization is an indispensable nexus point in bone defect repair and its dysregulation is detrimental to osteogenesis.

For a successful bone defect repair, conversion of the M1 phenotype to the M2 phenotype is essential.[83] Experiments have shown that anti-inflammatory M2 macrophages mediate a significant increase in bone volume and bone matrix mineralization.[15,87] M2 macrophages can secrete bone morphogenetic protein (BMP)-2, IL-10, TGF-β, *etc.*, which are key cytokines that promote osteoblastic differentiation and new bone formation.[72,83] In the study by Zhang et al,[88] the transformation of M1 macrophages into M2 macrophages upon stimulation by IL-4 significantly enhanced the osteogenic differentiation of pre-osteoblastic MC3T3 cells, owing to the increased secretion of osteogenic cytokines such as BMP-2, TGF-β, and vascular endothelial growth factor (VEGF). Furthermore, the activation of macrophage scavenger receptor 1 (MSR1) on the macrophage membrane maintained the M2 polarization of macrophages by activating the phosphoinositide 3-kinase (PI3K)/protein kinase B (PKB or AKT)/glycogen synthase kinase-3beta (GSK3β)/β-catenin signaling pathway, and the pro-osteogenic cytokines released by M2 macrophages acted on BMSCs to promote their osteogenic differentiation.[89] M2 macrophages also inhibit osteoclastic differentiation by releasing IL-4 and IL-13.[72] Collectively, most of the current studies suggest that M2 macrophage polarization is an important step in promoting osteogenesis, which provides an avenue to improve bone defect repair. Currently, strategies to regulate macrophage polarization toward M2 phenotype have been used to treat bone defects, such as biomaterials that promote bone regeneration.

Biomaterials accelerate bone regeneration by promoting M2 macrophage polarization

Biomaterials have been widely used in tissue engineering because of their unique physical and chemical surface properties and their ability to serve as carriers for regulatory substances such as cells and molecules.[90,91] Among them, many biomaterials affect osteogenesis by modulating macrophage polarization.

Some biomaterials promote osteogenesis through direct contact with tissues, due to their excellent surface properties. Titanium (Ti) implants with improved surface properties are common biomaterials widely used to promote bone formation. Ti dental implants with increased surface roughness and hydrophilicity contribute to M2 macrophage polarization and up-regulation of anti-inflammatory cytokines, which are advantageous for tissue repair and osseointegration.[92] In addition, Zhu et al[93] found that the HC-90-type TiO_2 honeycomb-like structure on the Ti surface

