Mechanism of Action of Icariin in Bone Marrow Mesenchymal Stem Cells

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Received 6 December 2018; Revised 28 February 2019; Accepted 12 March 2019; Published 4 April 2019

Academic Editor: Philippe Bourin

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Osteoporosis, femoral head necrosis, and congenital bone defects are orthopedic disorders characterized by reduced bone generation and insufficient bone mass. Bone regenerative therapy primarily relies on the bone marrow mesenchymal stem cells (BMSCs) and their ability to differentiate osteogenically. Icariin (ICA) is the active ingredient of Herba epimedii, a common herb used in traditional Chinese medicine (TCM) formulations, and can effectively enhance BMSC proliferation and osteogenesis. However, the underlying mechanism of ICA action in BMSCs is not completely clear. In this review, we provide an overview of the studies on the role and mechanism of action of ICA in BMSCs, to provide greater insights into its potential clinical use in bone regeneration.

1. Introduction

Conventional treatments for orthopedic disorders like osteoporosis, femoral head necrosis, bone defects, and nonunion disorders [1–7] have poor clinical efficacy due to their inability to ameliorate the loss in bone mass. Therefore, the current focus of treating bone disorders is tissue regeneration using bone marrow mesenchymal stem cells (BMSCs) [7, 8]. A number of studies have investigated the effects of various drugs, mechanical stress, physical stimuli, and scaffolds on BMSCs [9–11], in order to clinically translate its regenerative capacity [12]. Traditional Chinese medicine (TCM) has also garnered considerable interest in recent years due to its minimal toxicity [13]. According to the principles of TCM, bone function is closely associated with the balance of kidney yin and yang. Herbs such as Herba epimedii, Fructus psoraleae, Drynaria fortunei, and Radix dipsaci are known to invigorate the kidney and restore the balance and are therefore frequently used to treat bone disease. Currently, Herba epimedii is the most deeply studied among those herbs in the bone regeneration field [14]. Icariin (ICA) is the main active ingredient of Herba epimedii, which has been used in TCM formulations to strengthen the muscles and bones [15]. Although Herba epimedii is still used to treat orthopedic disorders, its mechanism of action remains unclear. Several studies have analyzed the effects of ICA in BMSCs and other cells and provided new insights into its therapeutic role in orthopedic disorders. In this review, we have summarized the recent findings on the role and mechanism of action of ICA in BMSCs.

2. Basic Properties of ICA

Herba epimedii (Yin Yang Huo in Chinese) is the dried leaf of Epimedium brevicornum Maxim as recorded in the Chinese Pharmacopoeia [16–18], as well as the 400-year-old Chinese medical classic Shennong Ben Cao Jing. It nourishes the
kidney and significantly reinforces yang [18]. More than 20 flavonoids have been identified by chemical reaction and spectral analysis and isolated from Herba epimedii by systematical separation technology [17]. ICA ($C_{33}H_{40}O_{15}$, molecular weight: 676.66, Figure 1) [17] is one of the primary active constituents which is also considered as the chemical marker for quality control components of Herba epimedii [18]. Specifically, the contents of ICA and the total flavonoids are no less than 0.5% and 5.0% of the components, respectively [16]. The main isolation methods for ICA include water boiling extraction, ethanol reflux extraction [19–22], and an ultrasonic-assisted ethanol extraction method developed by Zhang et al. [21]. The optimum ultrasonic-assisted extraction conditions were determined by an orthogonal test as follows: 50% (v/v) ethanol solution, 30 ml/g liquid-solid ratio, ultrasonic duration for 30 minutes, 50°C extraction temperature, and extraction for 3 times. Compared with the traditional water boiling extraction method, this kind of method has higher efficiency. In addition, microwave, high pressure, and vacuum reflux extraction methods have also been tested [17, 21, 23, 24]. Herba epimedii is widely used for the treatment of osteoporosis in China [18, 25, 26], and results from clinical trials [27, 28] show similar anti-osteoporotic effects of its flavonoid extracts, as well as those of Epimedium total flavone capsules. The effects of ICA have largely been studied in animal or in vitro models [2, 4, 5, 9, 29, 30], and its potential clinical applications are rarely reported.

3. Role of BMSCs in Bone Regenerative Therapy

The common culture method of mouse BMSCs was as described in several studies [31–34]. Bone marrow is extracted from the femur and tibia of mice using an aseptic technique. The bone marrow is then cultured in vitro and subcultured to the third passage. As for human BMSCs, the proximal femur or posterior iliac crest is the common part from which to extract human bone marrow [30, 32, 33, 35]. The bone marrow is cultured in vitro and usually cultured to the third passage [33]. It should be noted that the cell phenotype identification is important in BMSC culture processes [32]. The sorted mouse CD29⁺Sca-1⁺CD45⁻CD11b⁻ BMSCs and human CD146⁺STRO-1⁺CD45⁻ BMSCs are cultured for 1-2 weeks to reach 80%-85% confluence [32, 33]. Then, first-passage BMSCs are detached and seeded in culture flasks for enrichment of cell populations.

The bone is a kind of mineralized connective tissue which exhibits four types of cells: osteoclasts, osteoblasts, osteocytes, and bone lining cells. Osteoblasts, which comprise 4-6% of the total bone cells, are located along the bone surface and are widely known for their role in bone formation [36]. The osteocytes accounting for 90-95% of the total bone cells are located within lacunae surrounded by a mineralized bone matrix wherein they exhibit a dendritic morphology [37]. The morphology of embedded osteocytes varies independently of bone types. Osteoclasts derive from mononuclear cells of the hematopoietic stem lineage which are multinucleated and terminally differentiated. Though it exhibits an inert appearance, bone tissue is constantly resorbed by osteoclasts and reformed by osteoblasts in a highly dynamic way. The process of bone remodeling is greatly complicated which is in a cycle comprising three stages: (1) initiation of bone resorption by osteoclasts, (2) transition between resorption and reformation, and (3) formation of new bone by osteoblasts. This bone remodeling process requires coordinated actions of osteocytes, osteoclasts, osteoblasts, and bone lining cells which together form the temporary anatomical structure called the basic multicellular unit [38, 39]. Osteoblasts are the main functional cells of bone formation, which are mainly responsible for the synthesis, secretion, and mineralization of the bone matrix. Osteoblasts can produce extracellular matrix proteins and mineralization regulators [38], during which period it undergoes significant proliferation and

Figure 1: Chemical structure of ICA.
differentiation. Osteoclasts are the only cells with bone resorption function. Many cytokines such as interleukin-6 (IL-6), tumor necrosis factor-a (TNF-a), and cathepsin K can provide signals for osteoclast differentiation and bone resorption, promote the recruitment of osteoclast precursors, and drive osteoclast differentiation and bone resorption [40–43]. The osteoblasts and the differentiation of osteoclasts are regulated by many signaling pathways, among which the bone morphogenetic protein-drosophila mothers against decapentaplegic protein (BMP-Smad) signaling is important [44]. The bone mass can be increased by promoting the directional differentiation of BMSCs into osteoblasts, which is driven by the runt-related transcription factor 2 (Runx2) and Osterix (Osf) [36, 38, 45, 46]. Since osteocytes are derived from the BMSC lineage through osteoblast differentiation [38], so finding potential drugs prompting the differentiation of BMSCs into osteoblasts may be a promising strategy for bone regeneration.

