Biomedicine & Pharmacotherapy 162 (2023) 114356

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Review

Enhancers of mesenchymal stem cell stemness and therapeutic potency

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ARTICLE INFO

Keywords:
Mesenchymal stem cell
Stemness
Metformin
Resveratrol
Antioxidants
mTOR Inhibitors
Cytokines
Growth factors
Hormones
Vitamins

ABSTRACT

Mesenchymal stem cells (MSCs) are multipotent stromal cells that can differentiate into a range of cell types, including osteoblasts, chondrocytes, myocytes, and adipocytes. Multiple preclinical investigations and clinical trials employed enhanced MSCs-dependent therapies in treatment of inflammatory and degenerative diseases. They have demonstrated considerable and prospective therapeutic potentials even though the large-scale use remains a problem. Several strategies have been used to improve the therapeutic potency of MSCs in cellular therapy. Treatment of MSCs utilizing pharmaceutical compounds, cytokines, growth factors, hormones, and vitamins have shown potential outcomes in boosting MSCs' stemness. In this study, we reviewed the current advances in enhancing techniques that attempt to promote MSCs' therapeutic effectiveness in cellular therapy and stemness in vivo with potential mechanisms and applications.

1. Introduction

Mesenchymal stem cells (MSCs) are mesodermal progenitors that can be isolated from all vascularized tissues. Many human tissues, such as bone marrow [1], adipose tissue [2], dental pulp [3], and some embryonic tissues [4], have been demonstrated to be preferred sources of MSCs (Fig. 1). MSCs are a cell population that adheres spontaneously to plastic; they have a particular immunophenotypic profile (express a certain collection of surface CDs markers), and they develop into osteocytes, adipocytes, and chondrocytes [5]. Because of their exceptional anti-inflammatory, immunosuppressive, immunomodulatory, and regenerative characteristics, MSCs have been studied in cell-based therapeutics [6,7]. The promising therapeutic effects of MSCs are also due to their capacity to undergo lineage-specific differentiation, influence the immune system, and release essential bioactive molecules [5,8,9]. MSCs are thus very desirable candidates for cell-based therapy in inflammatory and degenerative diseases [6,10]. MSCs-based therapies, including their secretomes and conditioned media, have attracted attention for several therapeutic applications due to their distinct anti-inflammatory profile. In the last decade, several preclinical investigations and more than 5000 clinical trials involved MSCs have been registered, and more than 1500 have been completed (source:

Abbreviations: AMPK, Adenosine monophosphate-activated protein kinase; EGCG, Epigallocatechin-3-gallate; eNOS, Endothelial nitric oxide synthase; ERK, Extracellular signal-regulated kinase; FGF, Fibroblast growth factors; G-CSF, Granulocyte-colony stimulating factor; HGPS, Hutchinson-Gilford progeria syndrome; IL-1R1, IL-1 receptor type1; IDO, Indoleamine 2,3-dioxygenase; LAMF, Lyophilized aqueous extracts of Mori Fructus; LAMR, Lyophilized aqueous extracts of Mori Ramulus; MHC, Major histocompatibility complex; mTOR, Mammalian target of rapamycin; MSCs, Mesenchymal stem cells; MMPs, Metalloproteinase; MAPK, Mitogen-activated protein kinases; MCP1, Monocyte chemoattractant protein 1; NAC, N-acetylcysteine; Nrf2, Nuclear factor erythroid 2- related factor 2; NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B Cells; PPARγ, Peroxisome proliferator-activated receptor gamma; PI3k, Phosphatidylinositol 3-kinase; PDGF, Platelets derived growth factor; PRDX4, Peroxiredoxin 4; PAR1, Protein-activator receptor-1; ROS, Reactive oxygen species; Runx2, Runt-related transcription factor 2; SASP, Senescence-associated secretory phenotype; STAT3, Signal transducer and activator of transcription 3; SOD2, Superoxide dismutase 2; TGF-β, Transforming growth factor- β; TTS, Tribulus terrestris sapoinin; TRAF6, Tumor necrosis factor-associated factor 6; VEGF, Vascular endothelial growth factor.

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https://doi.org/10.1016/j.biopha.2023.114356
Received 8 December 2022; Received in revised form 24 January 2023; Accepted 31 January 2023
Available online 9 April 2023

http://www.clinicaltrials.gov). It was updated in 2016 that around two thousand individuals have received autologous or culture-expanded allogeneic MSCs to treat various disorders during the last 20 years [11]. Enhancing MSCs stemness has been widely used to induce their therapeutic efficacy in treating several diseases. The concept of increasing the stemness of MSCs is to stimulate MSCs in vitro prior to their use in vivo or sometimes to use an enhancer together with MSCs simultaneously as an adjuvant. A lot of preclinical studies have used several biomolecules such as IL-1α, IL-1β, IFN-γ, TNF-α, IGF-1, FGF-2, IL-17A, Poly I:C, and LPS for enhancing MSCs [12,13]. Current cellular therapy research focuses on elucidating the molecular processes that regulate or influence the immunomodulatory capacity of MSCs. Indeed, MSCs' immunoregulatory effectiveness is diminished by cellular aging [14] and other impediments [15]. Thus, the researchers attempted to identify the solutions for overcoming these suppressors and enhancing the MSCs' ability to tackle age-related disorders and inflammatory diseases. Of these solutions, stimulation the immunomodulatory potency of MSCs by changing the culture environment condition, priming by TLRs ligands or inflammatory biomolecules, or incubation at hypoxic atmosphere or in 3D-culture [12,13]. This updated review provides a comprehensive discussion about the recent trends of enhancers (Fig. 1) used to induce the stemness of MSCs in the research field. These enhancers are metformin, resveratrol, antioxidants, mTOR inhibitors, and miscellaneous pharmacological compounds. Cytokines, growth factors, hormones, and vitamins are also included. Additionally, this review suggests potential MSCs' enhancers' strategies that could be used in vivo if translated well. More important, the mode of action for all included enhancers is discussed.

2. Pharmacological compounds

As a result of their understanding of the processes that affect the proliferation, differentiation, and paracrine secretions of MSCs, researchers can recommend specific pharmacological modulators for improving the therapeutic efficacy of MSCs. In this study, we discuss the effects of numerous drugs, including metformin, resveratrol, antioxidants, and mammalian target of rapamycin (mTOR) inhibitors.