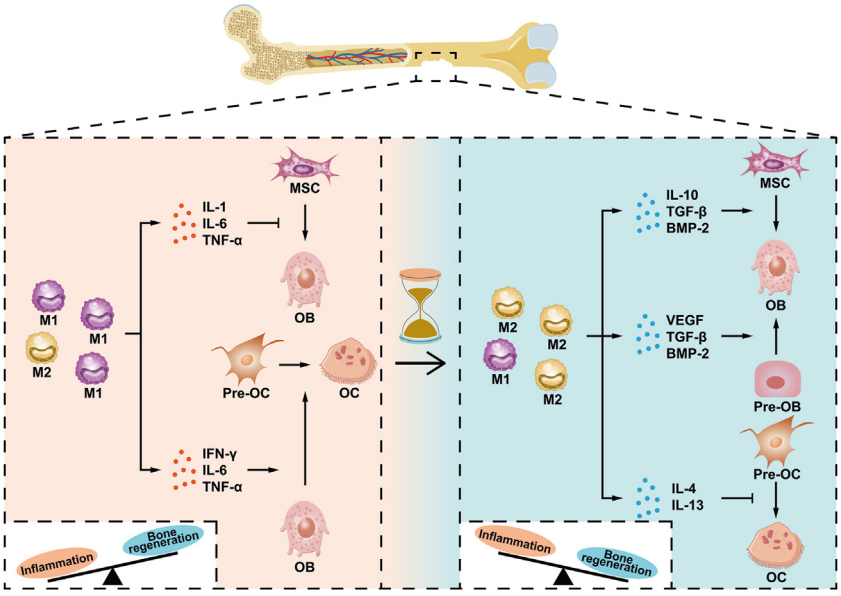

Figure 3 The role of macrophage polarization in bone defect repair. Bone defect repair is a dynamic and continuous process and M1/2 macrophages play different roles in it. In the early phase, M1 macrophages mediate inflammatory responses by releasing inflammatory cytokines. IL-1, IL-6, and TNF-α secreted by M1 macrophages inhibit the osteoblastic differentiation of MSCs. At the same time, M1 macrophages secrete TNF-α, IFN-γ, and IL-6 to promote the expression of RANKL in OBs, and RANKL can bind to RANK of pre-OCs to promote the differentiation and maturation of OCs. With the transition from M1 phenotype to M2 phenotype, inflammation gradually diminishes and the amount of bone formation increases. Pro-regenerative M2 macrophages promote the osteoblastic differentiation of MSCs and pre-OBs by secreting pro-osteogenic cytokines such as BMP-2 and TGF-β. They also secrete IL-4 and IL-13 to inhibit osteoclastic differentiation.

induced M2 macrophage polarization and pseudopodia formation by up-regulating guanosine triphosphatases (GTPases) and further activating the RhoA/ROCK signaling pathway. Activated M2 macrophages promoted MSCs osteogenic differentiation, resulting in better implant osseointegration compared to control.[93]

Some biomaterials are carriers of bioactive substances and cells through which bone defect repair can be improved. For example, platelet-rich plasma (PRP)-gelatin methacryloyl (GelMA) scaffolds mediate better bone regeneration compared to natural healing, which is attributed to PRP-GelMA hydrogels that inhibit transition from M0 macrophages to M1 macrophages, and promote M2 macrophage polarization, up-regulating the expression of osteogenic genes in MSCs.[94] In addition, cytokines closely associated with M2 polarization are often carried by biomaterials. BMP-calcium phosphate cement (CPC) gradually increases M2 macrophages and promotes their release of IL-10, TGF-β1, insulin-like growth factor-1 (IGF-1), and VEGF, which effectively relieve surrounding tissue inflammation and promote the osteogenic function of MSCs.[95] Similarly, under diabetes mellitus-mimicking conditions, hydrogels loaded with IL-10 and BMP-2 increase M2 polarization of BMDMs and enhance the activity of OBs, which is favorable to improving diabetic bone regeneration.[96]

With the continuous intersection of nanotechnology, material science, and life science, nano-biomaterials are becoming a research hotspot of modern biological and medical materials, including research on bone regeneration. Like other materials, nanomaterials can also carry certain molecules to promote bone formation. Zhao et al[97] designed a biphasic calcium phosphate loaded with gold nanocage (BCP-GNC), a dual-targeting nano-in-micro scaffold. BCP-GNC can promote M2 macrophage polarization, which is one of its targets, by releasing the loaded IL-4, and further facilitate new bone formation.[97] Likewise, Sun et al[98] designed transplantable three-dimensional (3D) bioprinted scaffolds which contained mesoporous silica nanoparticles (MSNs), BMSCs, and RAW264.7 macrophages. MSNs released the loaded BMP-4 to induce polarization of RAW264.7 toward the M2 phenotype and directly stimulated osteogenesis of BMSCs.[98] BMP-2 released by M2 macrophages further enhanced the osteogenic differentiation of BMSCs and suppressed the inflammatory response, resulting in the promotion of bone defect repair.[98] Additionally, there are also materials that modulate macrophage polarization through direct contact. For example, bone mimetic nanoparticles (NPs), thought to act as a driver of M2 phenotype, increase the expression of macrophage M2 markers such as the cluster of differentiation (CD)206, CD163, and C—C motif chemokine ligand (CCL) 13[20]. MSCs, which are cultured in media containing M2 macrophage secretions, express more BMP-2 and ALP, which enhanced their osteogenic function.[20] Other literature suggests that certain nanomaterials require special conditions to function. In type 2 diabetic rats, the polarized nanocomposite membranes, which form a biomimetic electrical microenvironment in the bone defect area, transformed M1 macrophages into M2 macrophages by down-regulating AKT2-IRF5 signaling and facilitated the differentiation of MSCs into OBs and bone regeneration.[99] Consistently, Fu et al[100] fabricated novel microwave-responsive engineered pseudo-

macrophages (M-Fe_3O_4/Au NPs), which consist of Fe_3O_4/Au NPs as the core and macrophage membrane as the envelope. The engineered macrophage can not only kill bacteria but also activate the M2 polarization of RAW 264.7 macrophages under microwave irradiation, significantly attenuating the inflammatory response and enhancing the osteogenic differentiation of MSCs.[100]