Hematopoietic stem cells (HSCs) and BMSCs are the two pluripotent cell types found in the bone matrix [47, 48]. BMSCs were first isolated from the adult bone marrow [49] and can differentiate into the adipocytes, chondrocytes, osteoblasts, and myoblasts [29, 50, 51]. Therefore, BMSCs are a highly promising therapeutic option for cardiovascular, orthopedic, and joint degenerative diseases [52–57]. Several studies have examined the ability of BMSCs to improve bone formation and prevent bone loss and necrosis, in addition to ameliorating congenital bone defects and osteoporosis [58, 59]. However, the engrafted BMSCs have poor survival and a low rate of differentiation at the site of transplantation, which significantly reduce the efficacy of BMSC-based regenerative therapy. Therefore, it is essential to develop new drugs to enhance BMSC proliferation and differentiation.

BMSC osteogenesis is the key step in bone regeneration and is affected by several factors including hormones, growth factors, environmental factors, and microRNAs [11]. BMSCs not only give rise to bone tissues but can also differentiate into adipose cells or osteoblasts [60]. Under physiological conditions, a dynamic balance exists between the osteogenic and the adipogenic potential of BMSCs [61–63] and is primarily regulated by Runx2 and the peroxisomal proliferator-activated receptor gamma (PPARγ) [30, 64]. Runx2 is regulated by BMP-2 and is a key modulator of osteogenic differentiation, whereas PPARγ promotes adipogenesis and inhibits osteogenesis [65–67]. Both signaling pathways concurrently regulate different cytokines to determine the fate of BMSC differentiation [68, 69]. The extracellular signal-regulated kinase-mitogen-activated protein kinase (ERK-MAPK) signaling pathway is also a key player in regulating BMSC differentiation [70], whereas the platelet-derived growth factor (PDGF) pathway is an essential proosteogenic pathway [71]. Cao et al. showed that Notch and BMP-9/Smad signaling synergistically enhanced osteogenic differentiation [72], and Li et al. found that miR-21 directly acted on Smad7 in the Smad7-Smad1/5/8-Runx2 pathway to modulate osteogenic differentiation [73]. Long et al. demonstrated that miR-139-5p regulated osteogenic differentiation of BMSCs via the Wnt/β-catenin pathway [74]. Furthermore, the transforming growth factor-β/bone morphogenetic protein (TGF-β/BMP) [75], phosphatidylinositol 3-kinase/protein kinase B/glycogen synthase kinase-3 (PI3K/Akt/GSK-3) [9], extracellular regulated kinase (ERK), PI3K/Akt [75], and insulin-like growth factor 1 (IGF1) [76] pathways also play important roles in osteogenic differentiation and bone formation (Figure 2). Since the two differentiation pathways are competing [68, 69], interregulatory, and interconvertible, certain growth factors can be used to promote the osteogenic differentiation of BMSCs ex vivo for bone tissue engineering.

Migration of BMSCs to the site of bone defect is a critical step in the treatment of orthopedic disorders [77]. Previous studies [78–81] have shown that the C-X-C motif chemokine ligand 12/C-X-C chemokine receptor type 4 (CXCL12/CXCR4) axis modulates BMSC homing and promotes angiogenesis, and the BMP-2/Smads/Runx2/Osterix axis modulates BMSC osteoblastic differentiation. The crosstalk between these two signaling axes is mediated by CXCR4, which modulates the migration [82] and osteogenic differentiation of BMSCs. Some studies have demonstrated that BMSC migration can also occur via the BMP-Smad1/5/8-twist-related protein 1/activating transcription factor 4 (Twist1/Atf4) [83, 84] and CXCR4/stromal-derived factor 1 (SDF-1) [85–87] axes and the Smad pathway [88].

4. Mechanisms of BMSC Regulation by ICA

4.1. BMSC Proliferation and Osteogenesis Promoted by ICA. ICA has multiple pharmacological activities, including hormone-like, antitumor, immunomodulatory, and antioxidative effects [89–94]. Studies [95, 96] show that ICA-mediated osteogenesis is associated with its hormone-like function. It can induce BMP-2 and BMP-4 mRNA expression in osteoblasts and significantly upregulates Osf at low doses [97, 98]. In addition, ICA facilitates bone formation by inducing proosteoblastic genes like Osf, Runx2, alkaline phosphatase (ALP), and collagen type I. It also inhibits bone resorption by regulating the osteoprotegerin/receptor activator of nuclear factor-κB ligand (OPG/RANKL) signaling in the osteoclasts [99]. Zhang et al. found that ICA inhibits the adipogenic differentiation of BMSCs and promotes osteoblastic differentiation [100]. Fan et al. found that ICA promoted not only BMSC proliferation in vitro in a dose-dependent manner but also osteoblastic differentiation at very low doses (10⁻⁹ M to 10⁻⁸ M). However, a higher concentration of 10⁻⁵ M was toxic and suppressed osteoblastic differentiation [30]. Using a rat model of bone fracture, Cao et al. [2] showed that intragastric administration of ICA significantly increased osteotylus formation and accelerated bone healing within 5 months of treatment. These findings demonstrate that ICA administration following bone fracture can accelerate mineralization and osteogenesis and significantly improve bone healing. Therefore, ICA can also be an alternative treatment for postmenopausal osteoporosis and bone fracture.

The imbalance between BMSC adipogenesis and osteogenesis is considered the primary cause of femoral head
necrosis [101]. The two processes are normally at an equilibrium under physiological conditions, which can be disrupted by external factors such as steroids and alcohol. Huang et al. [4] showed that ICA can effectively prevent femoral head necrosis, improve prednisolone-induced BMSC proliferation, enhance osteoblastic differentiation, and inhibit adipogenic differentiation. In addition, low concentrations of ICA (10^{-9} \text{ M} \text{ to } 10^{-5} \text{ M}) significantly increased BMSC proliferation, especially at 10^{-6} \text{ M} [4, 30].

Sun et al. [29] found that ICA restored the balance between osteogenic and adipogenic differentiation of mesenchymal stem cells in patients with osteonecrosis of the femoral head via ATP-binding cassette subfamily B member 1- (ABCB1-) promoter demethylation. In addition, ICA inhibited the differentiation of mesenchymal stem cells into adipocytes by inhibiting PPARγ, CCAAT/enhancer binding protein α (C/EBPα), and fatty acid-binding protein 4 (FABP4) mRNA via the Notch signaling pathway [102]. Zheng et al. [103] also found that daily oral administration of ICA (0.3 mg/g) to osteoprotegerin knockout male mice for 8 weeks increased the trabecular bone volume and trabecular number, indicating an important role of osteoprotegerin in ICA-mediated osteogenic effects. In addition, osteocalcin and osteopontin also mediate ICA-induced osteogenic differentiation by increasing ALP activity and collagen type I levels [104]. These results [29, 102–104] indicated that ICA plays an important role in bone synthesis and metabolism. Furthermore, ICA significantly promoted bone healing by increasing BMSC proliferation and osteoblastic differentiation in a New Zealand rabbit model of bone defect [105]. ICA can also induce BMSC osteoblastic differentiation under various pathological conditions such as osteoporosis [5] and bone necrosis [29]. Estrogen and epigenetic regulation are currently the research focus of ICA-induced osteogenesis under pathological conditions [5, 29]. Sun et al. showed that ICA improved BMSC viability and osteoblastic differentiation by upregulating ABCB1, indicating a demethylating function as well [29]. In addition to promoting osteogenic differentiation of BMSCs, ICA can also promote bone regeneration by promoting angiogenesis [106], since vascularization is a key step in bone regeneration which recruits the BMSCs and essential factors to the site of trauma [107].

Icaritin is a biologically active metabolite of ICA [108] and can be easily extracted from various sources. Wu et al. [12] showed positive effects of icaritin on BMSC osteoblastic differentiation in vitro. It promotes osteogenic differentiation and inhibits adipogenic differentiation of BMSCs by inactivating GSK-3β and suppressing PPARγ expression [102, 109, 110]. In addition, the BMPs (BMP-2, BMP-4, and BMP-7) and the MAPK/ERK pathway are also involved in icaritin-mediated osteogenic differentiation [12, 110].

