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Review

Hypoxia inducible factor-1α related mechanism and TCM intervention in process of early fracture healing

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ABSTRACT

As a common clinical disease, fracture is often accompanied by pain, swelling, bleeding as well as other symptoms and has a high disability rate, even threatening life, seriously endangering patients' physical and psychological health and quality of life. Medical practitioners take many strategies for the treatment of fracture healing, including Traditional Chinese Medicine (TCM). In the early stage of fracture healing, the local fracture is often in a state of hypoxia, accompanied by the expression of hypoxia inducible factor-1α (HIF-1α), which is beneficial to wound healing. Through literature mining, we thought that hypoxia, HIF-1α and downstream factors affected the mechanism of fracture healing, as well as dominated this process. Therefore, we reviewed the local characteristics and related signaling pathways involved in the fracture healing process and summarized the intervention of TCM on these mechanisms, in order to inspirit the new strategy for fracture healing, as well as elaborate on the possible principles of TCM in treating fractures based on the HIF molecular mechanism.

Contents

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1. Introduction

With the acceleration of the mechanization of productive forces and the aging of the population in modern society, the incidence of traumatic and pathological fracture is on the rise (Trajanoska et al., 2018). In the nearly 100 years since Franz Konig first reported the use of screw fixation for fracture in 1875, people have continuously tried various methods of fracture fixation until 1958, when the association for the study of internal fixation (ASIF) was established in Switzerland and the ASIF's theory was put forward, making internal fixation surgery become one of the main methods for fracture treatment (Beltran, Collinge, & Gardner, 2016; Hoekzema & Brambila, 2021). With the continuous progress of modern medicine, many scholars and clinicians have devoted themselves to opening up new ideas and methods for fracture treatment. New internal fixation brackets, biological implant materials, minimally invasive manipulation and other inventions have accelerated the cycle of fracture healing to a certain extent (Beale & McCally, 2020; Zhang, Ding, & Ruan, 2019), but it still cannot avoid postoperative complications of fracture, such as infection, delayed union or nonunion of fracture, local thrombus and so on (Luo et al., 2015). We are attempted to find and sum up the local characteristics of fracture healing and the cellular signaling pathways involved in fracture healing.

The use of traditional Chinese medicine (TCM) in the treatment of fractures has a long history and has the characteristics of significant curative effect, few side effects, and no obvious contraindications. In the process of fracture treatment and healing, *Drynariae Rhizoma* (Gusuibu in Chinese), *Rehmanniae Radix* (Dihuang in Chinese), *Epimedii Folium* (Yinyanghuo in Chinese), *Angelicae Sinensis Radix* (Danggui in Chinese) and so on are the most commonly used. These herbs often play the role of activating blood circulation, removing blood stasis, dredging channels and collaterals, relieving pain, strengthening tendons and bones, and improving microcirculation (Liao et al., 2021; Xu et al., 2019). From this, it can be seen that TCM can be used for the treatment of fractures, however, why can TCM treat fracture? Its specific molecular mechanism is not clear.

Fracture healing is a complex process, which requires recruiting suitable cells and giving full play to their functions at the right time and at the injured part, which involves signal transduction and biological effects among a variety of cells (Einhorn & Gerstenfeld, 2015). From the perspective of injury mechanism, direct or indirect violence will inevitably lead to varying degrees of injury in local tissue and blood vessels of the fracture. Bleeding, inflammatory exudation, insufficient tissue perfusion and other characteristics make the broken end of the fracture show a state of hypoxia (Kolar, Gaber, Perka, Duda, & Buttgereit, 2011; Teh et al., 2021; Zhang et al., 2021). This hypoxic state lasts for 1–2 weeks or even longer until the hematoma is absorbed, the inflammatory response is controlled, and a new microcirculatory system is locally established. During this period, the body will make an adaptive response to hypoxia and establish a protective mechanism. Its physiology is main characterized by the enhancement of cardiovascular function, the improvement of blood oxygen-carrying capacity and the enhancement of tissue oxygen utilization efficiency of multi-system and multi-level coordination effect. The occurrence of this coordination effect depends on the gene expression of HIF-1α, and its multiple downstream cytokines play an important role in angiogenesis, bone regeneration and bone remodeling (Miclau et al., 2017; Wilson, Wong, Toupadakis, & Yellowley, 2015). In recent years, studies have reported that HIF-1α contributes to the healing of osteoporotic fractures and the recovery of various bone diseases (Ahmed et al., 2021; Hirai, Furusho, Hirota, & Sasaki, 2018; Silagi, Schipani, Shapiro, & Risbud, 2021; Tian et al., 2022; K.L. Wang et al., 2021; P. Wang et al., 2021; Z.Y. Wang et al., 2021; Zhang et al., 2022; Zhao, Yeersheng, Xia, Kang, & Wang, 2021). At the same time, it can promote bone regeneration and protect bone cells (Xu et al., 2021; Zhao, Yeersheng, Xia, Kang, & Wang, 2021). Studies have reported that the activation of HIF can promote fracture healing (Chen et al., 2022; Xu et al., 2021). However, the mechanism of action of HIF-1α has not yet been systematically reviewed. Although some scholars have found the differences in the function of osteocytes in different physiological states, and the effects of various cytokines, miRNA and exosomes on bone metabolism, it is far from enough to be limited to a certain signal pathway or a certain factor, which may lead to a one-sided understanding of the mechanism of fracture healing (Haffner-Luntzer, 2021; Lang et al., 2019; Li et al., 2019; Liu et al., 2019; Sun et al., 2021; Teng, Ji, & Zhao, 2018; L. Zhang et al., 2020; W. Zhang, Zhao, Chen, & Huang, 2020; X.Y. Zhang et al., 2020). Therefore, on the basis of the existing experimental research and literature, we believe that hypoxia, HIF-1α and downstream factors affect the mechanism of fracture healing, as well as dominate this process (Chen, Yan, Qiu, Geng, & Wang, 2021; Li, Gu, Lin, Ma, & Zhang, 2020; Muinos-Lopez et al., 2016; Qiao, Huang, Zhou, Cao, & Shen, 2019; Ye et al., 2020; Zhao, Yeersheng, Xia, Kang, & Wang, 2021; Zhu et al., 2019).

Therefore, we introduced the effects of TCM formulas, single herbs and monomer components on these mechanisms and their help in fracture healing. Based on the recent studies on the effects of hypoxia and HIF-1α on the mechanism of bone healing, we systematically reviewed the biological function and activity of HIF-1α related pathways and cytokines to different osteoblasts (OBs), and TCM intervention through these mechanisms, in order to inspirit the new strategy for fracture healing.

2. Hypoxia, HIF-1α, and fracture

TCMs have already been utilized to cure various types of orthopedic illnesses, notably osteoporosis, bone fractures and rheumatism with great success (Peng, Xu, & You, 2022). From the perspective of TCM, they promote blood circulation and remove blood stasis, but their specific molecular mechanisms have not been clearly elucidated. The hypoxic environment at the fracture site has attracted our attention (Zhang et al., 2020). Therefore, based on the HIF-1α molecular mechanism, this review specifically elaborates on how TCMs promote fracture recovery and how TCM formulas that are not used for fracture treatment are expected to be used for fracture treatment in the future based on these molecular mechanisms.