Collectively, whether in the field of traditional biomaterials or novel nanomaterials, the regulation of macrophage polarization is a significant way to improve the therapeutic effect of bone defects. The mechanism by which these biomaterials promote osteogenesis is the promotion of M2 macrophage polarization through various stimuli. They utilize anti-inflammatory cytokines secreted by M2 macrophages to promote the osteogenic function of MSCs and OBs, resulting in the acceleration of new bone formation.

Novel implications of Hippo-YAP/TAZ signaling-mediated macrophage polarization in bone defect repair

Hippo-YAP/TAZ signaling regulates macrophage polarization in multiple ways

Macrophage polarization is influenced by multiple factors and it is necessary to clarify its molecular mechanism to facilitate the design of targeted therapeutic measures. As transcriptional co-stimulators, YAP/TAZ play a critical role in regulating multiple signaling pathways in macrophage polarization. Most studies show that YAP/TAZ activation can promote the polarization of M2 phenotype and inhibit the polarization of M1 phenotype in certain tumor diseases, while YAP inhibition does the opposite.[101] Rehrauer et al[102] revealed the progressive activation of YAP/TAZ and high expression of Arg1 during the development of mesothelioma. In colon cancer, the inhibition of YAP by siRNA attenuated the ability of THP-1 monocytes to polarize toward M2 macrophages, as seen from the results of up-regulation of IL-4, TGF-β1, and Ym2, and down-regulation of iNOS.[103] Consistently, in triple-negative breast cancer (TNBC), YAP deficiency converted tumor-promoting M2 macrophages into tumor-suppressing M1 macrophages, while M2 macrophages with YAP overexpression enhanced cancer cell invasiveness through CCL2/C—C chemokine receptor type 2 (CCR2) pathway, leading to poor prognosis in TNBC.[104] Furthermore, intracellular signal transduction upstream and downstream of YAP/TAZ has been extensively studied. Feng et al[105] found that upstream Wnt5a augmented TGF-β1-induced M2 polarization by up-regulating YAP/TAZ expression, resulting in more severe renal fibrosis in mice. Additionally, MSCs can inhibit MST1/2 and LATS phosphorylation in macrophages by secreting prostaglandin E2 (PGE2), making YAP translocated from the cytoplasm to the nucleus.[106] YAP in the nucleus interacts with downstream β-catenin to up-regulate the expression of the target gene X-box binding protein 1 (XBP1), leading to more M2 phenotypes and fewer M1 phenotypes.[106]

Conversely, Hippo-YAP/TAZ signaling can also promote M1 polarization and inhibit M2 polarization. For instance, LPS/TLR signaling promoted M1 polarization and suppressed M2 polarization by up-regulating activator protein 1 (AP-1)-mediated YAP expression in Kupffer cells, resulting in the massive release of proinflammatory cytokines and driving the progression of nonalcoholic hepatitis (NASH).[107] Furthermore, some targets downstream of YAP/TAZ have been revealed and studies have reported the respective roles of YAP and TAZ. Under stimulation by gut bacteria and IFN-γ, overexpression of YAP at both mRNA and protein levels up-regulated p53 gene expression and promoted M1 polarization, manifested by higher IL-6, iNOS, and TNF-α expression.[108] Consistent with p53, inhibited interaction of YAP with NF-κB in lactate-treated macrophages reversed the up-regulation of inflammatory mediators TNF-α and IL-6 upon LPS stimulation, indicating that YAP inactivation inhibits M1 polarization of macrophages.[109] El Ouarrat et al[110] found that adipocyte-specific TAZ knockout mice showed much fewer M1-like macrophages in adipose tissue, which was associated with the target gene peroxisome proliferator-activated receptor-γ (PPARγ). In addition, YAP/TAZ may have a synergistic effect on downstream targets, as Mia et al[111] revealed that after myocardial infarction (MI), YAP/TAZ promoted M1 phenotype by directly regulating IL-6 promoter activity or through the p38-dependent mitogen-activated protein kinase (MAPK) pathway.