Taken together, ICA promotes BMSC proliferation and osteoblastic differentiation and inhibits adipogenic differentiation, indicating its potential as a bone regenerative drug.
ICA promotes BMSC osteogenesis via signaling pathways such as RhoA-TAZ, JNK, Wnt/β-catenin, ERα-Wnt/β-catenin, and PI3K/Akt/eNOS/NO/cGMP/PKG. ICA promotes BMSC angiogenesis via PI3K/Akt/eNOS, EGF-EGFR, PI3K signaling, and ERK1/2 signaling pathways. ICA promotes migration of BMSCs through the MAPK signaling pathway.

**Figure 3:** Signaling pathways involved in ICA-mediated BMSC proliferation, osteogenesis, angiogenesis, and migration. ICA promotes proliferation of BMSCs through signaling pathways such as MAPK, ERK, p38, Wnt/β-catenin, RhoA-TAZ, and ERα-Wnt/β-catenin. ICA promotes BMSC osteogenesis via signaling pathways such as RhoA-TAZ, JNK, Wnt/β-catenin, ERα-Wnt/β-catenin, and PI3K/Akt/eNOS/NO/cGMP/PKG. ICA promotes BMSC angiogenesis via PI3K/Akt/eNOS, EGF-EGFR, PI3K, and ERK1/2 signaling pathways. ICA promotes migration of BMSCs through the MAPK signaling pathway.

### 4.2. Signaling Pathways Involved in ICA-Mediated BMSC Proliferation and Osteogenesis

The MAPK pathway consists of the ERK, p38 kinase (p38), and Jun amino-terminal kinases/stress-activated protein kinase (JNK) factors. It regulates essential cellular functions, such as growth, proliferation, differentiation, and apoptosis. In addition, MAPKs also mediate the biological functions of ICA [111], indicating a possible role in BMSC proliferation as well. Qin et al. [112] found that ICA-induced rat BMSC proliferation in vitro was positively correlated with ERK levels and p38 phosphorylation and significantly upregulated Elk1 and c-Myc, the transcription factors downstream of the MAPK pathway.

A study using BMSCs extracted from SD rat bone marrow showed that 0.05-2.0 mg/l ICA significantly facilitated BMSC proliferation by activating the Wnt/β-catenin pathway [113]. Ye et al. [114] found that low doses of ICA (10⁻⁸ M to 10⁻⁶ M) promoted the proliferation and osteoblastic differentiation of rat adipose-derived stem cells (ADSCs), and 10⁻⁷ M ICA significantly upregulated RhoA (ras homolog gene family, member A) and p-MYPT1 (a ROCK or Rho-associated protein kinase substrate). This indicates that ICA promotes ADSC proliferation and osteoblastic differentiation by activating the RhoA-transcriptional coactivator with the PDZ-binding motif (TAZ) signaling pathway. Furthermore, Zhai et al. [115] showed the involvement of the PI3K/Akt/eNOS/NO/cGMP/PKG signaling pathway in the ICA-mediated osteogenesis of BMSCs. As already mentioned, any imbalance between BMSC osteoblastic and adipogenic differentiation impairs bone stability and leads to bone loss and increased bone marrow adipogenesis [116], resulting in osteoporosis and bone necrosis [117, 118]. TAZ is a β-catenin-like transcriptional coactivator involved in modulating this balance [119, 120]. It activates Runx2-mediated transcription to regulate BMSC differentiation and stimulate osteoblastic differentiation and also interacts with PPARγ to suppress adipogenic differentiation. Furthermore, Wei et al. [121] demonstrated that ICA promotes BMSC proliferation and osteogenesis via activation of the estrogen receptor (ERα)-Wnt/β-catenin signaling pathway. There is considerable ambiguity regarding the interaction between TAZ and Wnt/β-catenin. One study [122] indicated an upstream regulatory role of TAZ, while another study [123] showed that TAZ lies downstream of the Wnt/β-catenin cascade. Nevertheless, TAZ is an important regulator of ICA-mediated BMSC osteoblastic differentiation.

Kammerer et al. [124] reported that the ERα signaling pathway transcriptionally regulates Runx2, while Cai et al. [125] showed that the Wnt/β-catenin pathway directly targeted Runx2 to promote osteoblastic differentiation and the calcification of vascular smooth muscle cells. Both studies indicated a close association of the ERα and Wnt/β-catenin signaling pathways with the Runx2 expression. Another study [126] found that ICA stimulated BMSC osteoblastic differentiation by upregulating TAZ and the downstream osteogenic genes, and blocking the aforementioned pathways abrogated ICA-induced TAZ upregulation (Figure 3). These findings point to a TAZ/ERα/Wnt/β-catenin axis that mediates ICA-induced BMSC osteoblastic differentiation. In one study, Wu et al. [104] demonstrated the involvement of the JNK pathway in the osteoblastic differentiation of BMSCs or periodontal ligament stem cells [127, 128].

Multiple signaling pathways, including the BMP, nitric oxide (NO), MAPK, and Wnt/β-catenin pathways, are likely activated in the osteoblasts due to the estrogen-like properties of ICA and ICA-induced estrogen production [90, 129, 130]. Shi et al. [131] showed that ICA promoted osteogenesis in rat cranial osteoblasts and in an in vivo rat model of bone growth by activating the cAMP/PKA/CREB signaling pathway.

### 4.3. ICA Promotes BMSC Migration and Angiogenesis

ICA not only activates endothelial angiogenesis in vitro but also directly stimulates angiogenesis in vivo, through the PI3K/Akt/eNOS-dependent signaling pathways [106]. ICA
can activate the epidermal growth factor-epidermal growth factor receptor (EGF-EGFR) pathway to promote endothelial NOS synthesis, thereby facilitating vascular regeneration [132]. In addition, ICA can directly stimulate angiogenesis by activating various angiogenic factors like ERK, PI3K, and Akt [133, 134]. An in vitro study by Liu et al. [135] showed that ICA upregulated angiogenesis-related genes like vascular endothelial growth factor (VEGF) and fibroblast growth factors (FGF). In addition, ICA upregulated brain-derived neurotrophic factor (BDNF) and VEGF via the PI3K and ERK1/2 signaling pathways [134], which in turn promoted the angiogenic differentiation of BMSCs. Furthermore, Jiao et al. [136] found that ICA enhanced the migratory ability of BMSCs in vitro and in vivo, via the MAPK signaling pathway.

In summary, the primary effects of ICA in BMSCs are to promote proliferation and osteogenesis and are mediated by multiple signaling pathways including the MAPK/ERK/p38, Wnt/β-catenin, PI3K/Akt/eNOS/NO/cGMP/PKG, RhoA-TAZ, and ERα-Wnt/β-catenin pathways. In addition, ICA can also act on osteoblasts through the BMP/Runx2, NO, MAPK, Wnt/β-catenin, cAMP/PKA/CREB, and JNK pathways. Furthermore, ICA promotes angiogenesis via the PI3K, ERK1/2, and EGF-EGFR pathways and BMSC migration via the MAPK pathway. The angiogenic effect of ICA is favorable for osteogenesis, although their exact relationship as well as that between angiogenesis and migration still needs to be elucidated.