Researchers have found that the average content of oxygen in human tissues under normal physiological conditions is about 3%, but the oxygen concentrations required by different organs

and tissues are also different. The oxygen concentration is between 10%−14% in lung, liver and arterial blood (Hendgen-Cotta, Kelm, & Rassaf, 2014; Semenza, 2014), about 7% in brain tissue and 5% in venous blood. However, the oxygen concentration on the bone surface and in the medullary cavity is only about 1%. Interestingly, short-term external hypoxia does not seem to affect the change of oxygen partial pressure in the body, and the activated protective mechanism enhances the efficiency of various organs in the application of oxygen. Although under hypoxia and resting state, serum calcium and phosphorus metabolism, bone mineral density and cartilage growth did not change significantly (McDonnell, Eiken, Mekjavic, Žlak, & Drobnič, 2020; Yellowley & Genetos, 2019). In the short term, the body seems to offset the negative effects of hypoxia by enhancing oxygen utilization and compensation mechanisms, but can the damage to the body be avoided by similar effects in a long-term hypoxic environment? Adults show two completely different situations in dealing with long-term hypoxia. The study found that the crowd who lives for a long time under high altitude hypoxia state has genotypic and phenotypic variation lines as well as reduced mitochondrial oxygen demand and inhibition of adenosine triphosphate (ATP) demand pathways, such as reducing erythropoiesis, inhibiting fatty acid metabolism involved in peroxisome proliferator-activated receptor α (PPARα), and improving oxygen utilization efficiency in order to resist the pressure of hypoxia and adapt to this environment (Avellanas Chavala, 2018; Murray, Montgomery, Feelisch, Grocott, & Martin, 2018). Individuals who do not adapt to the climate and are exposed to hypoxia at high altitude for a long time or suffer from sudden diseases with severe lack of oxygen intake, because of hypoxic stress, excessive production of reactive oxygen species (ROS) in tissues and accumulation of large amounts of free radicals, often lead to abnormal metabolic diseases such as pulmonary edema, brain edema and cardiovascular diseases (Gaur, Prasad, Kumar, Sharma, & Vats, 2021; Hartman-Ksycińska, Kluz-Zawadzka, & Lewandowski, 2016).

The possible damage caused by hypoxia lets body build up a protective mechanism based on the enhancement of cardiovascular function, blood oxygen carrying capacity and tissue oxygen utilization efficiency, but this process depends on the expression of HIF. HIF includes HIF-1, HIF-2 and HIF-3. HIF-1 is expressed in almost all cells of human body, while HIF-2 and HIF-3 are widely expressed in liver, spleen, kidney and other internal organs. Their stable expression depends on the concentration of oxygen and can respond to hypoxia signals at the same time. Compared with the other two, the expression of HIF-2 in endothelial cells is specific. It has been reported that HIFs are closely related to the establishment of collateral vessels in tumor, however HIF-1 can not only affect angiogenesis, but also mediate glycolysis, cell proliferation, apoptosis and other biological behaviors. HIF-1 could specifically bind to the hypoxia response element (HRE) of *erythropoietin* (*EPO*) gene and express stably in hypoxic environment. Its structure is a heterodimer, including α and β subunits. HIF-1β is the basic expression protein and HIF-1α is an oxygen regulatory protein, which consists of 826 amino acids with molecular weight of about 120 kD. The stability and transcriptional activity of HIF-1α protein are affected by oxygen-dependent hydroxylation. Hydroxylation of amino acid residues requires to utilize O_2 to produce succinate and CO_2. Hydroxylation is inhibited when the body tissue is anoxic, thus ensuring the stable expression of HIF-1α. Under normal physiological conditions, the proline residue of the HIF-1α protein molecule is hydroxylated by prolyl hydroxylase (PHDs), and the hydroxylated HIF-1α can be rapidly degraded by proteases in the cytoplasm. In addition, factor inhibiting hypoxia inducible factor-1 (FIH-1) could regulate the activity of HIF-1α. FIH-1 participated in the degradation of HIF-1α through hydroxylation of asparagic acid residues, but could not completely block the expression of downstream target genes of HIF-1α, which reason may be that FIH-1 can only specifically hydroxylate with asparagic acid residues in the functional region of HIF-1α subunits.

Fracture is defined as a disease with disruption or abnormality of bone physiological structure, but usually fracture contains not only bone tissue injury, but also soft tissue injury, vascular rupture and inflammation are the most common complications of fracture. Although the partial pressure of oxygen on the periosteal surface and medullary cavity of normal people is very low, it does not seem to affect the oxygen content in bone tissue. It has been reported that bone marrow samples from iliac bone were extracted for blood gas analysis, measured that the oxygen saturation in bone marrow is as high as 87.5% (Harrison, Rameshwar, Chang, & Bandari, 2002). However, after the occurrence of fracture, the local partial pressure of oxygen can be reduced to less than 1%. Unfortunately, tissue hypoxia is further aggravated by tissue swelling and bleeding, and some tissue oxygen partial pressure is even 0% (Brighton & Krebs, 1972). From this, it is not difficult to see that the injured part of the fracture is in an environment of extreme hypoxia, which just provides conditions for the stable expression of HIF-1α. The expression of HIF-1α in serum after fracture, increased in the early stage of fracture healing, and reached the highest level in the stage of bone formation (Sang, Wang, Qin, & Li, 2017). HIF-1α was positively correlated with bone mineral density, callus formation and angiogenesis during fracture healing, and it can significantly promote fracture healing. These results indicate that the hypoxia environment at the broken end of the fracture promotes the expression of HIF-1α, however, there seems to be insufficient evidence to show that HIF-1α can directly promote fracture healing, but it is certain that HIF-1α is closely related to this process.

TCMs have many unique effects, and play an important role in anti-tumor, anti-inflammatory, and autophagy regulation (Du, Guo, Chen, Miao, & Huang, 2022; K.L. Wang et al., 2021; P. Wang et al., 2021; Z.Y. Wang et al., 2021). Among them, TCMs have a long history in the treatment of bone-related diseases, such as fractures and osteoporosis (Wang, Wu, Li, & Lin, 2021; Zhang et al., 2016). Many *in vivo* experiments have shown that TCMs such as *Epimedii Folium* and *Drynariae Rhizoma* could promote bone regeneration to a certain extent, inhibit bone resorption, and then promote the healing of fracture wounds (Lee et al., 2015; Wang et al., 2018). In addition, *in vitro* experiments showed that chemical compounds from *Epimedii Folium* and *Drynariae Rhizoma* including flavonoids and alkaloids increased the expression of OB-related factors and genes in cells, thereby inducing OB differentiation, stimulating OB proliferation, bone nodule formation and matrix mineralization. At the same time, they stimulated the expression of intracellular osteoclast-related cytokines and genes, playing an important role in the balance of bone metabolism (Zhang, Niu, Yuan, & Liu, 2017).

3. TCMs exert therapeutic effects on fractures by affecting three types of bone marrow cells

3.1. Specific differentiation ability of bone marrow mesenchymal stem cells (MSCs) is a necessary condition for early fracture healing

Bone marrow MSCs are a subgroup of MSCs. MSCs, derived from the mesoderm of embryonic development, are a kind of stem cells with multi-directional differentiation. It widely exists in bone marrow, periosteum, skeletal muscle, fat, peripheral blood, umbilical cord blood, liver and pancreas and its function is to differentiate into OBs, adipocytes, chondrocytes, neurons, endothelial cells and so on (Chen, Zhuo, Duan, & Lu, 2020). In animal experiments, it was found that there were a large number of bone marrow

mesenchymal stem cells (BMSCs) around the broken end in the stage of bone regeneration, most of which came from bone marrow cavity and injured periosteum, while peripheral blood and skeletal muscle could only provide part of them. Under specific conditions, BMSCs can directionally play the functions of osteogenesis, adipogenesis and chondrogenesis, such as bone morphogenetic protein 2 (BMP2) and bone morphogenetic protein 6 (BMP6) in the bone morphogenetic protein family (BMPs) directionally induce BMSCs ossification by binding to type I and type II serine and threonine receptors on the cell membrane to produce transcription factor, or differentiate into adipocytes under the action of cell surface antigen spinocerebellar ataxia type 1 (SCA1) and cluster of differentiation 44 (CD44) (Valorani et al., 2010). When the continuity of bone is interrupted, BMSCs form callus and differentiate into OBs under the action of special extracellular environment and a variety of cytokines, and then complete the repair of bone physiological structure after OB maturation and osteoclast (OC) remodeling. It can be said that the function of BMSCs runs through the whole stage from fracture occurrence to fracture healing, and there is sufficient evidence to show that the function of BMSCs is related to bone mineral density, osteogenic ability, and thus promoting fracture healing. Therefore, the number of BMCSs and its specific differentiation ability are very important in the process of early fracture healing.