2.1. Metformin

Metformin is a well-known drug that is clinically approved for the treatment of type 2 diabetes. Studies have extensively revealed a novel effect of metformin on the augmentation of the potency of MSCs (Fig. 2) via the activation of osteogenic and neuronal differentiation and the upregulation of certain stemness markers. Metformin maintained the signaling pathways involved in the normal activities of MSCs, delayed replicative senescence, reduced apoptosis, and induced superoxide dismutase 1 (SOD1), SOD2, CAT, GSTP1, and GLRX anti-oxidant proteins [16]. Metformin can also increase the therapeutic efficacy of MSCs by enhancing the release, abundance, and quality of exosomes, one of the stemness' primary features. These effects were mediated by an autophagy-dependent pathway. Metformin promotes proteins of cell development, which can exert a positive effect on the senescence of intervertebral disc cells in vitro and in vivo, according to the proteomic study [17]. Gu et al. reported a novel activity for metformin on MSCs generated from human chorionic villous in which metformin can stimulate osteogenesis activation and inhibit adipogenesis. In osteogenic differentiation media containing metformin, alkaline phosphatase (ALP)

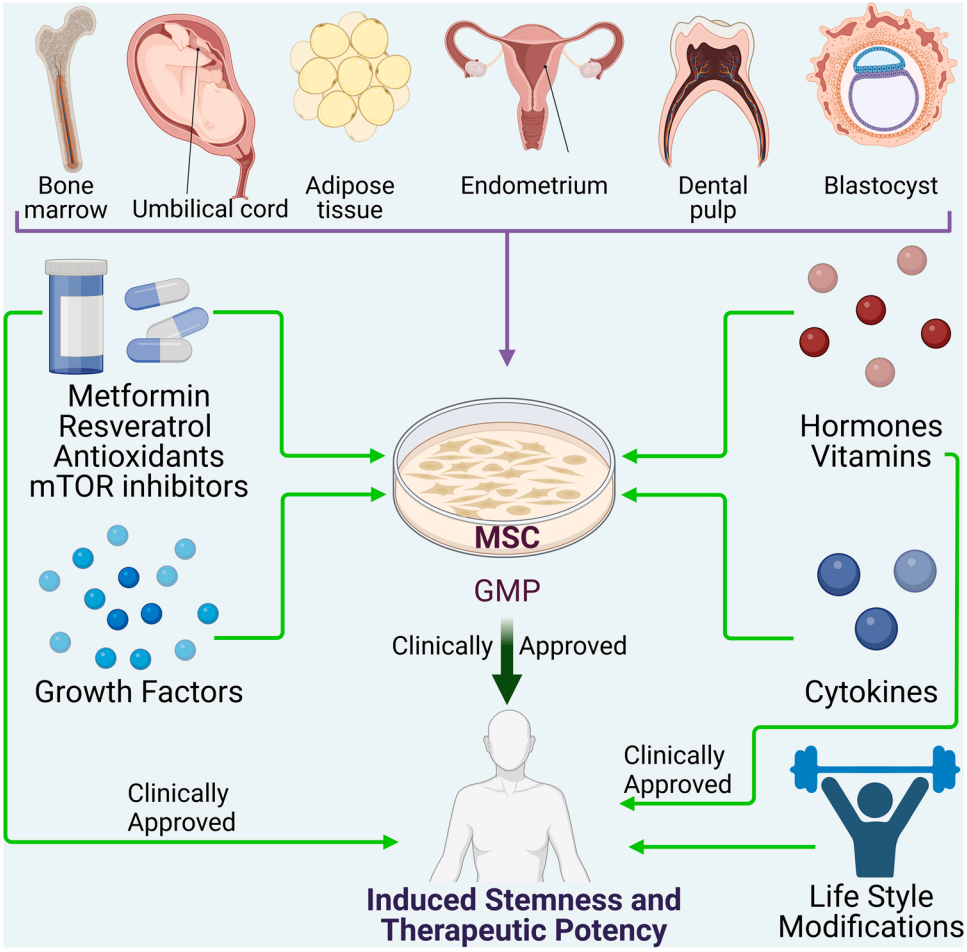

Fig. 1. An overview of MSCs' stemness' enhancers. MSCs isolated from bone marrow, adipose tissues, umbilical cord, dental pulp, endometrium, and blastocyst could be enhanced by a wide variety of biomolecules, including pharmacological medications, cytokines, growth factors, hormones and vitamins to enhance (green) their stemness. In case of human clinical usage of enhanced MSCs, the procedures of in vitro MSCs cultivation and preparation for transplantation shall follow Good Manufacturing Practices (GMP). Lifestyle modifications and clinically approved medications could be considered to enhance MSCs' stemness in human body.