5. Prospects

ICA can significantly promote BMSC proliferation and osteoblastic differentiation and inhibit adipogenic differentiation, making it a reliable option for bone regenerative therapy. Mechanistic studies show that multiple signaling pathways mediate these processes, indicating the potential of multiple therapeutic targets. Above all, ICA could be made into a liquid state at suitable concentration in the future and be applied in bone regeneration. Besides, the evidence indicated that the optimal concentration for ICA which can perform better effects in BMSCs is 1 μM [4, 30], while others reported 0.1 μM [114, 121, 126]. However, further studies are needed to figure out both a safe and an effective concentration of ICA [14]. For patients with fractures, bone defects, nonunion disorders, and osteonecrosis of the femoral head, a mixture of ICA and autologous BMSCs can be injected locally into the lesion to facilitate bone regeneration. For patients with osteoporosis, ICA can be delivered through intravenous administration. Since ALP (an early marker of osteogenic differentiation) levels peak on the 14th day of the in vitro BMSC culture with ICA [121, 126, 135], it is reasonable to consider 14 days of intravenous ICA administration for the treatment regimen. ICA also promotes the regeneration of periodontal tissue [137], peripheral nerves [138], neural stem cells [139], and endometrium [140], although the optimal concentration of ICA differs across tissues.

There are still several questions that need to be addressed in future studies. For example, although the pathways involved in BMSC migration are well-known, the mechanism(s) underlying ICA-mediated BMSC migration remain to be elucidated. At present, it is not clear whether there is a synergistic or antagonistic crosstalk or upstream and downstream relationship among the signaling pathways involved in ICA-mediated osteogenic differentiation of BMSCs. Most studies on BMSC osteogenesis and migration have been carried out under normoxic conditions. The oxygen levels in ischemic lesions, such as in femoral head necrosis, can be less than 1% [141], and severe hypoxia affects the osteogenic differentiation and migration of BMSCs in vivo and in vitro [142, 143]. Therefore, it is necessary to simulate the hypoxic conditions in the in vitro studies.

In conclusion, a better understanding of the role and mechanism of action of ICA in BMSCs can provide new therapeutic strategies for various orthopedic disorders such as osteoporosis, femoral head necrosis, and bone defects.

Abbreviations

| BMSCs: | Bone marrow mesenchymal stem cells |
| ICA: | Icaritin |
| IL-6: | Interleukin-6 |
| TNF-1: | Tumor necrosis factor |
| PPARγ: | Peroxisomal proliferator-activated receptor gamma |
| Runx2: | Runt-related transcription factor 2 |
| BMP-2: | Bone morphogenetic protein-2 |
| ERK-MAPK: | Extracellular signal-regulated kinase-mitogen-activated protein kinase |
| ALP: | Alkaline phosphatase |
| OPG/RANKL: | Osteoprotegerin/receptor activator of nuclear factor-kb ligand |
| C/EBPα: | CCAAT/enhancer-binding protein α |
| FABP4: | Fatty acid-binding protein 4 |
| PDGF: | Platelet-derived growth factor |
| CXCL12: | C-X-C motif chemokine ligand 12 |
| CCR4: | C-X-C chemokine receptor type 4 |
| SDF-1: | Stromal-derived factor 1 |
| Smad: | Drosophila mothers against decapentaplegic protein |
| Twist1: | Twist-related protein 1, also known as class A basic helix-loop-helix protein 38 (bHLHa38) |
| Atf4: | Activating transcription factor 4 |
| TGF-β/BMP: | Transforming growth factor-β/bone morphogenetic protein |
| PI3K/Akt/GSK-3: | Phosphatidylinositol 3-kinase-α |
| ERK: | Extracellular signal-regulated kinase |
| IGF1: | Insulin-like growth factor 1 |
| Osx: | Osterix |
| EGFR-EGFR: | Epidermal growth factor-epidermal growth factor receptor |
| NO: | Nitric oxide |
| VEGF: | Vascular endothelial growth factor |
| FGFR: | Fibroblast growth factors |
| MAPK: | Mitogen-activated protein kinase |
p38: p38 kinase
JNK: Jun amino-terminal kinases/stress-activated protein kinase
RhoA: Ras homolog gene family, member A
p-MYPT1: A ROCK (Rho-associated protein kinase) substrate molecule
ERα: Estrogen receptor α
PI3K/Akt/eNOS/NO/cGMP/PKG: Phosphatidylinositol 3-kinase/protein kinase B/endothelial nitric oxide synthase/nitric oxide/cyclic guanosine monophosphate/protein kinase-G

cAMP/PKA/CREB: cAMP/protein kinase A/cAMP response element-binding protein
GSK-3: Glycogen synthase kinase-3
ABCB1: ATP-binding cassette subfamily B member 1
TAZ: Transcriptional coactivator with PDZ-binding motif
BDNF: Brain-derived neurotrophic factor.

Disclosure
The funders had no role in the decision to publish or in the preparation of the manuscript.

Conflicts of Interest
The authors declare that they have no competing interests.

Authors’ Contributions
Aofei Yang and Chaochao Yu contributed equally to this manuscript. Qilin Lu and Hao Li helped illustrate the figures. Zhanghua Li and Chengjian He contributed to the conception and helped revise the manuscript.

Acknowledgments
This study was supported by the 6th National Famous and Experienced Chinese Medicine Experts’ Academic Inheritance Project funded by the Chinese Government’s State Administration of Traditional Chinese Medicine (No. 292017), the National Natural Science Foundation of China (No. 81472103), the “HuangheYingcai” Project of Wuhan City of China, and the Health and Family Planning Commission of Wuhan Municipality Research Foundation (WX18M01).

References
[1] A. D. Algarni and H. M. Al Moallem, “Clinical and radiological outcomes of extracorporeal shock wave therapy in early-stage femoral head osteonecrosis,” Advances in Orthopedics, vol. 2018, Article ID 7410246, 6 pages, 2018.
[2] H. Cao, Y. Zhang, W. Qian et al., “Effect of icariin on fracture healing in an ovariectomized rat model of osteoporosis,” Experimental and Therapeutic Medicine, vol. 13, no. 5, pp. 2399–2404, 2017.
[3] Z. H. Dailiana, N. Stefanou, L. Khalidi et al., “Vascular endothelial growth factor for the treatment of femoral head osteonecrosis: an experimental study in canines,” World Journal of Orthopedics, vol. 9, no. 9, pp. 120–129, 2018.
[4] Z. Huang, C. Cheng, B. Cao et al., “Icariin protects against glucocorticoid-induced osteonecrosis of the femoral head in rats,” Cellular Physiology and Biochemistry, vol. 47, no. 2, pp. 694–706, 2018.
[5] Z. Luo, M. Liu, L. Sun, and F. Rui, “Icariin recovers the osteogenic differentiation and bone formation of bone marrow stromal cells from a rat model of estrogen deficiency-induced osteoporosis,” Molecular Medicine Reports, vol. 1, no. 1, pp. 382–388, 2015.
[6] J. Moya-Angeler, A. L. Gianakos, J. C. Villa, A. Ni, and J. M. Lane, “Current concepts on osteonecrosis of the femoral head,” World Journal of Orthopedics, vol. 6, no. 8, pp. 590–601, 2015.
[7] A. Sanghani-Kerai, D. McCreary, H. Lancashire, L. Osagie, M. Coathup, and G. Blunn, “Stem cell interventions for bone healing: fractures and osteoporosis,” Current Stem Cell Research & Therapy, vol. 13, no. 5, pp. 369–377, 2018.
[8] Y. Z. Jin and J. H. Lee, “Mesenchymal stem cell therapy for bone regeneration,” Clinics in Orthopedic Surgery, vol. 10, no. 3, pp. 271–278, 2018.
[9] F. Song, D. Jiang, T. Wang et al., “Mechanical stress regulates osteogenesis and adipogenesis of rat mesenchymal stem cells through PI3K/Akt/GSK-3β/β-catenin signaling pathway,” BioMed Research International, vol. 2017, Article ID 6027402, 10 pages, 2017.
[10] S. Heydari Asl, H. Hosseinpoor, K. Parivar, N. Hayati Roodbari, and H. Hanaceh-Ahvaz, “Physical stimulation and scaffold composition efficiently support osteogenic differentiation of mesenchymal stem cells,” Tissue & Cell, vol. 50, pp. 1–7, 2018.
[11] Z. Yan, Y. Guo, Y. Wang, Y. Li, and J. Wang, “MicroRNA profiles of BMSCs induced into osteoblasts with osteoinductive medium,” Experimental and Therapeutic Medicine, vol. 15, no. 3, pp. 2589–2596, 2018.
[12] T. Wu, T. Shu, L. Kang et al., “Icariin, a novel plant-derived osteoinductive agent, enhances the osteogenic differentiation of human bone marrow- and human adipose tissue-derived mesenchymal stem cells,” International Journal of Molecular Medicine, vol. 39, no. 4, pp. 984–992, 2017.
[13] A. X. Lin, G. Chan, Y. Hu et al., “Internationalization of traditional Chinese medicine: current international market, internationalization challenges and prospective suggestions,” Chinese Medicine, vol. 13, no. 1, p. 9, 2018.
[14] Z. Wang, D. Wang, D. Yang, W. Zhen, J. Zhang, and S. Peng, “The effect of icarin on bone metabolism and its potential clinical application,” Osteoporosis International, vol. 29, no. 3, pp. 535–544, 2018.
[15] Y. Liu, H. Zuo, X. Liu, J. Xiong, and X. Pei, “The antosteoporosis effect of icariin in ovariectomized rats: a systematic review and meta-analysis,” Cellular and Molecular Biology, vol. 63, no. 11, pp. 124–131, 2017.
[16] H. Huang, M. Liang, X. Zhang, C. Zhang, Z. Shen, and W. Zhang, “Simultaneous determination of nine flavonoids and qualitative evaluation of Herba Epimedii by high performance liquid chromatography with ultraviolet detection,” Journal of Separation Science, vol. 30, no. 18, pp. 3207–3213, 2007.
[17] Z. Zhou, J. Luo, J. Wang, L. Li, and L. Kong, “Simultaneous enrichment and separation of flavonoids from Herba Epimedi- dii by macroporous resins coupled with preparative chromatographic method,” Natural Product Research, vol. 29, no. 2, pp. 185–188, 2015.