TCMs can promote BMSCs proliferation and improve BMSC function. They could directly repair liver damage from multiple perspectives by promoting BMSCs function or regulating the colonization of transplanted cells and the immune system (Wang, Zhang, & Wang, 2021). In addition, it was found that ligustrazine could promote the proliferation of BMSCs, and the appropriate concentration of ligustrazine could significantly improve the function of BMSCs to a certain extent (Wang et al., 2016). The tortoise shell extract at a specific concentration could increase the proportion of BMSCs in the proliferation phase of the cell cycle and promote the proliferation of BMSCs (Chen et al., 2007). *Astragalus* polysaccharide has been shown to promote the proliferation of BMSCs *in vitro*, it could improve the cell morphology of BMSCs, inhibit cell cycle arrest, and then increase cell proliferation and cell migration (Zhang et al., 2019). Astragaloside IV could promote the proliferation of human BMSCs *in vitro*, and the mechanism may be related to the promotion of the expression of stem cell factor (*SCF*), vascular endothelial growth factor (*VEGF*) and stromal cell-derived factor 1 (*SDF1*) mRNA in human BMSCs (Cao, Lv, & Li, 2021).

3.2. OBs are essential for bone formation

The largest number of cells in human bones are bone cells, accounting for 90%–95% of the total number of cells in bones. The source of OCs is generally believed that OBs continue to secrete osteoid and mineralize extracellular matrix, and finally form mature OCs and embed in bone lacunae, it can be said that OCs are the final state of OB differentiation (Franz-Odendaal, Hall, & Witten, 2006; Titorencu, Pruna, Jinga, & Simionescu, 2014). For OBs, once they stop secreting bone matrix, they will develop in three aspects: mature OCs, resting bone lining cells, and programmed apoptosis (Buenzli, 2015). At present, the specific genetic and molecular mechanisms in the process of OB differentiation and bone matrix maturation have not been fully understood. The bone tissue itself is in a normal hypoxic environment, and the partial pressure of oxygen is about 1%–6%, while the fracture can reduce the local partial pressure of oxygen to less than 1%, which provides a stable expression of HIF-1α, resulting in an external environment different from the physiological state of OB differentiation (Hannah, McFadden, McNeilly, & McClean, 2021). By summarizing the accumulated relevant literature, we proposed that oxygen as a necessary basic metabolite as well as HIF-1α plays the key regulatory role in the process of OB differentiation.

The active ingredients of TCMs that can promote the proliferation and differentiation of OBs include flavonoids, glycosides, coumarins, terpenoids (sesquiterpenes, monoterpenes, diterpenes) (An et al., 2016). It was found that *Rehmannia Radix* could promote the proliferation and differentiation of MC3T3-E1, and promote the production of insulin-like growth factor I (IGF1) and BMP and related proteins in PI3K/mTOR signaling pathway, enhance bone formation (Gong et al., 2019). Piperine enhanced OB differentiation by inducing adenosine monophosphate activated protein kinase (AMPK) phosphorylation in MC3T3-E1 cells (Kim, Kim, & Jang, 2018). Puerarin as well as stimulated OB proliferation and differentiation prevented the death of MG-63 through estrogen receptor (ER)-dependent MEK/ERK and PI3K/Akt activation (Wang et al., 2013). Icariin could promote the proliferation of MC3T3-E1 OBs and reduce apoptosis by regulating cycle-related cytokines, and enhance the differentiation and mineralization of MC3T3-E1 cells (Song, Zhao, Zhang, Li, & Zhou, 2013).

3.3. OCs maintain normal physiological morphology of bone

As a kind of metabolically active connective tissue, bone has been in a dynamic balance. Since embryonic growth, bone regeneration and bone remodeling have been pressing continuously. Particularly in adolescence, regeneration and remodeling can maintain bone mass, construct bone mechanical structure and optimize bone strength (Kim, Lin, Stavre, Greenblatt, & Shim, 2020). However, abnormal bone metabolism will break this balance, for example, the proliferation and function of OCs can lead to osteoporosis and rheumatoid arthritis (Madel et al., 2019; Steffen, Schett, & Bozec, 2019). OCs are CD14 monocytes or macrophage precursors fused in the presence of macrophage colony stimulating factor (M−CSF) and receptor activator of nuclear factor-kappa B ligand (RANKL) to form a diameter of 20–100 μm, containing 2–20 nuclei, which cytoplasm is rich in organelles such as mitochondria, lysosomes, ribosomes and Golgi bodies, and irregular cells with pseudopodia and processes. It is also the only cell that has been found to have the function of bone resorption so far (Jurdic, Saltel, Chabadel, & Destaing, 2006; Knowles, 2015). Its bone resorption function is through the combination of OC with bone tissue, the cytoskeleton reorganizes and produces obvious polarization, forming a microfilament ring attached to the bone surface. In acidic environment, under the combined action of protein kinase K, matrix metalloproteinases (MMPs), tartrate-resistant acid phosphatase and other enzymes, OC finally degrades the organic matter and inorganic minerals in bone tissue, producing a large number of calcium, phosphorus and collagen fragments, which are released by endocytosis (Georgess, Machuca-Gayet, Blangy, & Jurdic, 2014; Takito, Inoue, & Nakamura, 2018). In this process, the formation of microfilament ring is closely related to the migration and function of OC. It is generally believed that integrin vitronectin receptor α and β play a key role in regulating the polarization and activation of OC. Hypoxia does not seem to affect this expression, but may inhibit the adhesion of OC (Arnett et al., 2003; Faccio, Novack, Zallone, Ross, & Teitelbaum, 2003).

During fracture healing, bone formation and resorption are balanced and continuous process in which OCs are involved. Er-Zhi-Wan (EZW) was a traditional formulation that could inhibit OC differentiation and promote the proliferation of OBs (Zhang et al., 2008). In addition, some other TCM ingredients could have an effect on OCs, and to a certain extent regulate fracture healing by inhibiting the proliferation and function of OCs. Studies showed that high glucose environment could induce and enhance bone resorption by OCs. Aconitine could inhibit RANKL-induced OC formation in a dose-dependent manner by inhibiting the activation of

nuclear factor κB (NF-κB) and nuclear factor of activated T cell cytoplasmic 1 (NFATc1) and reducing the expression of dendritic cell-specific transmembrane protein (DC-STAMP). Artesunate reduced osteoclast production by inhibiting lipopolysaccharide (LPS)-stimulated NFATc1 protein expression by inhibiting toll like receptor 4 (TLR4), TNF receptor-associated factor 6 (TRAF6) and phospholipase Cγ1-Ca^{2+} (PLCγ1-Ca^{2+}) (Zeng et al., 2016; Zeng et al., 2020).

4. Effects of hypoxia and HIF-1α on three kinds of bone lineage cells

4.1. BMSCs

4.1.1. Hypoxia avoid BMCSs apoptosis through various signaling pathways

Hypoxia can directly or indirectly induce the expression of pro-apoptotic gene Bcl-2 19-kDa interacting protein 3 (*BNIP3*) and tumor suppressor gene *p53*, activate pro-apoptotic Bcl2 associated X protein (*Bax*) and Bcl2-associated agonist of cell death (*Bad*) genes and then activate Caspase 3 and Caspase 9 to make cells enter the process of apoptosis. However, it was found that BMSCs cultured *in vitro* at 1% and 5% oxygen concentration could significantly observe cell proliferation for one week, and its ability of proliferation was much greater than that of differentiation (Hung, Ho, Shih, Lo, & Lee, 2012). The reason for this phenomenon may be that there are some regulatory mechanisms at the fracture site in the hypoxic environment to avoid excessive apoptosis or even promote cell proliferation. This may be closely related to the activation of PI3K/Akt signal pathway (Lee et al., 2013; Li et al., 2017). Hypoxia weakens hydroxylation of HIF-1 α by inhibiting the activity of PHDs. The stable expression of HIF-1 α prevents apoptosis by regulating a variety of downstream cytokines, such as VEGF, fibroblast growth factor (FGF), NF-κB and so on. Activated PI3K stimulated Akt expression by inducing phosphatidylinositol 4,5-bisphosphate (PIP2) to produce phosphatidylinositol 3,4,5-trisphosphate (PIP3). As a proto-oncogene, Akt can regulate cell proliferation, growth, survival, metabolism and other behaviors. Activated Akt can activate the anti-apoptosis gene B-cell lymphoma-2 (*Bcl2*) and B-cell lymphoma-extra large (*Bcl-xL*) related promoter and inhibit Caspase 9 to resist hypoxia-induced apoptosis through phosphorylation, and mix autophagy mechanism to avoid cell damage caused by glucose deprivation (Chen, Crawford, Chen, & Xiao, 2013; Lv et al., 2017).