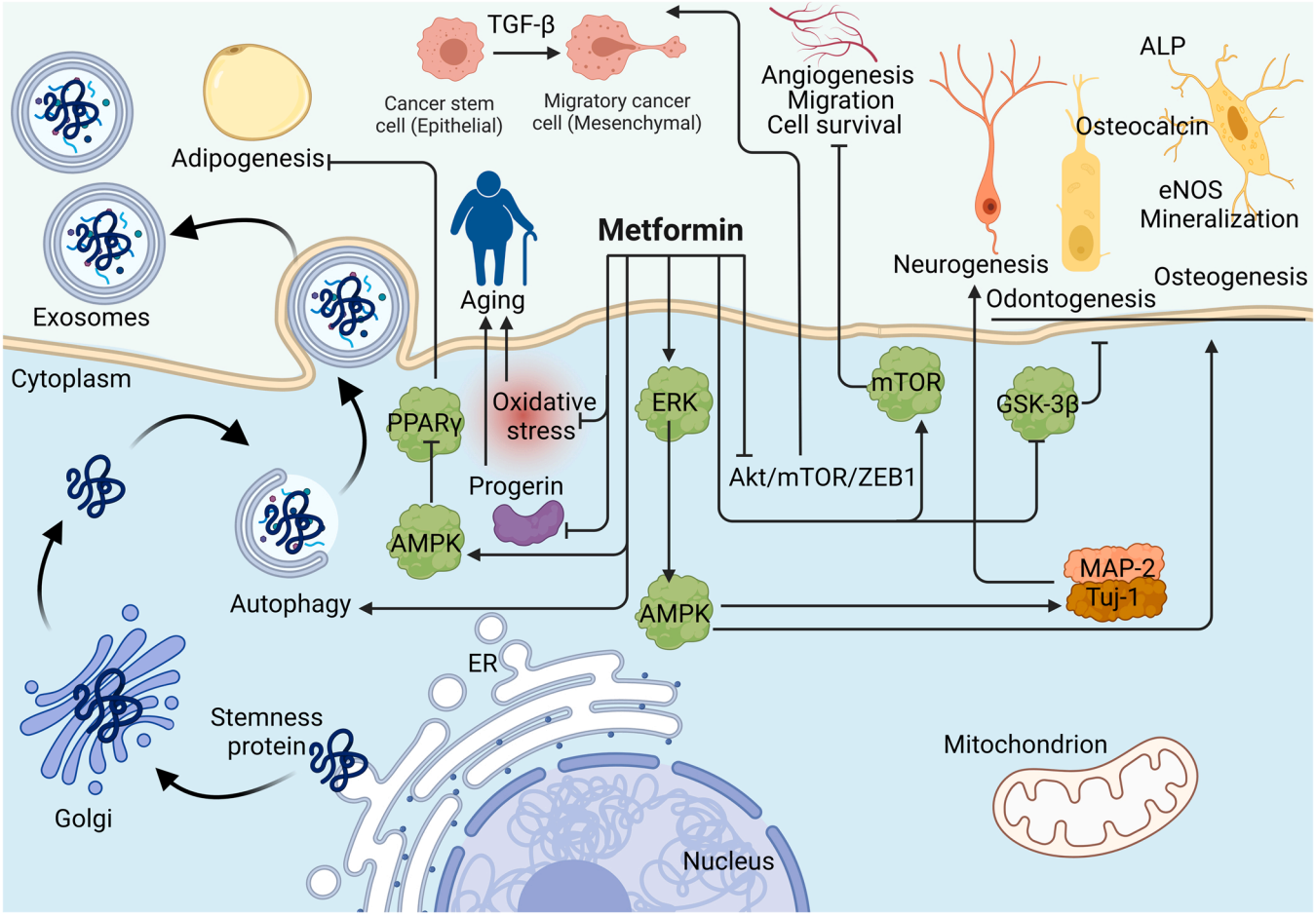

Fig. 2. Metformin enhances MSCs' stemness. Metformin can induce MSCs' therapeutic potency through activating AMPK, ERK, and autophagy pathways. Thanks to regulating these pathways, metformin activating MSCs' osteogenesis and odontogenesis through modulating GSK3β and neurogenesis by MAP-2 and Tuj-1 overexpression. Additionally, it can inhibit MSCs' adipogenesis via inhibiting PPAR-γ and aging by antioxidant effects or decreasing progerin expression. Through activating autophagy, metformin can improve the quantity and ingredients of MSCs' exosomes, and promote anti-tumor action by inhibiting migration, cell survival, angiogenesis TGF-β-mediated EMT of cancer stem cell. Activate or regulate (⟶), Inhibit (⟞).

activity and calcium mineralization were elevated in MSCs. In the meantime, metformin stimulated the expression of the bone formation component, endothelial nitric oxide synthase (eNOS). However, treatment of MSCs with metformin reduced adipocyte development in both adenosine monophosphate-activated protein kinase (AMPK)-dependent and -independent ways via down-regulation of peroxisome proliferator-activated receptor gamma (PPARγ) [18,19]. In addition, metformin contributed to the development of MSCs from the dental pulp into odontoblasts via an extracellular signal-regulated kinase (ERK)/AMPK-dependent pathway, as evidenced by an increase in osteocalcin, dentin sialophosphoprotrin, and other odontoblastic markers [20,21]. Moreover, adipose-derived MSCs exhibited an enhanced osteogenic effectiveness in bone production and resilience to the oxidative stress after metformin treatment [22].

MSCs derived from human induced pluripotent stem cells improve their osteogenic differentiation potential in response to metformin [23]. The effect of metformin on the osteogenic differentiation of MSCs is mediated via inhibition of glycogen synthase kinase-3 β (GSK3β) [24]. It has also been demonstrated that metformin promotes neural development in MSCs. The presence of metformin in the neural development environment hastens the differentiation of MSCs, resulting in an increase in neurite length. Further exploration demonstrated that metformin increased neuronal proteins such as MAP-2 and Tuj-1 by activating AMPK [25]. These results indicate the potential relevance of metformin and its derivatives in the treatment of age-related diseases, particularly bone-loss diseases and neurodegenerative disorders. In another field, metformin treatment of MSCs-derived from Hutchinson-Gilford progeria syndrome (HGPS) induced pluripotent stem cells revealed positive results in vitro as an anti-aging agent by modulation of decreased expression of progerin [26]. Indeed, animal experiments in mice with diabetes mellitus type 2 revealed a synergistic effect of injected metformin-preconditioned adipose tissue-derived MSCs on decreasing hyperglycemia, hyperinsulinemia, and triglyceridemia [27]. It has also been reported that metformin regulates Yes-associated protein (YAP) activity and block the stemness-related marker, CD133 in glioma stem cells [28], or halts the transforming growth factor-β (TGF-β)-dependent epithelial-mesenchymal transition via Akt/mTOR/ZEB1 [29] as well as in breast cancer [30]. In the same context, the resistance of cancer stem cell-like HepG2 for sorafenib was ameliorated by a low dose of metformin through reversing epithelial-mesenchymal transformation [31].

Although the positive impact of metformin in boosting the potency of MSCs is emphasized, a negative effect was also demonstrated in diabetic mice with myocardial infarction. Experiments demonstrated that metformin inhibited the therapeutic effectiveness of MSCs by activating apoptosis through AMPK [32]. Through regulation of the mTOR pathway, chronic metformin administration impaired angiogenic and differentiation capacity, migration, and cell survival of MSCs [33]. Taking together, metformin enhances the therapeutic efficacy of MSCs, particularly in illnesses of bone loss, neurologic degenerative diseases, and cancer, but in other age-related diseases, the topic remains

debatable.