[18] M. Chen, J. Wu, Q. Luo et al., “The anticancer properties of Herba Epimedi and its main bioactive componentisecaritin and icariside II,” Nutrients, vol. 8, no. 9, 2016.

[19] N. Wang, X. Huang, X. Wang, Y. Zhang, R. Wu, and D. Shou, “Pipette tip solid-phase extraction and high-performance liq- uid chromatography for the determination of flavonoids from Epimedi herba in rat serum and application of the tech- nique to pharmacokinetic studies,” Journal of Chromatogra- phy B, vol. 990, pp. 64–72, 2015.

[20] X. Sun, Q. Li, J. Zhang et al., “The reason leading to the increase of icariin in Herba Epimedi by heating process,” Journal of Pharmaceutical and Biomedical Analysis, vol. 149, pp. 525–531, 2018.

[21] H. F. Zhang, T. S. Yang, Z. Z. Li, and Y. Wang, “Simultaneous extraction of epimedin A, B, C and icarin from Herba Epi- medi by ultrasonic technique,” Ultrasonics Sonochemistry, vol. 15, no. 4, pp. 376–385, 2008.

[22] N. Wang, H. Xin, Q. Zhang et al., “Carbon nanotube-polymer composite for effervescent pipette tip solid phase microex- traction of alkaloids and flavonoids from Epimedi herba in biological samples,” Talanta, vol. 162, pp. 10–18, 2017.

[23] X. J. Chen, H. Ji, Q. W. Zhang et al., “A rapid method for simultaneous determination of 15 flavonoids in Epimedium using pressurized liquid extraction and ultra-performance liquid chromatography,” Journal of Pharmaceutical and Biomedical Analysis, vol. 46, no. 2, pp. 226–235, 2008.

[24] H. B. Li and F. Chen, “Separation and purification of epime- din A, B, C, and icarin from the medicinal herb Epimedium brevicornum Maxim by dual-mode HSCCC,” Journal of Chromatographic Science, vol. 47, no. 5, pp. 337–340, 2009.

[25] F. Xie, C. F. Wu, W. P. Lai et al., “The osteoprotective effect of Herba Epimedi (HEP) extract in vivo and in vitro,” Evidence-Based Complementary and Alternative Medicine, vol. 2, no. 3, 361 pages, 2005.

[26] L. Wang, Y. Li, Y. Guo et al., “Herba Epimedi: an ancient Chinese herbal medicine in the prevention and treatment of osteoporosis,” Current Pharmaceutical Design, vol. 22, no. 3, pp. 328–349, 2016.

[27] G. Zhang, L. Qin, and Y. Shi, "Epimedium-derived phytoes- tenogen flavonoids exert beneficial effect on preventing bone loss in late postmenopausal women: a 24-month randomized, double-blind and placebo-controlled trial,” Journal of Bone and Mineral Research, vol. 22, no. 7, pp. 1072–1079, 2007.

[28] M. Lu, L. H. Wang, Y. W. Luo et al., “Treatment of primary osteoporosis with epimedium total flavone capsule: a multi- center clinical observation on 360 cases,” Chinese Journal of Osteoporosis, vol. 19, no. 3, pp. 279–282, 2013.

[29] Z. B. Sun, J. W. Wang, H. Xiao et al., “Icariin may benefit the mesenchymal stem cells of patients with steroid-associated osteonecrosis by ABCB1-promoter demethylation: a prelimi- nary study,” Osteoporosis International, vol. 26, no. 1, pp. 187–197, 2015.

[30] J. J. Fan, L. G. Cao, T. Wu et al., “The dose-effect of icariin on the proliferation and osteogenic differentiation of human bone mesenchymal stem cells,” Molecules, vol. 16, no. 12, pp. 10123–10133, 2011.

[31] P. Sreejit, K. B. Dilip, and R. S. Verma, “Generation of mesenchymal stem cell lines from murine bone marrow,” Cell and Tissue Research, vol. 350, no. 1, pp. 55–68, 2012.

[32] C. J. Li, Y. Xiao, M. Yang et al., “Long noncoding RNA Bmncr regulates mesenchymal stem cell fate during skeletal aging,” The Journal of Clinical Investigation, vol. 128, no. 12, pp. 5251–5266, 2018.

[33] C. J. Li, P. Cheng, M. K. Liang et al., “MicroRNA-188 regula- tes age-related switch between osteoblast and adipocyte differ- entiation,” The Journal of Clinical Investigation, vol. 125, no. 4, pp. 1509–1522, 2015.

[34] W. Zhang, N. Liu, H. Shi et al., “Upregulation of BMSCs osteo- genesis by positively-charged tertiary amines on polymeric implants via charge/iNOS signaling pathway,” Scientific Reports, vol. 5, no. 1, article 9369, 2015.

[35] J. F. Blanco, J. García-Brion, L. Benito-Garzón, D. Pescador, S. Muntián, and F. Sánchez-Guijo, “Human bone marrow mesenchymal stromal cells promote bone regeneration in a xenogeneic rabbit model: a preclinical study,” Stem Cells International, vol. 2018, Article ID 7089484, 10 pages, 2018.

[36] M. Capulli, R. Paone, and N. Rucci, “Osteoblast and osteo- cyte: games without frontiers,” Archives of Biochemistry and Biophysics, vol. 561, pp. 3–12, 2014.

[37] T. A. Franz-Odendaal, R. K. Hall, and P. E. Witten, “Buried alive: how osteoblasts become osteocytes,” Developmental Dynamics, vol. 235, no. 1, pp. 176–190, 2006.