Polygoniside and parthenin could promote the proliferation and osteogenic differentiation of BMSCs through the Wnt/β-catenin signaling pathway (Chen et al., 2019; Hong et al., 2019). *Juglandis Semen* (Hetao in Chinese) leaf extract could also regulate the osteogenic differentiation and autophagy of BMSCs through the BMP2/Smad/Runx2 and Wnt/β-catenin pathways (Pang, Zhong, Jiang, Yang, & Nie, 2022). In recent years, researchers have been particularly interested in the Wnt/β-catenin signaling pathway. It has been reported that active ingredients of TCMs such as icariin, neohesperidin, and berberine could promote fracture healing through this signaling pathway (Wei et al., 2016; Yuan, Zhang, & Wang, 2022). These indicated that TCM played a role in the molecular mechanism of local hypoxia in the early stage of fracture formation, and provided help for the early healing of fractures.

In fact, in the HIF-1α/PI3K/Akt signal pathway, what enhanced cell survival is not only by the anti-apoptotic mechanism of Bcl2 and Bcl-xL activated by Akt, but also the activated Akt can also play an anti-apoptotic effect by preventing the activation of *Bax* and *Bad* genes. It has been reported psoralen or estradiol treatment down-regulated the expression of inosital-requiring enzyme-1 (IRE1), phosphorylated apoptosis signal-regulating kinase (P-ASK), phosphorylated c-Jun N-terminal kinase (P-JNK) and Bax, while the expression of Bcl2 was up-regulated. And some studies have found that inhibitor of apoptosis proteins (IAPs) are also closely related to apoptosis (Greijer & van der Wall, 2004). IAPs are a kind of endogenous anti-apoptosis proteins, and in its family, IAPs are regulated by hypoxia-related genes. Experimental studies have shown that 2% oxygen concentration can significantly stimulate the expression of IAP2, and this effect will increase with the decrease of oxygen concentration. HIF-1α does not directly regulate the production of IAP2 in this process. However, it can indirectly activate the internal ribosome entry site (IRES) by inducing NF-κB pathway to promote the expression of X-linked inhibitor of apoptosis proteins (XIAP) in the IAPs family. NF-κB plays an important role in fracture healing. γ-Bufalin could inhibit bone loss in mice by inhibiting the RANKL-induced NF-κB and mitogen-activated protein kinase (MAPK) pathways. Neobavaisoflavonoid inhibited OC formation by blocking the RANKL signaling pathway mediated TRAF6 and proto-oncogene tyrosine-protein kinase SRC (C-SRC) recruitment and NF-κB pathway, thus promoting new bone formation in fracture healing. All these indicate the importance of NF-κB signaling pathway (Dong et al., 2001; Galbán & Duckett, 2010; Jost & Vucic, 2020; Kilic, Kasperczyk, Fulda, & Debatin, 2007). Activated NF-κB can not only play an anti-apoptotic effect by promoting the expression of IAP2, but also prevent apoptosis by antagonizing the promoter of BNIP3 structural domain activated by HIF-1α with E2 promoter binding factor 1 (E2F1), blocking the transcription of *BNIP3* gene and inhibiting the expression of downstream *Bax* and *Bad* genes. In addition, HIF-1α-mediated miRNA can also affect BMSCs through different ways, such as: miRNA-210 enhances the activity of PI3K/Akt and Erk to improve cell survival; miRNA-429 enhances cell oxygen tolerance; miRNA-126 activates sprouty-related EVH1 domain-containing protein 1 (SPRED1) and Ras/Erk pathway to promote cell proliferation and migration; HIF-1α inhibits exosome PC12 and BMSCs apoptosis (Ge et al., 2018; Liu et al., 2020; Luo et al., 2019).

4.1.2. HIF-1α maintains cell cycle and promotes mitosis to increase number of BMSCs

Under hypoxia, BMSCs is basically at rest. Christina found that at 1% concentration, only 1.37% of BMSCs entered the mitotic G2/M phase, while G1 phase was significantly prolonged, and the ability of osteogenic differentiation could be restored when the oxygen concentration was raised to 3% (Holzwarth et al., 2010). This means that hypoxia inhibits the differentiation of BMSCs to preserve the multidirectional differentiation of stem cells and constantly renew themselves, thus maintaining the cell cycle (Buravkova & Anokhina, 2008). This inhibition of differentiation is very necessary in the early stage of fracture healing. The cells that stop differentiation in the early stage of new fracture seem to be beneficial to fracture healing, because it can provide a certain guarantee for differentiation into OBs in the later stage. Hypoxia activates PI3K/Akt pathway through HIF-1α and a variety of growth factors, which can promote the synthesis of organic cation/carnitine transporter 4α (Oct4α), homeobox protein NANOG (Nanog) and SYR box transcription factor 2 (Sox2) to maintain cell diversity (Zhang et al., 2015). On the other hand, the prolongation of cell cycle G1 is the result of the widespread expression of cyclin-dependent kinase (CDK) inhibitor p21 (Mansilla, de la Vega, Calzetta, Siri, & Gottifredi, 2020). Through the transforming growth factor-β (TGF-β) pathway mediated by HIF-1α, its mediator R-drosophila mothers against decapentaplegic protein 2 (R-Smad2) and Smad3 bind to TGF-β type Ⅰ receptor and polymerize Co-Smad4 to form p21 protein after transcription and translation. P21 can prolong the cell cycle by inhibiting the phosphorylated kinase activity of CDK complex. Many phenomena suggest that TGF-β/Smad2 play a crucial role in improving fracture union, and *Rehmanniae Radix*

enhances the formation of Gli1$^+$ cells to bone and cartilage and promotes fracture healing through TGF-β-dependent pathway (Moustakas, Pardali, Gaal, & Heldin, 2002; Samarakoon, Higgins, Higgins, & Higgins, 2019).

The effects of these mechanisms seem to hinder the proliferation of BMSCs, but obvious proliferation was still observed in BMSCs cultured *in vitro* at 1% oxygen concentration (Hung, Ho, Shih, Lo, & Lee, 2012), indicating that hypoxia promotes cell proliferation. In the course of the experiment, it was found that hypoxia activated a variety of mitogens, such as VEGF and IGF, through paracrine or autocrine, which played an important role in promoting cell proliferation. Many TCM treatments involve the regulation of VEGF in fracture. Naringin could regulate VEGF/VEGFR2 signaling pathway in osteoporotic rats and promote fracture healing by stimulating angiogenesis. Salidroside and Tao Hong Si Wu Decoction could promote fracture healing by regulating the expression of VEGF (Hefka Blahnova, Dankova, Rampichova, & Filova, 2020; Liao, Chen, Jiang, & Tian, 2014). VEGF binds to the receptor VEGFR2 to activate Ras pathway, and after Akt cascade amplification, Raf1 activates MAPK pathway, and phosphorylation activates MEK and its downstream only target gene *ERK*, which regulates cell proliferation and differentiation (Hefka Blahnova, Dankova, Rampichova, & Filova, 2020). While TGF-β can maintain the cell cycle, it can also activate MAPK subfamily JNK and p38 to continue to activate MAPK pathway, coactivate MEK, and enhance the ability of cell proliferation. The effect of hypoxia and HIF-1α on BMSCs during fracture healing, and intervention of TCMs were summarized in Fig. 1.