2.2. Resveratrol

Resveratrol is a polyphenol with antioxidant properties that is found in the skin of red grapes, peanuts, Japanese knotweed, and blueberries. Due to its impact on AMPK, Sirtuin, autophagy, and oxidative stress pathways, resveratrol has generated increased interest in the scientific community. These pathways are also important for the stemness of MSCs, suggesting that resveratrol may enhance the therapeutic efficacy of MSCs (Fig. 3). Recent studies have demonstrated that resveratrol is one of the optimal adjuvants for treating diabetes mellitus using MSCs. This positive effect of resveratrol is attributed to its capacity to improve glucose metabolism and promote cell viability [34]. MSCs pretreated with resveratrol promoted wound healing in type 1 diabetes by suppressing tumor necrosis factor-associated factor 6 (TRAF6) via extracellular vesicle's miR-129 [35]. Acceleration of wound healing by resveratrol-treated MSCs is also attributed to increased secretion of growth factors, TGF-β1, platelets derived growth factor (PDGF), EGF, and HGF [36]. Resveratrol may induce the hermetic response of promoting proliferation, osteogenic differentiation [37,38], and resilience to cellular stress can improve the stemness of MSCs [39]. Interaction between miR-139a and Sirt7 [40], modulation of the miR 320c/Runt-related transcription factor 2 (Runx2) axis [41], or activation of the Wnt/β-catenin pathway [42] underlie resveratrol-induced osteogenesis in MSCs. In the same context, to avoid bone loss in osteoporosis, bone marrow MSCs can be treated by resveratrol to suppress TNF-α inflammatory niche, thereby regulating nuclear YAP [43], or upregulate miR-146a which in turn regulates Wnt/FOXO and Sirt1/nuclear factor kappa-light-chain-enhancer of activated B Cells (NF-κB) pathways [44]. It has also been observed that resveratrol can promote osteogenesis in MSCs generated from periosteum and increase mitochondrial biogenesis [45]. Intriguingly, resveratrol exhibited anti-aging effects on MSCs by reducing premature senescence via modulation of RELA/Sirt1 pathway [46], and by enhancing hematopoiesis via induction of Sirt1 overexpression [47]. In rats with hyperglycemia-induced cardiomyopathy, MSCs from adipose tissue treated with resveratrol served as antioxidants via Sirt1/Akt pathway [48]. Resveratrol also rejuvenate MSCs after hyperglycemia-induced senescence and promote their therapeutic potency to heart recovery after myocardial infarction in diabetic rats [49]. Combined treatment of atorvastatin and resveratrol protects MSCs from rapamycin-induce apoptosis and promotes cell migration by Akt pathway [50].

Exosomes obtained from MSCs following treatment with 5-azacytidine and resveratrol inhibit aging and apoptosis and alleviate endoplasmic reticulum stress in cells derived from animals with equine metabolic syndrome [51]. In addition, neuronal differentiation and neuronal progenitor makers, NES and SOX1, were observed after priming of human stem cell from apical papilla by resveratrol [52]. Indeed, treatment of MSCs by resveratrol is promising in the treatment of rats severe acute pancreatitis via modulation of the PI3K/Akt/VEGFA pathway in pancreatic cells and human umbilical vein endothelial cells,

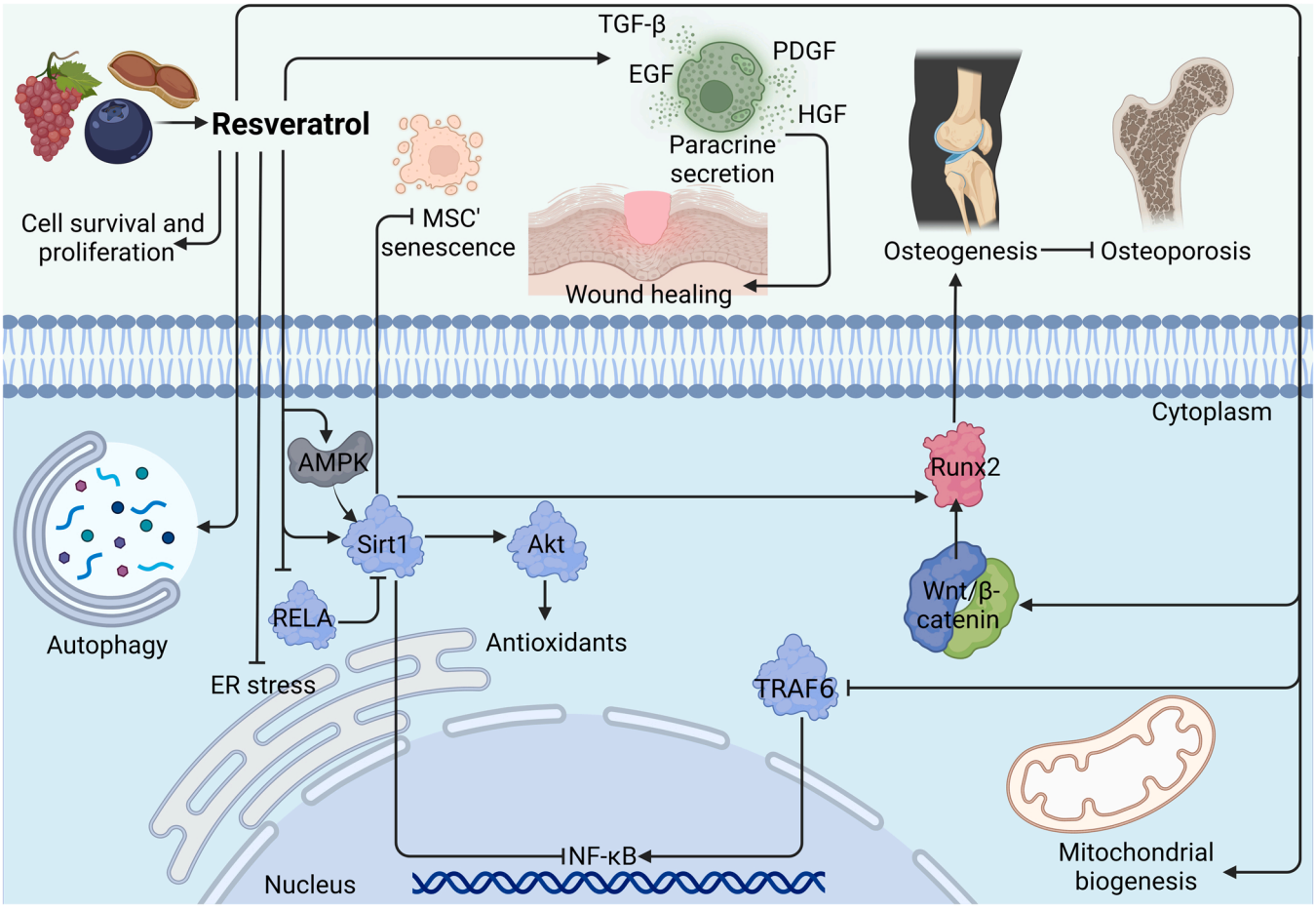

Fig. 3. Resveratrol enhances MSCs' stemness. Resveratrol extracted from grapes, blueberries, or peanuts can induce MSCs' therapeutic potency by activating AMPK, sirtuin, Wnt/β-catenin, and autophagy pathways as well as mitochondrial biogenesis. It can inhibit osteoporosis through Wnt/β-catenin/Runx2-mediated osteogenesis and promote wound healing via inducing expressions of growth factors, TGF-β, PDGF, EGF, and HGF in paracrine secretion. Resveratrol can also inhibit MSCs' senescence via Sirt1 and inflammation via regulating NF-κB as well as inducing cell survival and relieving endoplasmic reticulum stress. Activate or regulate (⟶), Inhibit (⟞).

thereby protecting from cell death and inducing regeneration of damaged tissues [53].