[38] R. Florencio-Silva, G. R. Sasso, E. Sasso-Cerri, M. J. Simões, and P. S. Cerri, "Biology of bone tissue: structure, function, and factors that influence bone cells," BioMed Research International, vol. 2015, Article ID 421746, 17 pages, 2015.

[39] S. Huang, P. P. Eleniste, K. Wayakanon et al., “The Rho-GEF Kalirin regulates bone mass and the function of osteoblasts and osteoclasts,” Bone, vol. 60, pp. 235–245, 2014.

[40] Q. Wu, X. Zhou, D. Huang, Y. Ji, and F. Kang, “IL-6 enhances osteocyte-mediated osteoclastogenesis by promoting JAK2 and RANKL activity in vitro,” Cellular Physiology and Biochemistry, vol. 41, no. 4, pp. 1360–1369, 2017.

[41] J. Teramachi, H. Zhou, M. A. Subler et al., “Increased IL-6 expression in osteoclasts is necessary but not sufficient for the development of Paget’s disease of bone,” Journal of Bone and Mineral Research, vol. 29, no. 6, pp. 1456–1465, 2014.

[42] W. Y. Cheung, C. A. Simmons, and L. You, “Osteocyte apop- tosis regulates osteoclast precursor adhesion via osteocytic IL-6 secretion and endothelial ICAM-1 expression,” Bone, vol. 50, no. 1, pp. 104–110, 2012.

[43] W. Feng, H. Liu, T. Luo et al., “Combination of IL-6 and sIL-6R differentially regulate varying levels of RANKL-induced osteoclastogenesis through NF-κB, ERK and JNK signaling pathways,” Scientific Reports, vol. 7, no. 1, article 41411, 2017.

[44] M. Bustamante, X. Nogués, A. Enjuanes et al., “COL1A1, ESR1, VDR and TGFB1 polymorphisms and haplotypes in relation to BMD in Spanish postmenopausal women,” Osteoporosis International, vol. 18, no. 2, pp. 235–243, 2007.

[45] A. E. Grigoriadis, J. N. Heersche, and J. E. Aubin, “Differentiation of muscle, fat, cartilage, and bone from progenitor cells present in a bone-derived clonal cell population: effect of dexamethasone,” The Journal of Cell Biology, vol. 106, no. 6, pp. 2139–2151, 1988.
[64] P. Ducy, R. Zhang, V. Geoffroy, A. L. Ridall, and G. Karsenty, "Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation," Cell, vol. 89, no. 5, pp. 747–754, 1997.

[65] S. P. Wong, J. E. Rowley, A. N. Redpath, J. D. Tilman, T. G. Fellous, and J. R. Johnson, "Pericytes, mesenchymal stem cells and their contributions to tissue repair," Pharmacology & Therapeutics, vol. 151, pp. 107–120, 2015.

[66] M. K. Majumdar, M. A. Thiede, J. D. Mosca, M. Moorman, C. Lu, S. Gao, and G. Xu, "Multilineage potential of cultures of marrow-derived mesenchymal stem cells (MSCs) and stromal cells," Journal of Cellular Physiology, vol. 176, no. 1, pp. 57–66, 1998.

[67] T. Matsumoto, K. Kano, D. Perrier, A. L. Ridall, and G. Karsenty, "Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation," Cell, vol. 89, no. 5, pp. 747–754, 1997.

[68] S. Kokabu, J. W. Lowery, and E. Jimi, "Cell fate and differentiation of bone marrow mesenchymal stem cells," Stem Cells International, vol. 2016, Article ID 3753581, 7 pages, 2016.

[69] A. Yang, C. Yu, F. You, C. He, and Z. Li, "Mechanisms of Zuoqiu pill in treating osteoporosis: perspective from bone marrow mesenchymal stem cells," Evidence-Based Complementary and Alternative Medicine, vol. 2018, Article ID 3717391, 8 pages, 2018.

[70] J. Justesen, K. Stenderup, E. N. Ebbesen, L. Mosekilde, T. Steiniche, and M. Kassem, "Adipocyte tissue volume in bone marrow is increased with aging and in patients with osteoporosis," Biogerontology, vol. 2, no. 3, pp. 165–171, 2001.

[71] M. Miller, J. D. Shuman, T. Sebastian, Z. Dauter, and P. F. Johnson, "Structural basis for DNA recognition by the basic region leucine zipper transcription factor CCAAT enhancer-binding protein α," Journal of Biological Chemistry, vol. 278, no. 17, pp. 15178–15184, 2003.

[72] P. Tontonoz, E. Hu, and B. M. Spiegelman, "Stimulation of adipogenesis in fibroblasts by PPARγ2, a lipid-activated transcription factor," Cell, vol. 79, no. 7, pp. 1147–1156, 1994.

[73] S. Peng, G. Zhou, K. D. K. Luk et al., "Strontium promotes osteogenic differentiation of mesenchymal stem cells through the Ras/PAK signaling pathway," Cellular Physiology and Biochemistry, vol. 23, no. 1-3, pp. 165–174, 2009.

[74] A. W. James, "Review of signaling pathways governing MSC osteogenic and adipogenic differentiation," Scientifica, vol. 2013, Article ID 684736, 17 pages, 2013.

[75] M. T. Valenti, U. Garbin, A. Pasini et al., "Role of ox-PAPCs in the differentiation of mesenchymal stem cells (MSCs) and Runx2 and PPARγ2 expression in MSCs-like of osteoporotic patients," PLoS One, vol. 6, no. 6, article e20363, 2011.

[76] X. Li, Q. Cui, C. Kao, G. J. Wang, and G. Balian, "Lovastatin inhibits adipogenic and stimulates osteogenic differentiation by suppressing PPARγ2 and increasing Cbfα1/Runx2 expression in bone marrow mesenchymal cell cultures," Bone, vol. 33, no. 4, pp. 652–659, 2003.

[77] Y. Mei, C. Bian, J. Li et al., "miR-21 modulates the ERK-MAPK signaling pathway by regulating SPRY2 expression during human mesenchymal stem cell differentiation," Journal of Cellular Biochemistry, vol. 114, no. 6, pp. 1374–1384, 2013.

[78] L. A. Goff, S. Boucher, C. L. Ricupero et al., "Differentiating human multipotent mesenchymal stromal cells regulate microRNAs: prediction of microRNA regulation by PDGF during osteogenesis," Experimental Hematology, vol. 36, no. 10, pp. 1354–1369.e2, 2008.

[79] J. Cao, Y. Wei, J. Lian et al., "Notch signaling pathway promotes osteogenic differentiation of mesenchymal stem cells by enhancing BMP9/Smad signaling," International Journal of Molecular Medicine, vol. 40, no. 2, pp. 378–388, 2017.

[80] X. Li, L. Guo, Y. Liu et al., "microRNA-21 promotes osteogenesis of bone marrow mesenchymal stem cells via the Smad7-Smad1/5/8-Runx2 pathway," Biochemical and Biophysical Research Communications, vol. 493, no. 2, pp. 928–933, 2017.
[74] H. Long, B. Sun, L. Cheng et al., “miR-139-5p represses BMSC osteogenesis via targeting Wnt/β-catenin signaling pathway,” DNA and Cell Biology, vol. 36, no. 8, pp. 715–724, 2017.

[75] B. Sen, C. Guilluy, Z. Xie et al., “Mechanically induced focal adhesion assembly amplifies anti-adipogenic pathways in mesenchymal stem cells,” Stem Cells, vol. 29, no. 11, pp. 1829–1836, 2011.

[76] C. G. T. Tahimic, R. K. Long, T. Kubota et al., “Regulation of ligand and shear stress-induced insulin-like growth factor 1 (IGF1) signaling by the integrin pathway,” Journal of Biological Chemistry, vol. 291, no. 15, pp. 8140–8149, 2016.