4.2. OBs

4.2.1. Hypoxia leads to decreased activity of OBs

OBs as a functional cell, its metabolic process is dependent on oxygen, no matter under physiological or pathological conditions, hypoxia may affect the healthy growth of bones (Yellowley & Genetos, 2019). In addition to the local extreme hypoxia caused by fracture, some scholars have investigated the related pathological hypoxia, such as high-altitude hypoxia, lack of oxygen intake in chronic obstructive pulmonary disease, anemia and so on, there is a correlation between oxygen concentration and bone mineral density (Czuba et al., 2017; Hartman-Ksycińska, Kluz-Zawadzka, & Lewandowski, 2016; Ramachandran, Mani, Gopal, & Rangasami, 2016). Wang exposed mice to high altitude (oxygen concentration 9.8%) for 21 d and observed that the trabecular volume of mice decreased by 33%, and histomorphometry showed a significant decrease in bone strength, increasing the risk of fracture (Hannah, McFadden, McNeilly, & McClean, 2021; Wang et al., 2007). This shows that the reason for the change of bone strength is that the osteogenic function of OBs is weakened under hypoxia, and the second is the enhancement of bone resorption capacity of OCs. No matter what the reason is, the final pathological manifestation is the decrease of bone unit and trabecular density (Hannah, McFadden, McNeilly, & McClean, 2021). The negative effect of hypoxia on OBs is almost catastrophic, OBs exposed to 2% oxygen concentration, bone formation decreased 10 times, OB function stopped and even OBs began to apoptosis at 0.2% oxygen concentration (Wang, Wan, Gilbert, & Clemens, 2007; Wu, Zheng, & Yang, 2017). And this damage is irreversible, reoxygenation after hypoxia cannot restore the activity of OBs, but aggravate the cell injury leading to apoptosis (Baik et al., 2015).

This result is not surprising. Oxidative stress under hypoxia significantly decreased the expression of specific transcription factors Runx2, BMP2 intracellular, as well as type I collagen in extracellular matrix, which restricted matrix mineralization (Liu et al., 2007; Pyo et al., 2013). The irisin in the rhizome of *Paeoniae Radix* (Baishao in Chinese) could up-regulate the expression of *BMP2*, *BMP4* and *Smad4* genes, enhance the expression of Runx2 and Osterix, and significantly promote the formation of new bone. And *in vitro* experiments showed that irisin could stimulate osteogenic differentiation and inhibit OC differentiation, and *in vivo* experiments showed that it could promote bone regeneration and inhibit inflammatory bone loss (Lee et al., 2018). These findings suggest that irisin may be helpful in the treatment of fractures. The tumor-related gene *metastasis-associated 1* (*MTA1*)

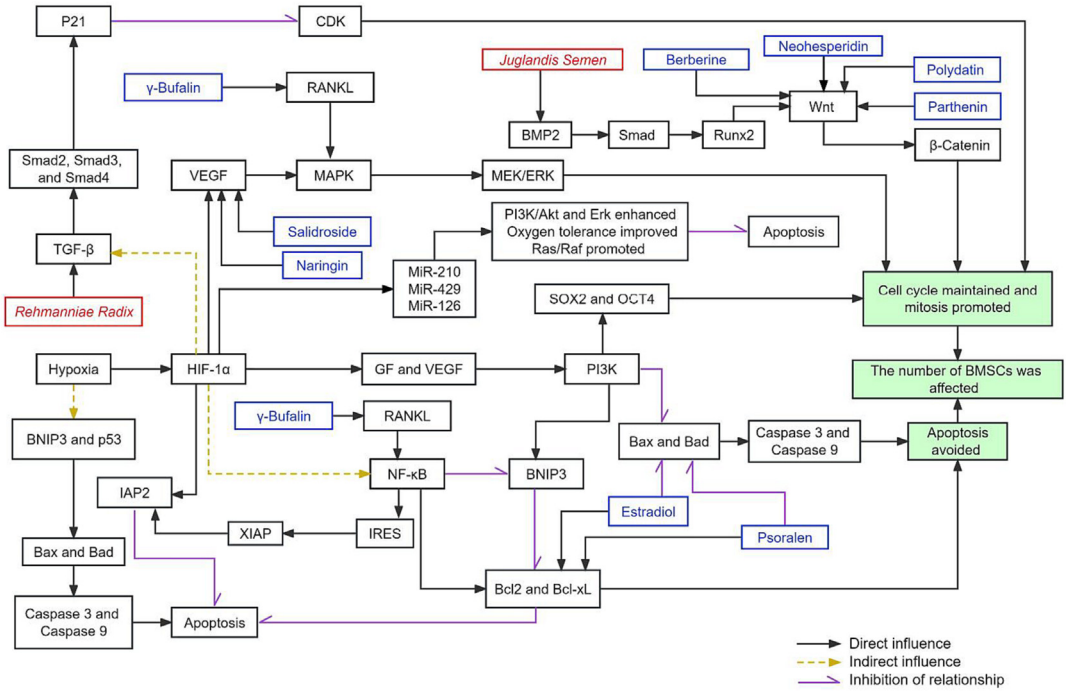

Fig. 1. Effect of hypoxia and HIF-1α on BMSCs during fracture healing, and intervention of TCMs on these signaling pathways.

induced by HIF-1α also decreased the expression of mineral deposition and osteogenic markers such as alkaline phosphatase (ALP) (Liu et al., 2015). Under the condition of hypoxia, ATP metabolism is slow, and OBs without sufficient energy supply will activate autophagy-related AMPK pathway, inhibit the negative regulation mechanism of mTOR pathway on autophagy, and activate serine at a specific position in human autophagy initiation protein ULK1 to initiate autophagy (Herzig & Shaw, 2018). And hypoxia-mediated combination of BNIP3 with Bcl2 and Bcl-xL will promote Beclin1-dependent autophagy, and under hypoxia conditions, a large amount of ROS is produced and accumulated due to oxidative stress. These negative factors further promote autophagy behavior and decrease cell activity. However, related studies have shown that artemisinin, an active ingredient of TCMs, could resist this increase in ROS to alleviate the oxidative stress effect and improve the survival rate of BMSCs (Cheng, He, Zhang, Zheng, & Yang, 2020; Zhang, He, Cheng, Zheng, & Yang, 2019). Other scholars have also found that the synergistic action of HIF-1α and Osterix inhibits the Wnt pathway and affects the number of OBs (Chen et al., 2012). After further exploring this mechanism, HIF-1α may further inhibit BMP receptor and LRP5/6 by activating *Sost* gene, blocking the downstream signal pathway and Wnt signal pathway induced by BMP, and then inhibit cell proliferation (Chen et al., 2013). Wnt signaling pathway and BMP signaling pathway are important for fracture healing. It has been reported that *Ginkgo biloba* (Yinxing in Chinese) extract could promote fracture healing through the Wnt/β-catenin pathway. Icariin could accelerate fracture healing by activating the BMP2/Smad5/Runx2 pathway, while Gukang Capsule could promote OB differentiation and fracture healing by triggering the Wnt/β-catenin and BMP/Smad signaling pathways.