There are also situations in which macrophage polarization is complex. In hepatocellular carcinoma (HCC), the up-regulation of YAP in hepatocytes exacerbated HCC progression by increasing CCL2 secretion to recruit macrophages and promote M2 polarization,[112] while Nogo-B (a member of the reticulon family of proteins) directly facilitated M2 polarization of tumor-associated macrophages via YAP/TAZ activation.[113] In contrast, Zhang et al[114] found that the matricellular protein spondin 2 (SPON2) inhibited LATS1 phosphorylation through F-actin accumulation and enhanced translocation of YAP into the nucleus, resulting in the promotion of M1 phenotype. However, one study came to a different conclusion that gene deletion of MST1/2, which released the suppression of YAP/TAZ in hepatocytes of mice, up-regulated the expression of CCL2, resulting in increased expression of both M1 markers (CD86, interleukin-1 receptor antagonist (IL-1RA)) and M2 markers (Ym1, CD206).[115] Generally, YAP/TAZ exerts diverse effects on the regulation of macrophage polarization in different conditions. The complex mechanism by which YAP/TAZ regulates macrophage polarization is worth exploring in the future.

Hippo-YAP/TAZ signaling exhibits diverse effects on osteogenesis by regulating macrophage polarization

Recent studies mainly focus on the role of Hippo-YAP-mediated macrophage polarization in osteogenesis instead of TAZ. During the process of osteogenesis, YAP also plays an intricate role in regulating macrophage polarization.

Currently, there is evidence that the downregulation of YAP promotes osteogenesis by mediating macrophage polarization. Some biomaterials utilize inhibited YAP-mediated reduction of M1 polarization and promotion of M2 polariza-

tion to alleviate inflammation, resulting in improved osteogenesis. For instance, periosteal bone scaffolds, a softer biomaterial than decalcified cortical bone, enabled less localization of YAP in the nucleus of BMDMs, resulting in a substantial reduction of the M1 marker iNOS compared to decellularized decalcified cortical bone group.[116] The reduction of M1 macrophages inhibited the inflammatory damage of the surrounding tissue of the biomaterial, which is of great significance for osteogenesis since the timely elimination of inflammation is an essential link to osteogenesis.[116] Cells can sense mechanical stimuli from the microenvironment, leading to altered levels of YAP activity, which can modulate cell behavior, and YAP-mediated mechanotransduction regulates macrophage inflammatory responses.[42] In *in vitro* experiments, stiff biografts up-regulated YAP expression in peripheral macrophages and increased iNOS $^{+}$ macrophages.[117] Similarly, under near-infrared light (NIR) irradiation, the culture platform doped with Ca^{2+} was changed from soft to hard and the high expression of YAP in the nuclei of BMDMs cultured in it promoted the transformation of M2 phenotype to M1 phenotype.[118] The mechanism of YAP regulated by stiffness is that the import and export of YAP through the nuclear pores in the cells around the soft substrates achieve a balance, while more YAP is transferred into the nucleus than it is transferred out with the stiff substrates.[119] This suggests that the stiffness of biomaterials has regulatory implications for the intensity of the inflammatory response in bone defect repair. Additionally, Ti2448 is another biomaterial widely used in bone repair with lower elastic modulus and better osseointegration activity than Ti6Al4V.[120] There are a large number of M2 macrophages in the surrounding tissue of Ti2488, and the intracellular YAP expression is inhibited, leading to the massive secretion of IL-4, IL-10, BMP-2, and platelet-derived growth factor-BB (PDGF-BB) into the extracellular space.[121] These pro-osteogenic factors can promote the osteogenic differentiation of MC3T3-E1 cells and new bone formation.[121] These results indicate that the inhibition of YAP, as a crucial mechanotransducer, may mediate the M2 macrophage polarization induced by direct mechanical stimulation of biomaterials, thereby promoting osteogenesis.