[77] P. Su, Y. Tian, C. Yang et al., “Mesenchymal stem cell migration during bone formation and bone diseases therapy,” International Journal of Molecular Sciences, vol. 19, no. 8, article 2343, 2018.

[78] Z. Li, W. Wang, H. Xu et al., “Effects of altered CXCL12/CXCR4 axis on BMP2/Smad/Runx2/Osterix axis and osteogenic genes expression during osteogenic differentiation of MSCs,” American Journal of Translational Research, vol. 9, no. 4, pp. 1680–1693, 2017.

[79] S. J. Zhang, X. Y. Song, M. He, and S. B. Yu, “Icaritin from Epimedium brevicornum Maxim promotes the biosynthesis of estrogen by aromatase (CYP19),” Journal of Ethnopharmacology, vol. 145, no. 3, pp. 715–721, 2013.

[80] A. Sanghani, L. Osagie-Clouard, S. Samizadeh et al., “Protective effects of icariin on brain dysfunction induced by lipopolysaccharide in rats,” Phytomedicine, vol. 17, no. 12, pp. 950–955, 2010.

[81] S. C. W. Sze, Y. Tong, T. B. Ng, C. L. Y. Cheng, and H. P. Cheung, “Herba Epimedii: anti-oxidative properties and its medical implications,” Molecules, vol. 15, no. 11, pp. 7861–7870, 2010.

[82] X. N. Ma, B. F. Ge, K. M. Chen et al., “Antiosteoporotic chemical constituents from Er-Xian decoction, a traditional Chinese herbal formula,” Journal of Ethnopharmacology, vol. 118, no. 2, pp. 271–279, 2008.

[83] H. Y. Ye and Y. J. Lou, “Estrogenic effects of two derivatives of icariin on human breast cancer MCF-7 cells,” Phytotherapy Research, vol. 12, no. 10, pp. 735–741, 2005.

[84] J. Zhao, S. Ohba, M. Shinkai, U. I. Chung, and T. Nagamune, “Icaritin induces osteogenic differentiation in vitro in a BMP- and Runx2-dependent manner,” Biochemical and Biophysical Research Communications, vol. 369, no. 2, pp. 444–448, 2008.

[85] X. X. Yin, Z. Q. Chen, Z. J. Liu, Q. J. Ma, and G. T. Dang, “Icaritine stimulates proliferation and differentiation of human osteoblasts by increasing production of bone morphogenetic protein 2,” Chinese Medical Journal, vol. 120, no. 3, pp. 204–210, 2007.

[86] X. N. Ma, B. F. Ge, K. M. Chen et al., “Mechanisms of icariin in regulating bone formation of osteoblasts and bone resorption of osteoclasts,” Zhongguo Yi Xue Ke Xue Yuan Xue Bao, vol. 35, no. 4, pp. 432–438, 2013.

[87] S. Zhang, P. Feng, G. Mo et al., “Icaritin influences adiogenic differentiation of stem cells affected by osteoblast-osteoclast co-culture and clinical research adipogenic,” Biomedicine & Pharmacotherapy, vol. 88, pp. 436–442, 2017.

[88] Y. Tang, X. Wu, W. Lei et al., “TGF-β1–induced migration of bone mesenchymal stem cells couples bone resorption with formation,” Nature Medicine, vol. 15, no. 7, pp. 757–765, 2009.

[89] Z. Wang, X. Zhang, H. Wang, L. Qi, and Y. Lou, “Neuroprotective effects of icarin against beta amyloid-induced neurotoxicity in primary cultured rat neuronal cells via estrogen-dependent pathway,” Neuroscience, vol. 145, no. 3, pp. 911–922, 2007.

[90] L. Yang, D. Lu, J. Guo, X. Meng, G. Zhang, and F. Wang, “Icaritin from Epimedium brevicornum Maxim promotes the biosynthesis of estrogen by aromatase (CYP19),” Journal of Ethnopharmacology, vol. 145, no. 3, pp. 715–721, 2013.

[91] S. Li, P. Dong, J. Wang et al., “Icaritin, a natural flavonol glycoside, induces apoptosis in human hepatoma SMMC-7721 cells via a ROS/JNK-dependent mitochondrial pathway,” Cancer Letters, vol. 298, no. 2, pp. 222–230, 2010.

[92] J. Zhou, J. Wu, X. Chen et al., “Icaritin and its derivative, ICT, exert anti-inflammatory, anti-tumor effects, and modulate myeloid derived suppressive cells (MDSCs) functions,” International Immunopharmacology, vol. 11, no. 7, pp. 890–898, 2011.

[93] J. Guo, F. Li, Q. Wu, Q. Gong, Y. Lu, and J. Shi, “Protective effects of icariin on brain dysfunction induced by lipopolysaccharide in rats,” Phytomedicine, vol. 17, no. 12, pp. 950–955, 2010.

[94] S. C. W. Sze, Y. Tong, T. B. Ng, C. L. Y. Cheng, and H. P. Cheung, “Herba Epimedii: anti-oxidative properties and its medical implications,” Molecules, vol. 15, no. 11, pp. 7861–7870, 2010.

[95] A. Abuelmula, R. Huang, Q. Pu, H. Tamamura, G. Morosan-Puopolo, and B. Brand-Saberi, “SDF-1 controls the muscle and blood vessel formation of the somite,” The International Journal of Developmental Biology, vol. 60, no. 1–2–3, pp. 29–38, 2016.

[96] J. F. Zhang, W. M. Fu, M. L. He et al., “MiRNA-20a promotes osteogenic differentiation of human mesenchymal stem cells by co-regulating BMP signaling,” RNA Biology, vol. 8, no. 5, pp. 829–838, 2011.

[97] A. Sanghani, L. Osagie-Clouard, S. Samizadeh et al., “CXCR4 has the potential to enhance bone formation in osteogenic rats,” Tissue Engineering. Part A, vol. 24, no. 23–24, pp. 1775–1783, 2018.

[98] H. B. Tan, P. V. Giannoudis, S. A. Boxall, D. McGonagle, and E. Jones, “The systemic influence of platelet-derived growth factors on bone marrow mesenchymal stem cells in fracture patients,” BMC Medicine, vol. 13, no. 1, p. 6, 2015.

[99] A. Li, X. Xia, J. Yeh et al., “PDGF-AA promotes osteogenic differentiation and migration of mesenchymal stem cell by down-regulating PDGFRα and derepressing BMP-Smad1/5/8 signaling,” PLoS One, vol. 9, no. 12, article e113785, 2014.

[100] A. Sanghani-Kerai, L. Osagie-Clouard, G. Blunn, and M. Coathup, “The influence of age and osteoporosis on bone marrow stem cells from rats,” Bone & Joint Research, vol. 7, no. 4, pp. 289–297, 2018.

[101] A. Sanghani-Kerai, M. Coathup, S. Samazidih et al., “Osteoporosis and ageing affects the migration of stem cells and this is ameliorated by transfection with CXCR4,” Bone & Joint Research, vol. 6, no. 6, pp. 358–365, 2017.

[102] C. Y. Ho, A. Sanghani, J. Hua, M. Coathup, P. Kalia, and G. Blunn, “Mesenchymal stem cells with increased stromal cell-derived factor 1 expression enhanced fracture healing,” Tissue Engineering Part A, vol. 21, no. 3–4, pp. 594–602, 2015.
in ovariectomized rats,” Experimental and Therapeutic Medicine, vol. 13, no. 4, pp. 1360–1368, 2017.

[103] D. Zheng, S. Peng, S. H. Yang et al., “The beneficial effect of icariin on bone is diminished in osteoprogerin-deficient mice,” Bone, vol. 51, no. 1, pp. 85–92, 2012.