4.2.2. HIF-1α antagonizes apoptosis induced by hypoxia and indirectly enhances osteogenic ability

Oxygen concentration has a great effect on OBs. Hypoxia or hyperoxia can inhibit the function of osteoblasts and induce apoptosis to a certain extent (Matsuo, Yamazaki, Takase, Aoyagi, & Uchinuma, 2008). Oxidative stress can induce apoptosis through mitochondrial-dependent and mitochondrial-independent pathways, which mainly affect the changes of mitochondrial membrane potential and release cytochrome *C* to activate apoptosis regulatory proteins Caspase 3 and Caspase 9 (Sinha, Das, Pal, & Sil, 2013). On the other hand, hypoxia stress causes the production of a large amount of ROS *in vivo*, resulting in mitochondrial dysfunction, resulting in irreversible damage to cells, and then accelerating apoptosis (Wang, Wu, Wu, & Wei, 2013). However, some studies have shown that HIF-1α may be a protective factor against apoptosis. Overexpression of HIF-1α can prevent hypoxia-induced damage to rat cardiomyocytes and fibroblasts. The mechanism is that HIF-1α reverses hypoxia-induced apoptosis genes expression of Bax, BNIP3 and Caspase by indirectly activating PI3K/Akt/mTOR pathway (Wang, Ma, & Qi, 2012; Yang et al., 2014). This protective effect is also reflected in OBs, which hypoxia reduces cell activity, while high expression of HIF-1α can antagonize apoptosis induced by hypoxia and glucose deprivation (Xu, Xue, Wang, & Xiang, 2015). But this is not the direct effect of HIF-1α, it is through a variety of ways to inhibit the expression of apoptosis-related genes, such as HIF-1α signal pathway downstream target gene *VEGF* expression to activate the anti-apoptosis mechanism of PI3K/Akt/mTOR. Icariin, timosaponin and ferulic acid could protect mouse OBs, and promote the expression of bone matrix by regulating BMP and Wnt/β-catenin signaling pathway in OBs to protect bone (Li et al., 2016). In addition, it was found that the expression of Notch signaling pathway was positively correlated with anti-apoptosis factor Bcl2. The activation of Notch ligand could significantly reduce the expression of Caspase 3 and inhibit apoptosis (Xu et al., 2018). HIF-1α may play its anti-apoptosis role by acting on Notch ligand and then stimulating jagged1, delta-like 4 ligand (DDL4) etc, together with activated PI3K/Akt/mTOR pathway to promote the expression of *Bcl2* gene. The echinacoside extracted from *Cistanches Herba* (Roucongrong in Chinese), *in vitro* experiments showed that it could cause the proliferation of OBs at a concentration of 1 nmol/L and the mineralization ability of bone was significantly increased. Echinacoside could stimulate the formation of OBs. In addition, echinacoside could also increase the ratio of OPG/RANKL to promote the regeneration of osteoblast MC3T3-E1 (Li et al., 2012).

The stable expression of HIF-1α at the same time activates the downstream factors of the hypoxia pathway, such as VEGF, TGF, FGF and EPO, which can promote bone regeneration and repair in different ways (Hankenson, Gagne, & Shaughnessy, 2015; Ying et al., 2020; Zhang et al., 2019). Several experiments of Liu showed that bone mineral density, bone trabecular structure and bone formation-related cytokines showed low levels in HIF-1α knockout mice (Liu et al., 2007). Neovascularization continuously provides oxygen, nutrition and growth factors for callus, and provides necessary conditions for the differentiation, proliferation and signal transduction of bone progenitor cells. VEGF plays an important role in angiogenesis (Ramasamy, Kusumbe, Wang, & Adams, 2014). *Salviae Miltiorrhizae Radix* et *Rhizome* (Danshen in Chinese), *Angelicae Sinensis Radix, Astragali Radix* (Huangqi in Chinese) and *Puerariae Lobatae Radix* (Gegen in Chinese), which were related to osteogenesis and angiogenesis, could enhance angiogenesis and osteogenesis, and they could promote bone formation to a certain extent, and the mechanism may be related to their promotion of blood vessel formation and osteoblast proliferation through the regulation of VEGF and other substances (Yang, Chin, Zhang, Lu, & Wong, 2014). VEGF is the main participant of angiogenesis, which can stimulate endothelial cell migration and proliferation, change vascular permeability and indirectly stimulate osteogenesis by regulating the release of osteogenic growth factor and paracrine signal (Kanczler & Oreffo, 2008). Subsequent studies have confirmed that conditional deletion of VEGF in bone progenitor cells induces osteoporosis-like phenotype with increased bone fat and decreased bone mass in bone marrow, while giving the exogenous VEGF significantly promotes angiogenesis and bone regeneration (Diomede et al., 2020). The effect of hypoxia and HIF-1α on OB during fracture healing, and intervention of TCMs were summarized in Fig. 2.

4.2.3. Hypoxia can promote OC formation without affecting OC activity

Oxygen concentration of OCs is closely related to the number and activity of OC. Arnett demonstrated the amount and activity of OC stimulated by hypoxia in mouse bone marrow cells cultured in an ivory disc, and found that the absorptive capacity of OC at 2% oxygen concentration was 21 times higher than that of normal OC, and still showed a stimulating response even at 0.2% oxygen concentration (Arnett et al., 2003). The effect of hypoxia on OC production is not limited to locals. when the oxygen concentration is less than 2%, the number of OCs formed in peripheral blood increases by 3 times, and the number of nuclei per OC increases by 2 times and the function of bone resorption enhances by 10 times when the oxygen concentration increases to 20% (Utting, Flanagan, Brandao-Burch, Orriss, & Arnett, 2010). There are many mechanisms of OC proliferation induced by hypoxia signaling pathway, such as HIF-1α expression promotes RANKL by activating JAK2/STAT3 pathway, stimulates the fusion of $CD14^+$ monocytes or macrophages into OCs (Zhu, Tang, Wu, Ji, & Kang, 2019), and hypoxia inhibits osteoprotegerin OPG production and avoids binding to RANKL and reduces OC production (Knowles, 2015).

Studies have shown that the supplementation of exogenous melon polypeptide could inhibit the OC differentiation induced by RANKL/M−CSF, thus alleviating osteolysis and promoting bone

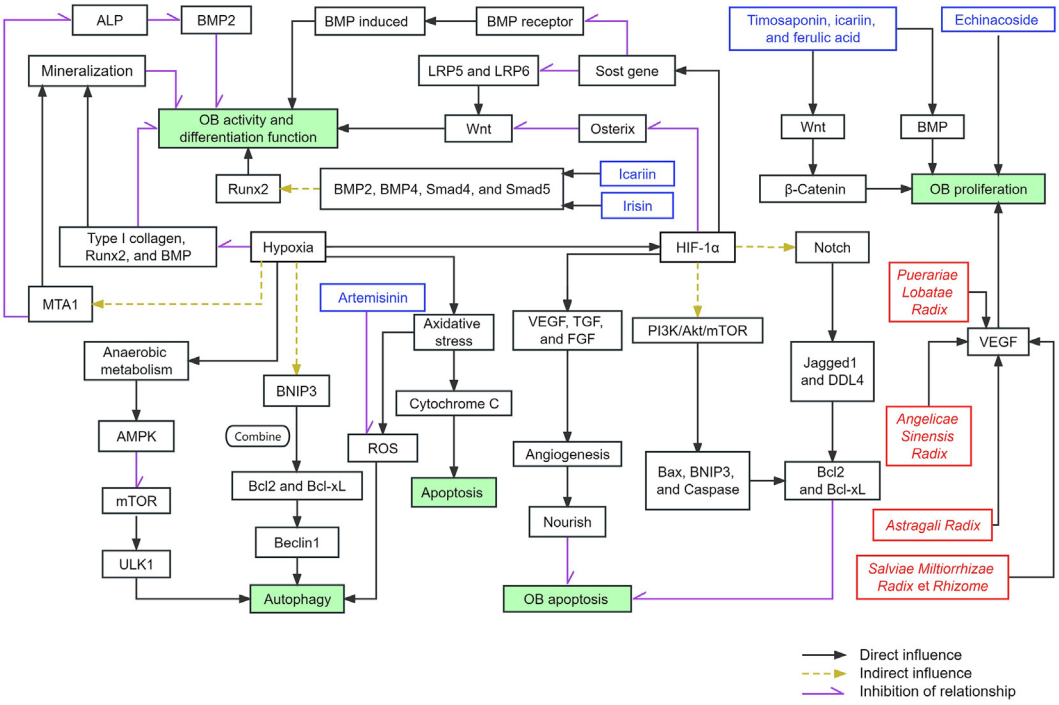

Fig. 2. Effect of hypoxia and HIF-1α on OB during fracture healing, and intervention of TCMs on these signaling pathways.

formation. RANKL seemed to be closely related to the growth of OCs. Galangin could inhibit RANKL-induced osteoclastogenesis by inhibiting the MAPK and NF-κB signaling pathways (Li et al., 2021). Curcumin could attenuate oxidative stress and osteoclastogenesis by regulating the Nrf2/NF-κB signaling pathway in RAW264.7 cells (Liu et al., 2021). Kanroin extract has also been shown to inhibit RANKL-induced osteoclastogenesis (Inagaki et al., 2021). Shikonin may promote the differentiation of BMSCs into OBs through the Wnt/β-catenin signaling pathway, and it may also inhibit the formation of OCs mediated by the RANK/RANKL/OPG pathway *in vitro* (Zhou, Wang, Zhao, Kuai, & Yang, 2020). In addition, *Schisandrae Chinensis Fructus* (Wuweizi in Chinese) is a relatively common single herb, which could maintain the balance of bone remodeling and play an important role in promoting OB differentiation and regulating OC activity (Jiang et al., 2020).