In treatment of cancer, resveratrol is also considered, especially in targeting MSCs residing at tumor microenvironment. The antitumor effect of resveratrol on gastric cancer MSCs was demonstrated by the downregulation of interleukin-6 (IL-6), IL-8, vascular endothelial growth factor (VEGF), and monocyte chemoattractant protein 1 (MCP1) expressions [54]. Collectively, at the preclinical level, resveratrol induces MSCs' stemness by inducing antioxidants, correcting skewed differentiation, preventing mitochondrial dysfunction, enhancing paracrine secretion, and promoting cell survival.

2.3. Antioxidants

The generation of reactive oxygen species (ROS) is one of the most powerful mechanisms for inhibiting MSCs' stemness and activating cellular senescence. Therefore, addressing oxidative stress or triggering antioxidant mechanisms is one of the preferred methods for researchers to improve the therapeutic efficacy of MSCs in clinical applications (Fig. 4). While there are many forms of antioxidants, including enzymatic and non-enzymatic varieties, the most important antioxidants are those found naturally in seafood, meats, fruits, and vegetables. Recent research has focused on identifying the most effective antioxidant to combat oxidative stress, a phenomenon that accelerates MSCs' senescence and inhibits MSCs' stemness. As an illustration, astaxanthin, a xanthophyll present in certain seafood such as shrimp and salmon, can protect MSCs from apoptosis and oxidative stress by boosting expression of nuclear factor erythroid 2- related factor 2 (Nrf2) [55]. After palmitate treatment, astaxanthin exerted anti-inflammatory effects on human bone marrow-derived MSCs [56]. Selenium nanoparticles promoted MSCs' viability and osteogenic differentiation by inducing antioxidant levels and activation of JNK/FOXO3 pathway [57]. The polyphenol in green tea, -(-) epigallocatechin-3-gallate (EGCG), functions as an alternative antioxidant that can induce MSCs' osteogenesis [58]. In a high-glucose environment, it can also promote MSCs' survival by increasing Akt phosphorylation [59]. In addition, *Cladophora glomerata* methanolic extract was reported to have an antioxidant impact on MSCs obtained from equine adipose tissues. The extract increased the MSCs' viability and the expression of antioxidant enzymes, SOD2 and catalase, while decreasing the expression of p53, p21, Bax, and caspase 9 [60]. Indeed, plant saponins from *Tribulus terrestris* performed admirably as antioxidants by reversing oxidative damage generated by H_2O_2 in adipose tissue-derived MSCs [61].

Moreover, the steric impact of sanggenons C and D antioxidants from the Chinese medicine Sang-bai-pi protected MSCs from oxidative stress. Comparing the two medications found that sanggenon C is more effective than sanggenon D [62]. Traditional Chinese herbal medicines *Mori fructus* and *Mori ramulus* have been shown to have an antioxidant effect on MSCs by protecting them from OH-induced damage [63]. Resveratrol, another anti-oxidant derived from food, has been thoroughly addressed above in this article.

N-acetylcysteine (NAC), a well-known ROS-scavenger, may potentially be utilized to improve the therapeutic efficacy of MSCs in the research field. Experiments on animals showed that the combination of MSCs and NAC lowered proinflammatory markers and raised antioxidant markers in rats with severe acute pancreatitis [64]. Interestingly, scientists have observed a synergistic antioxidant effect for the administration of α-lipoic acid and antihypertensive medications, amlodipine/perindopril, after MSCs' transfusion in experimental mice with isoproterenol-induced heart damage. This synergy increased cellular antioxidant levels [66]. In another context, CCR2-overexpressed MSCs triggered an antioxidant mechanism that increased the therapeutic

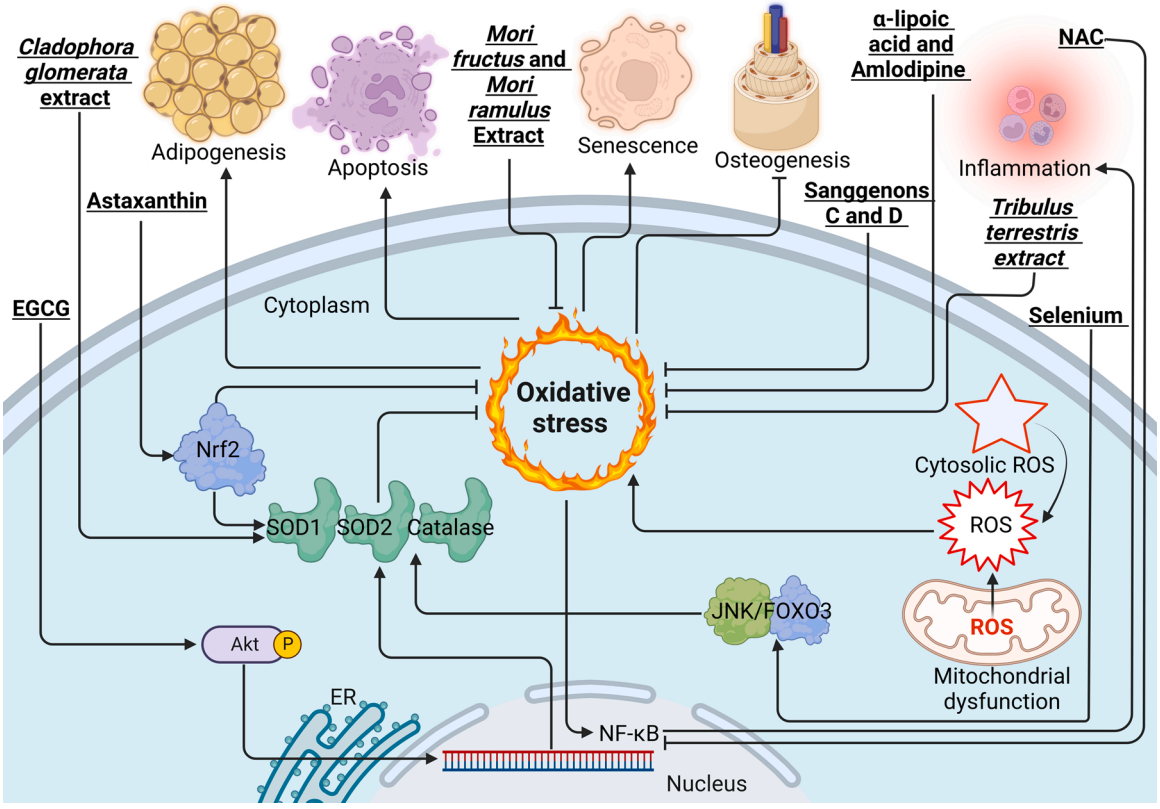

Fig. 4. Antioxidants enhance MSCs' stemness. Inhibition of oxidative stress induces MSCs' therapeutic potency by inhibiting skewed differentiation, apoptosis, senescence, and inflammation, and inducing antioxidants molecules, SOD1,2 and catalase. Among antioxidants, astaxanthin, ECGC, and selenium which downregulate oxidative stress through Nrf2, phosphorylated Akt, and JNK/FOXO3 pathways respectively. Activate or regulate (⟶), Inhibit (⟞), antioxidant (**bold** and underlined), living organism (*italic,* **bold**, and underlined).