[104] Y. Wu, L. Xia, Y. Zhou, Y. Xu, and X. Jiang, “Icariin induces osteogenic differentiation of bone mesenchymal stem cells in a MAPK-dependent manner,” Cell Proliferation, vol. 48, no. 3, pp. 375–384, 2015.

[105] J. Fan, L. Bi, T. Wu et al., “A combined chitosan/nano-size hydroxyapatite system for the controlled release of icariin,” Journal of Materials Science. Materials in Medicine, vol. 23, no. 2, pp. 399–407, 2012.

[106] B. H. Chung, J. D. Kim, C. K. Kim et al., “Icariin stimulates angiogenesis by activating the MEK/ERK- and PI3K/Akt/eNOS-dependent signal pathways in human endothelial cells,” Biochemical and Biophysical Research Communications, vol. 376, no. 2, pp. 404–408, 2008.

[107] E. Wernike, M. O. Montjovent, Y. Liu et al., “VEGF incorporated into calcium phosphate ceramics promotes vascularization and bone formation in vivo,” European Cells & Materials, vol. 19, pp. 30–40, 2010.

[108] R. Z. L. Lim, L. Li, E. L. Yong, and N. Chew, “STAT-3 regulation of CXC4R is necessary for the prenyllavonoid Icariin to enhance mesenchymal stem cell proliferation, migration and osteogenic differentiation,” Biochimica et Biophysica Acta (BBA) - General Subjects, vol. 1862, no. 7, pp. 1680–1692, 2018.

[109] H. Sheng, X. F. Rui, C. J. Sheng et al., “A novel semisynthetic molecule icariin stimulates osteogenic differentiation and inhibits adipogenesis of mesenchymal stem cells,” International Journal of Medical Sciences, vol. 10, no. 6, pp. 782–789, 2013.

[110] G. Luo, B. Xu, W. Wang, Y. Wu, and M. Li, “Study of the osteogenesis effect of icariside II and icariin on canine bone marrow mesenchymal stem cells,” Journal of Bone and Mineral Metabolism, vol. 36, no. 6, pp. 668–678, 2018.

[111] L. Ding, X. G. Liang, D. Y. Zhu, and Y. J. Lou, “Icariin promotes expression of PGC-1α, PPARα, and NRF-1 during cardiomyocyte differentiation of murine embryonic stem cells in vitro,” Acta Pharmacologica Sinica, vol. 28, no. 10, pp. 1541–1549, 2007.

[112] S. Qin, W. Zhou, S. Liu, P. Chen, and H. Wu, “Icariin stimulates the proliferation of rat bone mesenchymal stem cells via ERK and p38 MAPK signaling,” International Journal of Clinical and Experimental Medicine, vol. 8, no. 5, pp. 7125–7133, 2015.

[113] S. Fu, L. Yang, H. Hong, and R. Zhang, “Wnt/β-catenin signaling is involved in the icariin induced proliferation of bone marrow mesenchymal stem cells,” Journal of Traditional Chinese Medicine, vol. 36, no. 3, pp. 360–368, 2016.

[114] Y. Ye, X. Jing, N. Li, Y. Wu, B. Li, and T. Xu, “Icariin promotes proliferation and osteogenic differentiation of rat adipose-derived stem cells by activating the RhoA-TAZ signaling pathway,” Biomedicine & Pharmacotherapy, vol. 88, pp. 384–394, 2017.

[115] Y. K. Zhai, X. Y. Guo, B. F. Ge et al., “Icariin stimulates the osteogenic differentiation of rat bone marrow stromal cells via activating the PI3K-AKT-eNOS-NO-cGMP-PKG,” Bone, vol. 66, pp. 189–198, 2014.

[116] P. Xue, X. Wu, L. Zhou et al., “IGF1 promotes osteogenic differentiation of mesenchymal stem cells derived from rat bone marrow by increasing TAZ expression,” Biochemical and Biophysical Research Communications, vol. 433, no. 2, pp. 226–231, 2013.

[117] P. Astudillo, S. Rios, L. Pastenes, A. M. Pino, and J. P. Rodriguez, “Increased adipogenesis of osteoporotic human-mesenchymal stem cells (MSCs) characterizes by impaired leptin action,” Journal of Cellular Biochemistry, vol. 103, no. 4, pp. 1054–1065, 2008.

[118] K. T. Suh, S. W. Kim, H. L. Roh, M. S. Youn, and J. S. Jung, “Decreased osteogenic differentiation of mesenchymal stem cells in alcohol-induced osteonecrosis,” Clinical Orthopaedics and Related Research, no. 431, pp. 220–225, 2005.

[119] M. R. Byun, A. R. Kim, J. H. Hwang et al., “Phorbaketal A stimulates osteoblast differentiation through TAZ mediated Runx2 activation,” FEBS Letters, vol. 586, no. 8, pp. 1086–1092, 2012.

[120] M. R. Byun, C. H. Lee, J. H. Hwang et al., “Phorbaketal A inhibits adipogenic differentiation through the suppression of PPARγ-mediated gene transcription by TAZ,” European Journal of Pharmacology, vol. 718, no. 1-3, pp. 181–187, 2013.

[121] Q. Wei, J. Zhang, G. J. Hong et al., “Icariin promotes osteogenic differentiation of rat bone marrow stromal cells by activating the ERα-Wnt/β-catenin signaling pathway,” Biomedicine & Pharmacotherapy, vol. 84, pp. 931–939, 2016.

[122] X. Varelas, B. W. Miller, R. Sopko et al., “The Hippo pathway regulates Wnt/β-catenin signaling,” Developmental Cell, vol. 18, no. 4, pp. 579–591, 2010.

[123] L. Azzolin, F. Zanconato, S. Bresolin et al., “Role of TAZ as mediator of Wnt signaling,” Cell, vol. 151, no. 7, pp. 1443–1456, 2012.

[124] M. Kammerer, S. Gutzwiller, D. Stauffer, I. Delhon, Y. Seltenmeyer, and B. Fournier, “Estrogen receptor a (ERα) and estrogen related receptor a (ERRα) are both transcriptional regulators of the Runx2-1 isoform,” Molecular and Cellular Endocrinology, vol. 369, no. 1-2, pp. 150–160, 2013.

[125] T. Cai, D. Sun, Y. Duan et al., “WNT/β-catenin signaling promotes VSMCs to osteogenic transdifferentiation and calcification through directly modulating Runx2 gene expression,” Experimental Cell Research, vol. 345, no. 2, pp. 206–217, 2016.

[126] Q. Wei, M. He, M. Chen et al., “Icariin stimulates osteogenic differentiation of rat bone marrow stromal stem cells by increasing TAZ expression,” Biomedicine & Pharmacotherapy, vol. 91, pp. 581–589, 2017.

[127] Y. Wang, J. Li, W. Song, and J. Yu, “Mineral trioxide aggregate upregulates odonto/osteogenic capacity of bone marrow stromal cells from craniofacial bones via JNK and ERK MAPK signalling pathways,” Cell Proliferation, vol. 47, no. 3, pp. 241–248, 2014.

[128] Y. Yu, J. Mu, Z. Fan et al., “Insulin-like growth factor 1 enhances the proliferation and osteogenic differentiation of human periodontal ligament stem cells via ERK and JNK MAPK pathways,” Histochemistry and Cell Biology, vol. 137, no. 4, pp. 513–525, 2012.

[129] Z. B. Zhang and Q. T. Yang, “The testosterone mimetic properties of icarin,” Asian Journal of Andrology, vol. 8, no. 5, pp. 601–605, 2006.

[130] J. Liu, H. Ye, and Y. Lou, “Determination of rat urinary metabolites of icariin in vivo and estrogenic activities of its metabolites on MCF-7 cells,” Die Pharmazie, vol. 60, no. 2, pp. 120–125, 2005.