HIF-1α downstream regulatory factors VEGF, IGF and M−CSF can simultaneously activate PI3K/Akt and MAPK signal pathways to affect OC formation and survival (Knowles & Athanasou, 2008). In addition, hypoxia-induced autophagy did not affect the activity of OCs. The expression of Bcl2 and BNIP3 protein enhanced the autophagy gene *ATG4* and enhanced OC production, while the proliferation of cells with *HIF-1α* or *BNIP3* gene knockout was significantly inhibited (Zhao et al., 2012). The results of this kind of research prove to us that there is a close relationship between hypoxia and OCs. The proliferation and metabolism of osteoclasts do not seem to be critical to the demand for oxygen, and they are highly tolerant to the change of oxygen tension. Reoxygenation after acute hypoxia has no great effect on its function, on the contrary, it has been found that high concentration of oxygen will inhibit the number and activity of OCs (Al Hadi, Smerdon, & Fox, 2013). However, in terms of the function of OCs, they contain a large number of mitochondria and lysosomes, and the demand for oxygen in the process of metabolism is inevitable, such research results seem unreasonable. In fact, hypoxia did damage the structure of OCs, continuous hypoxia reduced the integrity of cell membrane by 21%, and the function of OCs was inhibited when exposed to 1% oxygen for a long time, but cell activity was not affected (Knowles & Athanasou, 2009; Ma et al., 2019). Hypoxia and HIF-1α also lead to OC apoptosis, which is related to the inhibition of Notch1 signal pathway by apoptosis-related genes *Caspase 3* and *miRNA34a-5p* (Kang et al., 2017; Song et al., 2020). We suspect that the continuous increase in the number of cells under hypoxia makes up for the damage of this function.

4.2.4. HIF-1α enhances OC bone resorption but inhibits OC differentiation

Although hypoxia has a clear effect on OC formation, the mechanism is not fully understood. The bone resorption capacity of OCs under hypoxia seems to depend on the expression of HIFs. Studies have shown that both HIF-1α and HIF-2α can regulate OCs, but HIF-2α seems to mainly regulate differentiation and OC formation (Knowles, 2020). In the process of OC formation induced by M−CSF and RANKL, it was observed that the expression of OC fusion-related genes increased when HIF-2α was overexpressed, while silencing HIF-2α in OCs had no effect on hypoxia-induced bone resorption (Knowles, 2015, 2020; Martinez-Guardado et al., 2019). It was found that naringin could increase the *in vitro* expression of BMP, and activate Wnt/β-catenin and extracellular signal-related kinase (Erk) pathways, thereby promoting OB proliferation and MSCs differentiate to form bone. In addition, naringin also inhibited osteoclastogenesis by altering RANK/RANKL interaction and induced OC apoptosis *in vitro* (Yu et al., 2020). HIF-1α plays a key role in the bone resorption function of OCs. It can act on phosphoglycerate kinase 1 (PGK1) to increase the utilization rate of ATP, and enhance the bone resorption function of OC to some extent by regulating glycolysis. When prolyl hydroxylase domain-2 (PHD2) significantly enhanced bone resorption function and inhibited HIF-1α, the expression of *Glut1* and *glycolysis* genes was inhibited, and the bone resorption capacity of OCs was significantly decreased (Indo et al., 2013). HIF-1α can also increase the utilization of ATP by increasing the level of angiopoietin-like protein 4 (ANGPTL4), as well as enhance the bone resorption function of OC, and enhance the proliferation and differentiation of OB

while promoting the function of OC (Knowles, 2015). In addition, HIF-1α can also promote bone resorption of OC by activating downstream *FGF11* gene expression (Knowles, 2017). Due to the lack of oxygen in the fracture site, this hypoxia state will lead to OC mitochondrial dysfunction and increase ROS production, and then inhibit JNK/MAPK and IκBα pathways, weaken RANKL-dependent phosphorylation, and finally inhibit OC differentiation. Excessive activation of OCs can lead to the difficulty of new bone formation. Studies have found that *Dendrobium Officinale Caulis* (Shihu in Chinese) inhibits OC formation by inhibiting ROS, p38-c-Fos and NFATc1-MMP9 *in vitro*, thereby attenuating inflammatory osteolysis *in vivo*, which helps new bone form better (Ma et al., 2019; Srinivasan et al., 2010). HIF-1α and HIF-2α play different roles in OC, and HIF-1α regulates OC function while HIF-2α mainly regulates OC production (Knowles, 2020). The effect of hypoxia and HIF-1α on OC during fracture healing, and intervention of TCMs were summarized in Fig. 3.

5. Discussion and prospects

At present, the studies on hypoxia and HIF-1α are mostly focused on the mechanism of tumor metabolism and angiogenesis, but the hypoxic microenvironment formed after fracture and the mechanism of core regulatory factor HIF-1α in promoting fracture healing are still worthy of our study. Fracture healing is an extremely complex process, which is affected by many factors, including age, nutritional status, endocrine and metabolism, growth factors, local injury, local environment and so on. These systemic or local, internal or external factors will promote or slow down the process of fracture healing in varying degrees. The cause of fracture injury and pathological changes after fracture will inevitably lead to local blood flow changes to form a hypoxic microenvironment, while MSCs and OBs, which are most closely related to osteogenesis, and only OCs can maintain bone physiology and remodeling in hypoxic microenvironment, so it is necessary to explore the regulatory mechanism of these three bone cells under hypoxia and HIF-1α, as well as what are the responsible compounds in TCM for what signal molecules in what cells. Current studies have shown that HIF-1α can directly or indirectly affect the apoptosis, proliferation and differentiation of MSCs, osteoblasts and OCs under hypoxia (Fig. 4). And a variety of active ingredients of TCMs such as psoralen and icariin have been reported to have the function of promoting fracture healing. Their effects on three kinds of OCs and the mechanisms of promoting fracture healing have also been revealed one by one.

The role of a single signaling pathway or the function of a single protein cannot form a complete molecular network, and the underlying mechanisms cannot be systematically explained. Fortunately, with the vigorous development of systems biology technology guided by the "system science perspective", the related omics research can help us to explain the changes of human physiological function. Proteins, as the direct carriers of life activities, need to be DNA transcribed, translated and modified before they can be expressed. Transcriptome technology can be used to study the relationship between genes and proteins, but it also has many limitations. The premise of gene-to-protein expression is that the level of mRNA in cells reflects the level of protein expression, and this process will be affected by transcriptional level, post-transcriptional level, translation level and post-translation level. And the correlation between mRNA abundance and protein abundance is not good, especially for low abundance proteins. More importantly, the complex post-translational modification, subcellular localization and protein–protein interaction of proteins can hardly be judged from the mRNA level. In the process of fracture healing, the regulation of related genes is static. Although its expression level is different in different tissues or cells, the genetic information involved has not changed. However, the expressed products will change according to the degree of expression, time, place and environmental factors, so the expression of the protein