Table 1
Antioxidants used in research to enhance the stemness of MSCs.

Source	Anti-oxidant	Mode of action	Outcome	Ref.
Carotenoids chemicals	Astaxanthin	Inhibits IL-6, VEGF, and MCP-1	Down-regulates inflammation and apoptosis in MSCs	[55, 56]
Fish and shellfish	Selenium	Inducing JNK/FOXO3 pathway	Activates MSCs' viability and differentiation	[57]
Green tea polyphenol	EGCG	Increasing ALP activity and mineralization	Promotes murine MSCs' osteogenesis	[58]
Cladophora glomerata	Methanolic extract	Decreases cell cycle inhibitors	Promotes the equine MSCs' viability.	[60]
Plant saponins	TTS	-	Decreases oxidative stress	[61]
Chinese drug, Sang-bai-pi	Sanggenons C and D	Inducing Fe^{2+}-binding	Protects MSCs' from oxidative stress	[62]
Mori Fructus and Mori Ramulus	LAMF and LAMR	Decreases ROS generation	Increase MSCs' viability	[63]
Modified amino acid	NAC	Increases antioxidant marker, SOD	Decreases markers of inflammation and serum amylase in rat with acute severe pancreatitis	[64]
Cellular proteins	CCR2 overexpression	Upregulates expression of PRDX4	Induces targeted migration of MSCs in the treatment of acute ischemic brain stroke in rats	[65]
- α-lipoic, Fatty acid - Amlodipine, antihypertensive drug	α-lipoic acid and amlodipine combination	ROS-scavenging	Synergistic antioxidant effect in rats cardiac injury	[66]

efficacy of MSCs in the treatment of rats' acute ischemic stroke of the brain [65]. In conclusion, addressing ROS signaling by antioxidants is important for enhancing the therapeutic efficacy of MSCs. Vitamins that function as antioxidants were described briefly below. In Table 1, we compiled a summary of representative antioxidants employed by researchers to stimulate the therapeutic efficacy of MSCs. Not very far from oxidative stress downregulation, hypoxic environment was reviewed as an enhancer for MSCs' therapeutic potency [12,13].

2.4. mTOR Inhibitors

It is generally recognized that the mTOR signaling pathway plays a crucial role in cellular functions such as autophagy and cell survival. Therefore, regulation of mTOR activity is a crucial step in improving the stemness of MSCs. Strong evidence suggests that mTOR signaling promotes senescence and stemness downregulation in MSCs. In this context, the direction of research is centered on exploring and improving the optimal mTOR inhibitors (Table 2), which may protect MSCs from early senescence and enhance their immunomodulatory potential (Fig. 5). Rapamycin has recently been found to reverse MSCs' replicative senescence and induce therapeutic efficacy in treating ischemia illness in mice by preserving proangiogenic factor overexpression, VEGFR2 [67]. In vitro, rapamycin was reported to promote the migration of MSCs from the umbilical cord via CXCR4. In vivo experiments revealed that preconditioning MSCs with rapamycin allows them to alleviate liver ischemia injury in mice [68]. Rapamycin treatment of stem cells derived from the apical papilla (SCAP) enhanced osteogenic and dentinogenic differentiation in vitro and in vivo by suppressing mTOR pathway [69]. A low dose of rapamycin strengthened the ability of MSCs to preserve allografts, which increased allograft survival in mice with major histocompatibility complex (MHC) incompatibility [70]. In addition, incubation of MSCs with rapamycin and its derivative, everolimus presented immunomodulatory potency enhancement [71]. A clinical trial was registered and hypothesized that the autonomous infusion of MSCs with everolimus maintaining renal structure and function in renal transplant recipients, but no results declared up to date [72]. A further rapamycin derivative, temsirolimus, was shown to inhibit cancer stem cells by inhibiting their stemness and epithelial-mesenchymal transition [73,74]. Research has led to the development of a new generation of mTOR inhibitors that block the downstream substrate of both mTOR complex 1 and 2 [75], sapanisertib, or INK128; a small-molecule drug that inhibits the mTOR pathway via the ATP site. It is demonstrated that INK128 has a function in promoting the differentiation and rejuvenation of MSCs [76]. In short, mTOR signaling modulation is critical for MSCs' stemness increase.

2.5. Miscellaneous pharmacological agents

Diverse pharmacological compounds were studied in the research field to test their action on MSCs' biological activities, particularly those related to immunomodulatory potency (Table 3). For instance, fullerol nanoparticles was observed to have anti-inflammatory and antioxidant effects on vertebral MSCs displayed by down-regulation of IL-1β-induced ROS, MMP1/2/13, and TNF-α [77], as well as inhibition of adipogenesis and stimulation of osteogenesis by alleviation dexamethasone-stimulated oxidative stress [78]. Another compound, fucoidan, has been shown to improve MSCs osteoblast differentiation by promoting the angiogenic factor, VEGF [79], especially when combined with the β-tricalcium phosphate-chitosan scaffold [80].

Isoproterenol-induced heart failure of the rat was improved after being treated by MSCs with nicorandil, suggesting a role for nicorandil in enhancing MSCs' therapeutic power [81]. It is reported previously that nicorandil could protect MSCs from oxidative stress and nutrients depletion-induced apoptosis [82]. Carvedilol promoted the therapeutic potency of MSCs in treatment of heart injury in animal model through caspase-3 down-regulation [83] and protected from oxidative stress-induced cell death by phosphorylation of PI3K-Akt pathway in vitro[84].