In contrast, the up-regulation of YAP can also accelerate osteogenesis in many studies by promoting M2 polarization and suppressing M1 polarization. Zhang et al[122] found that YAP-mediated CGRP-induced M2 macrophage polarization and up-regulation of BMP-2/6, OSM, and Wnt10b, which enhanced the expression of osteogenesis-related genes Runx2, osterix (OSX), and ALP in MC3T3 cells and promoted bone formation. Moreover, it has been shown that YAP knockdown up-regulated growth differentiation factor 15 (GDF15) levels in monocyte macrophages, and GDF15 activated NF-κB and contributed to M1 macrophage polarization and OC formation.[123] A large number of pro-inflammatory M1 macrophages and bone-resorbing OCs are detrimental to bone regeneration. In addition, indirect regulation of macrophage polarization can be mediated by YAP via interactions between cells. For instance, low-temperature deposition modeling (LDM) printed sponges with good osteogenic properties activated the focal adhesion kinase (FAK) signaling pathway of MSCs, which increased the expression of the downstream target molecule YAP and translocated a large amount of YAP into the nucleus, enhancing the paracrine function of MSCs.[124] The paracrine signal produced by MSCs inducedsurrounding macrophages to express high M2 markers (Arg1, IL-10, and CD206) and low M1 markers (iNOS, CD11c, CD80, and CD86), thereby inhibiting inflammation and promoting osteogenesis.[124]

In summary, YAP plays a complex and crucial role in macrophage polarization in bone tissue, depending on different environments and stimuli. Therefore, YAP is a potential therapeutic target for accelerating and improving bone defect repair. Although the role of TAZ in regulating macrophage polarization in osteogenesis alone or in synergy with YAP has hardly been investigated, the important role of TAZ in regulating macrophage polarization in other tissues has been demonstrated. Therefore, TAZ also has a non-negligible potential to regulate macrophage polarization to promote bone defect repair. We hope to promote the transition of M1 macrophages to M2 phenotype by regulating the level of YAP/TAZ because of the significant physiological functions of M2 macrophages such as facilitating inflammation elimination and new bone formation. However, the complex molecular mechanisms of Hippo-YAP/TAZ-mediated macrophage polarization on osteogenesis have not been fully resolved by current studies.

Conclusion and future perspectives

Hippo-YAP/TAZ signaling plays a key role in osteogenesis through multiple mechanisms. As transcriptional co-stimulators, YAP/TAZ regulates the expression of various osteogenesis-related genes in many cells, such as MSCs, OBs, OCs, and macrophages, which are major participants in the osteo-immune response.[125] In bone defect repair, M1 macrophages mediate the early inflammatory phase and their normal occurrence and regression play a crucial role in bone regeneration. M2 macrophages release a large number of pro-tissue repair factors, forming a microenvironment conducive to osteogenesis. Activated macrophages directly affected the osteogenic function of MSCs and the balance between OBs and OCs. In order to improve the efficacy of bone defect repair, many biomaterials apply the principle of regulating macrophage polarization, especially the manipulation of M2 phenotype. A variety of biomaterials with improved surface properties or special loading substances can accelerate bone regeneration, among which nanobiomaterials are emerging materials with great potential. Most of them accelerate bone regeneration by promoting M2 macrophage polarization or inhibiting M1 macrophage polarization, and their complex biological mechanisms have become a research hotspot.

There is a strong relationship between Hippo-YAP/TAZ signaling and macrophage polarization, but the regulation of macrophage polarization by Hippo-YAP/TAZ signaling in bone tissue is a novel and insufficient study. Several studies have applied YAP-mediated macrophage polarization to biomaterials. Soft periosteal bone scaffolds designed by Zhao et al[116] inhibited the entry of YAP into the nucleus of BMDMs through mechanical stimulation, thereby inhibiting M1 macrophage polarization and inflammatory responses mediated by M1 macrophages.