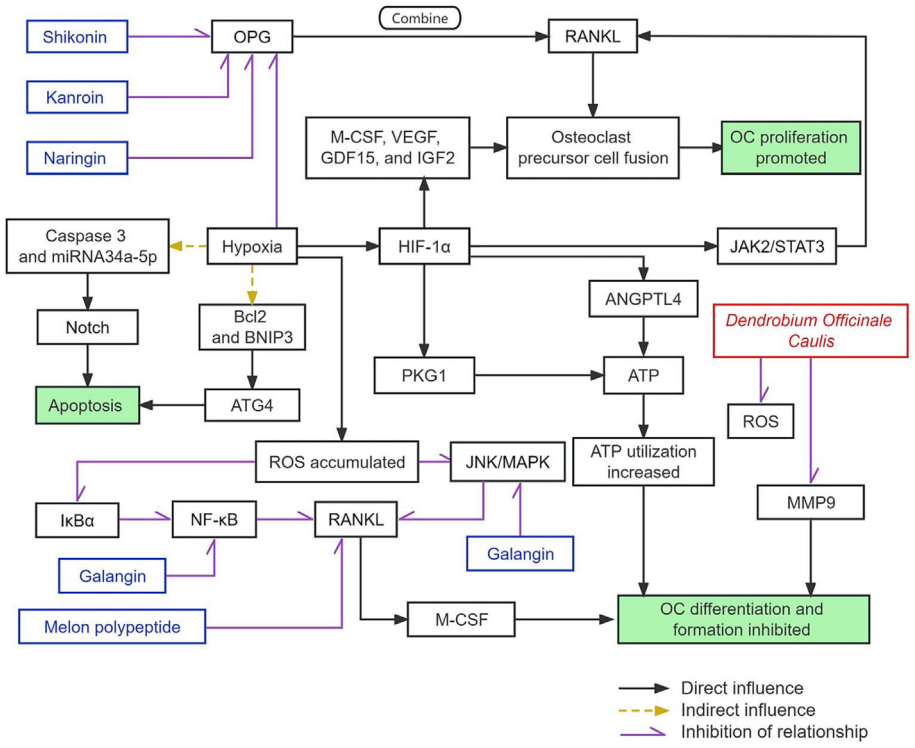

Fig. 3. Effect of hypoxia and HIF-1α on OC during fracture healing, and intervention of TCMs on these signaling pathways.

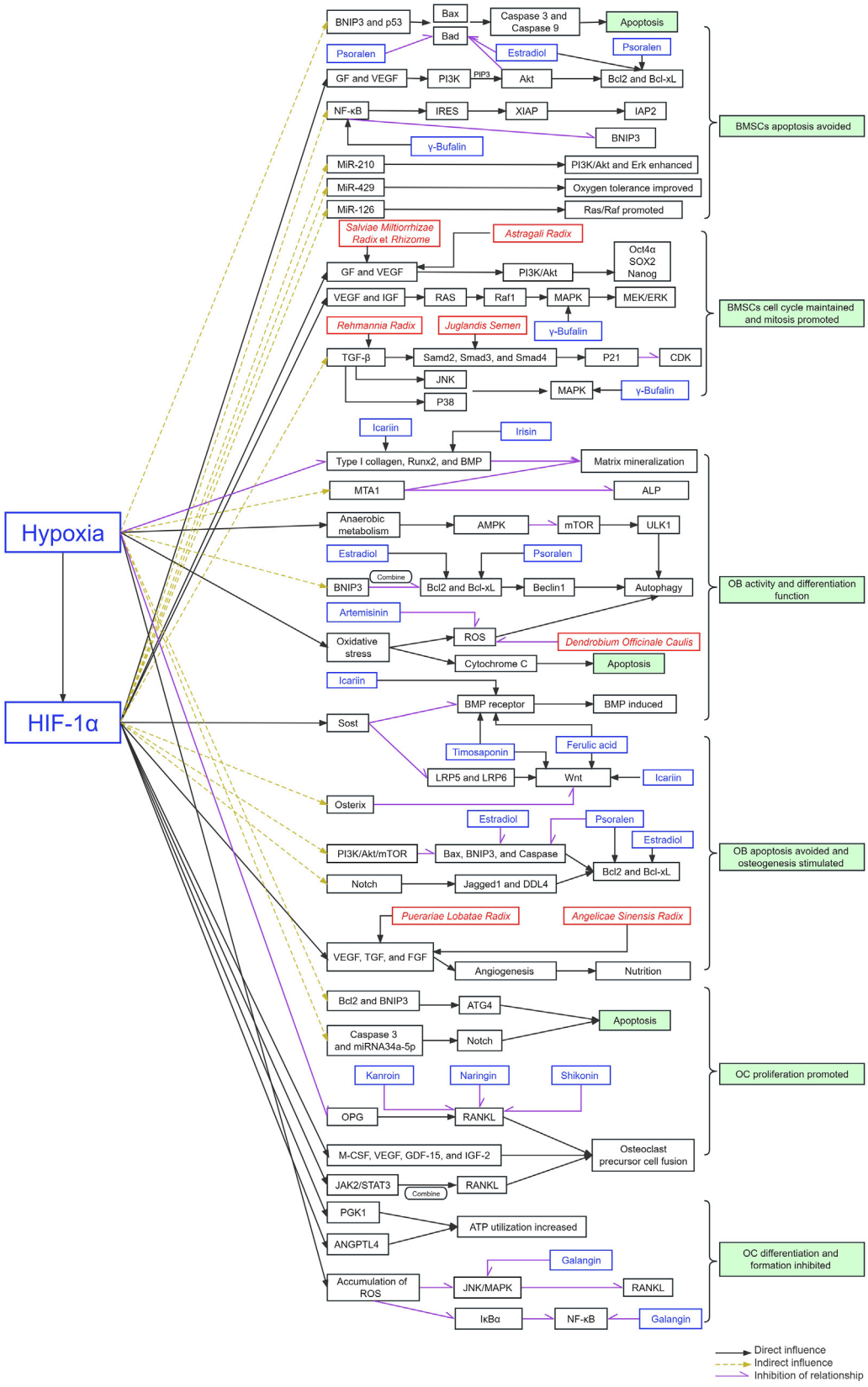

Fig. 4. Related mechanism of hypoxia and HIF-1α in process of early fracture healing, and intervention of TCMs on these signaling pathways.

is dynamic. Therefore, proteomics should be used to study the whole level of proteins, and the quantitative analysis of protein network can be carried out by stable isotope labeling technique. The changes of activity and function of these proteins under different biological phenotypes, states and conditions were compared and analyzed. In fact, it is impossible to build a bridge between

macro and micro by using a certain technology. Through the integrated analysis of genomics, proteomics and metabonomics, detailed studies on gene regulation, protein expression, functional changes and symbiotic interaction can reveal the changes of the whole biological network in the occurrence and development of fracture healing.

6. Conclusion

Fracture healing is closely related to the cell status and function of BMSCs, OB and OC. The hypoxic environment in the local fracture and its induced HIF-1α affect the process of fracture healing through a variety of signaling pathways, and TCMs such as *Psoraleae Fructus* (Buguzhi in Chinese) and *Epimedii Folium* can accelerate fracture healing through these pathways. The mechanism of hypoxia, HIF-1α and TCM in the process of fracture healing is still in the exploration stage. Although some progress has been made *in vitro* or animal experiments, a large number of clinical evidences are needed to verify it. Multi-group integrated analysis was used to clarify the changes of protein network with HIF-1α as the core under hypoxia environment, and finally confirmed its molecular mechanism by molecular biology and other techniques, and found a new target to promote fracture healing. Accelerating the healing cycle so as to reduce the burden of patients may even open up a new direction for clinical treatment of bone nonunion.

CRediT authorship contribution statement

Wenxian Zhang: Data curation, Formal analysis, Visualization, Writing – original draft. **Fusen Yang:** Supervision, Writing – review & editing. **Qikai Yan:** Supervision, Writing – review & editing. **Jiahui Li:** Supervision, Writing – review & editing. **Xiaogang Zhang:** Writing – review & editing. **Yiwei Jiang:** Writing – review & editing. **Jianye Dai:** Conceptualization, Data curation, Project administration, Validation, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

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

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