While isoquercitrin promoted osteogenesis differentiation of bone marrow MSCs [85], aspirin low-dose reduced some paracrine secretion of MSCs derived from decidua basalis of pregnant with preeclampsia [86]. Indeed, it is reported that MSCs' osteogenesis could be enhanced by multiple drugs, such as anthocyanidins, malvidin, delphinidin, and cyanidin; however, adipogenesis is inhibited by delphinidin [87]. Furthermore, treating adipose tissues MSCs with azacytidine and resveratrol mediated outstanding anti-inflammatory activities when incubated with PBMCs and macrophages, and associated with promoting T regulatory cells [88]. In summary, in addition to metformin,

Table 2
mTOR inhibitors used in research to enhance the stemness of MSCs.

mTOR inhibitor	Mode of action	Outcome	Ref.
Rapamycin	Inhibition of AKT/mTOR signaling	Promotes dentinogenic and osteogenic differentiation of SCAP	[69]
Rapamycin and everolimus	*In vitro* inhibition of induced T lymphocyte	Enhancing immunomodulatory potency of MSCs	[71]
Temsirolimus	Suppression of MSCs' oncogenic stemness	Attenuation of cancer stem cells	[73,74]
INK128	Inhibits phosphorylation of 4EBP1 and p70S6K1/2	Enhancing anti-aging mechanisms in MSCs	[76]

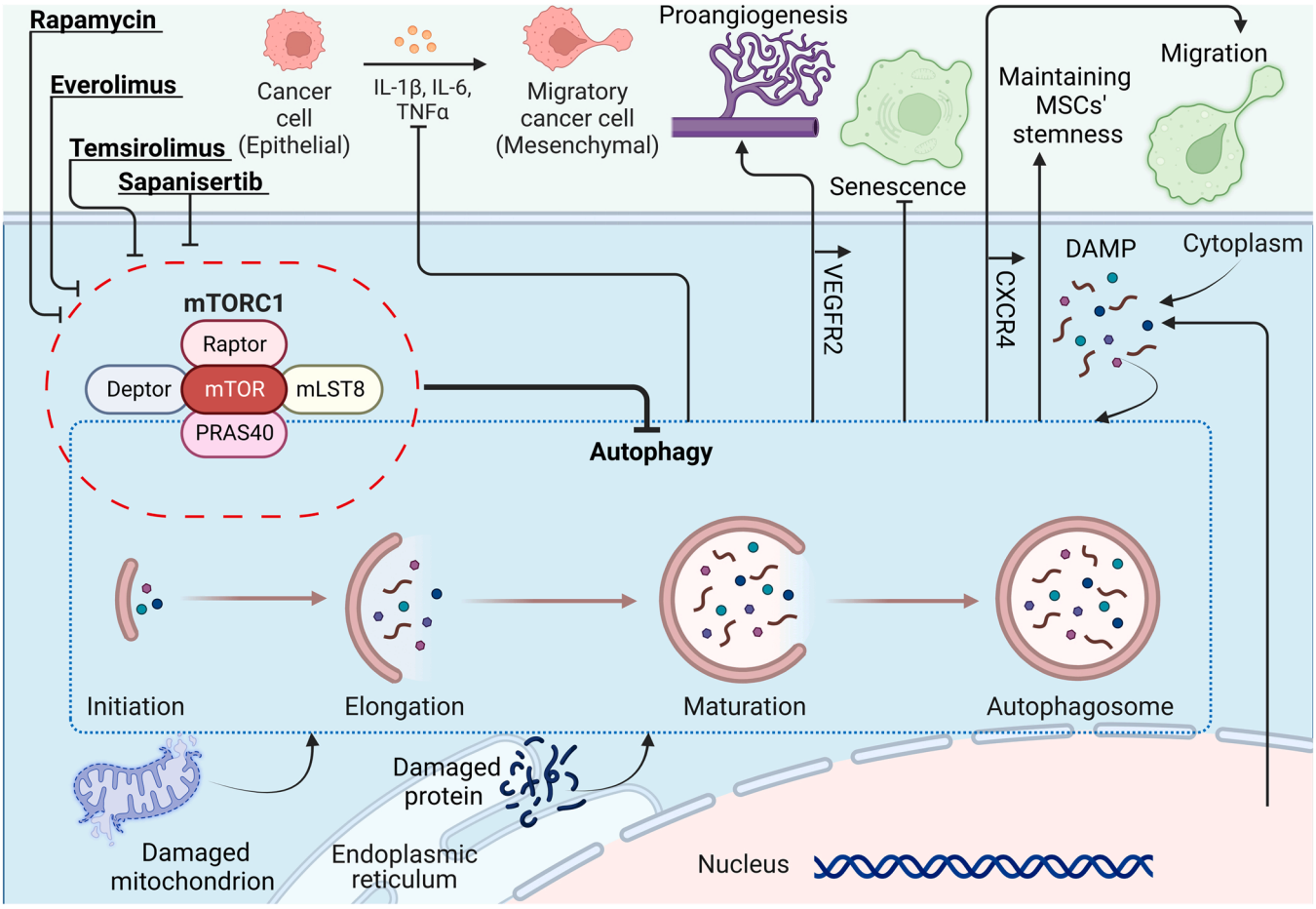

Fig. 5. mTOR inhibitors enhance MSCs' stemness. Inhibiting mTOR pathway induce MSCs' therapeutic potency by maintaining the right path of autophagy. Maintaining autophagy can activate migration and angiogenesis, and inhibit senescence and tumorigenesis via regulation of CXCR4, VEGF, processing DAMP and damaged mitochondria, and inhibiting IL-6, IL-1β, and TNF-α-mediated cancer stem cell EMT respectively. Activate or regulate (⟶), Inhibit (⟞), mTOR inhibitor (**bold** and underlined).

Table 3
Miscellaneous compounds used in research to enhance the stemness of MSCs.

Compound	Mode of action	Outcome	Ref.
Fullerol nanoparticles	Down-regulation of ROS, MMP1/2/13, and TNF-α	Anti-inflammatory and antioxidant effects	[77]
Fucoidan	Induces VEGF expression	Enhances osteogenesis	[79]
Nicorandil	Induces anti-oxidant and anti-apoptotic mechanisms	Induces MSCs' survival	[82]
Carvedilol	Caspase-3 down-regulation [83] and Phosphorylation of PI3K-Akt pathway [84]	Promotes the therapeutic potency of MSCs in animal model	[83, 84]
Isoquercitrin	Induces ALP activity	Promotes osteogenesis	[85]
Aspirin	Induces MSCs' adhesion	Modulates paracrine secretion of MSCs	[86]
Cyanidin and malvidin	Induce expression of Runx2 and BMP-2 genes	Enhance MSCs' osteogenesis	[87]
Delphinidin	Decreases expression of adiponectin genes and FABP4	Inhibits adipogenesis	[87]
Azacytidine and resveratrol	Promoting mitophagy	Anti-inflammatory effects	[88]

resveratrol, antioxidants, and mTOR inhibitors, there are a number of modulators that can improve the therapeutic potency of MSCs by controlling many of the stemness mechanisms (Table 3).