Reduction of M1 macrophages attenuated inflammation in bone tissue and creates a favorable microenvironment for bone formation.[116] Consistently, Ti2448 with better osseointegration properties can inhibit the expression of YAP and recruit a large number of M2 macrophages.[121] These M2 macrophages secrete abundant pro-osteogenic cytokines such as IL-10 and BMP-2 and promote the osteoblastic differentiation of MC3T3 cells.[121] The above studies imply that YAP/TAZ-mediated macrophage polarization is a potential therapeutic target for bone defects. However, at present, this potential treatment direction has only been proposed and there is little research on it, so its application and feasibility still need to be further studied.

Understanding its biological mechanism is helpful to promote the application of Hippo-YAP/TAZ signaling-mediated macrophage polarization in bone defect repair. The regulatory effect of YAP on macrophage polarization is influenced by the microenvironment, including LPS, IFN-γ, MSCs, as well as mechanical stimulation. Intracellular molecular mechanisms have also been reported. CGRP can trigger YAP-mediated macrophage polarization of M2 phenotype and promote the osteogenic function of MC3T3 cells.[122] FAK signaling increases YAP expression in MSCs and indirectly promotes M2 polarization of surrounding macrophages through paracrine signaling released by MSCs.[124] These intracellular molecules could be utilized to regulate YAP-mediated macrophage polarization. However, how YAP/TAZ regulate macrophage polarization in osteogenesis remains largely unknown. Li et al[123] found that YAP knockdown up-regulated the expression of GDF15, which activated NF-κB, in macrophages, thereby promoting M1 macrophage polarization and aggravating inflammatory damage. In addition, previous research on other tissues may be suggestive. A study by Zhou et al[108] demonstrated that YAP inhibited reparative macrophage polarization by promoting the expression of transcriptional factor p53. p53 expression is increased during both pro-inflammatory or reparative macrophage polarization but is more prominent in pro-inflammatory macrophages compared to reparative macrophages.[126] However, some studies have demonstrated that p53 negatively regulates activation of both pro-inflammatory (IL-6, TNF-α, *etc.*) and reparative (Arg1, found in inflammatory zone 1 (Fizz1), Irf4, *etc.*) marker genes.[126,127] Judging from the above results, p53 has intricate biological effects on YAP-mediated macrophage polarization and may have a similar role in osteogenesis. β-catenin is another important target of YAP. YAP can be colocalized and interact with β-catenin to down-regulate their target gene XBP1, resulting in the reduction of NLR family pyrin domain containing 3 (NLRP3) activity.[106] The down-regulation of XBP1-mediated NLRP3 activation leads to reprograming macrophage polarization toward an anti-inflammatory M2 phenotype.[106]

As the co-stimulator of YAP, the role of TAZ in macrophage polarization has received much less attention. TAZ deficiency can activate PPARγ and lead to fewer M1-like macrophages in adipose tissue, indicating that TAZ is a negative regulator of PPARγ activity.[110] PPARγ promotes M2 polarization by promotion of a metabolic switch to oxidative phosphorylation and induction of retinoic acid (RA) signaling, and it is essential for IL-4-induced M2 macrophage polarization.[128] However, the effects of TAZ-mediated macrophage polarization on bone regeneration and its mechanism remain unknown. TAZ may act synergistically with YAP or act independently to regulate downstream target molecules, which are required to be explored in future experiments.

In summary, Hippo-YAP/TAZ signaling shows great potential to influence bone defect repair by regulating macrophage polarization, and many potential target molecules of YAP/TAZ have been reported by previous studies or not. The elucidation of the mechanism by which Hippo-YAP/TAZ signaling mediates macrophage polarization will greatly advance the study of its therapeutic implications in bone defects.

Author contributions

Haochen Wang designed the outline and drafted the manuscript. Hui Yu contributed to figure construction. Tianyu Huang collected the references. Bin Wang revised the manuscript. Lin Xiang designed the frame of the manuscript and critically revised the manuscript. All authors read and approved the manuscript.

Conflict of interests

The authors declare no conflict of interests.

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