3. Cytokines

Because of their essential functions in cell autocrine, paracrine, and endocrine signaling, the stimulation of MSCs by cytokines is becoming a hot issue due to their beneficial effects on enhancing cellular therapeutic potency (Table 4) (Fig. 6). Pretreatment of MSCs from synovial tissue by IL-1β, for instance, boosted their proliferation and chondrogenic differentiation capacity [89], and enhanced their migration via the MMP1/protein-activator receptor-1 (PAR1) and G-protein-coupled pathway [90]. In addition, preconditioning MSCs with IL-1β inhibited LPS-induced inflammation in microglia cells via IL-1 receptor type1 (IL-1R1) and granulocyte-colony stimulating factor (G-CSF) [91]. Moreover, stimulation MSCs by IL-1β and IFN-γ mediated immunoregulatory enhancement on macrophage polarization and promoted anti-inflammatory ability [92].

IL-6 contributes to the enhancement of carcinogenesis by recruiting cancer stem cells [93]. In hypoxic environment, IL-8 was reported as an enhancer for MSCs because it promoted their proliferation and decreased the proportion of apoptotic cells via the Akt/ signal transducer and activator of transcription 3 (STAT3) pathway [94]. IL-8 also

Table 4
Cytokines used in research to modulate the stemness of MSCs.

Cytokine	Mode of action	Output	Ref.
IL-1β	Activating MMP1/PAR1 pathway [90]	Induces migration [90] and anti-inflammatory ability of MSCs [91]	[90,91]
IL-6	Induces MAPK, STAT3, and Akt signaling	Increases stemness of cancer stem cell	[93]
IL-8	Regulating Akt-STAT3 [94] and PI3K/Akt pathways [95]	Induces proliferation, cell survival [94], osteogenesis and chondrogenesis [95]	[94,95]
IL-18	Suppressing tumor cells proliferation through activating immune cells and cytokines	Improves the therapeutic effects of MSCs in breast cancer	[97,98]
TNF-α	Increasing IL-10 and TGF-β expression	Increases anti-inflammatory effects of MSCs	[100]
IFN-γ	Upregulation of TGF-β, HGF, and PGE2.	Improves the immunosuppressive effects of MSCs	[103]
IL-3	Activation of JAK/STAT pathway [109]	Modulates MSCs' osteogenesis and chondrogenesis	[109–112]
IL-4	Overexpression of IL-4	Increases anti-inflammatory power of MSCs	[113]
IL-7	Downregulation of the MAPK pathway	Suppresses MSCs' osteogenesis	[117]
IL-17	Improvement of immunoregulatory properties	Induces MSCs' therapeutic potency	[118,119]
IL-22	Induction of MSCs' viability	Promotes osteogenic differentiation	[121]
IL-25	Decreases expression of IL-17 and increase T regulatory lymphocytes	Increases MSCs' immunoregulation in treatment of of rats IBD	[122]
GM-CSF	Inducing MSCs' proliferation	Improves chondrogenesis	[123]

enhanced MSCs' therapeutic potency in bone formation and chondrogenesis by regulation PI3K/Akt pathway in CXCR2-dependent manner [95]. Worth mentioning, inhibiting IL-8 causes MSCs generated from placenta to age prematurely [96].

Increasing the expression of the proinflammatory cytokine IL-18 improved the therapeutic effect of MSCs in breast cancer in vitro [97] and in vivo [98], suggesting that the establishment of procedure to stimulate MSCs' IL-18 production may introduce a promising remedy in cellular therapy. Conversely, myocardial infarction in rats model was improved with IL-18 binding protein genetically modified MSCs [99]. Also, TNF-α activation of MSCs enhanced expressions of IL-10 and TGF-β [100], thereby recruiting MSCs for inflammation suppression. Consistently, after treatment with TNF-α, MSCs and their exosomes exhibited distinct microRNA expression [101]. In addition, preconditioning of murine MSCs with TNF-α facilitated bone regeneration and immune modulation [102].

IFN-γ pretreatment of MSCs mediated their immunosuppressive effect on activated lymphocytes through increased expression of immunosuppressive molecules, TGF-β, HGF, and PGE2 [103]. The IFN-γ-induced morphological alterations on MSCs have been considered when predicting immunosuppressive capacity [104]. It was shown that a cocktail of cytokines, IFN-γ, TGF-β, and retinoic acid stimulated MSCs stemness by activating indoleamine 2, 3-dioxygenase (IDO) and PD-L1 immunologic mediators [105]. In contrast, IFN-γ may have a detrimental effect on the therapeutic efficacy of mouse MSCs by producing aging-related characteristics [106] but it had no influence on the MSCs' inhibitory effect on lymphocyte proliferation in vitro [107].

CXCR2 receptor of CXCL1 chemokine may regulate the adipogenesis of MSCs via altered activation of the p38/ERK-ELK1 pathway in co-culture with macrophage [108]. Moreover, IL-3 has been identified as a promoter for MSCs in bone regeneration via JAK/STAT pathway [109], cartilage formation by decreasing MMP expressions [110], and enhancing MSCs' migration [111]. In the same context, Hong et al. also reported that IL-3 played dual roles related to osteoclastogenesis in which promoted osteoclast progenitors development but discourage the process of osteoclastogenesis [112]. NF-κB-mediated IL-4 increased the anti-inflammatory property of MSCs [113], as well as engineered MSCs were reported to have a beneficial effect on osteogenesis through co-overexpressing of PDGF-BB and IL-4 [114]. Although IL-7 promoted renal regeneration by enhancing the fusion ability of rat hypoxic-stressed MSCs [115], it is reported that IL-7 inhibited MSCs differentiation into osteoblast [116] through inhibition of the MAPK pathway [117].

IL-17-stimulated MSCs increased skin transplant survival from allogenic source [118], and increased therapeutic efficiency in renal diseases [119], whereas IL-17b modulated the immunoregulatory potency of MSCs to promote gastric cancer progression [120]. In addition, IL-22 promoted MSCs' migration, proliferation, and osteogenic differentiation [121]. Indeed, IL-25-primed MSCs alleviated autoimmune inflammatory intestinal diseases, IBD in rat model presents by low IL-17 and elevated T regulatory lymphocytes [122]. Moreover, a lymphokine, granulocyte macrophage-colony stimulating factor (GM-CSF) promoted cartilage rejuvenation by increasing numbers of MSCs in rabbits [123]. Furthermore, TL1A promoted the stemness-related biological behaviors of MSCs derived from bone marrow in order to enhance their immunoregulatory potency in alleviating inflammation in T lymphocytes and fibroblast-like synoviocytes of rheumatoid arthritis patients [124]. In other words, cytokines implicate in modulating the stemness of MSCs via diverse related pathways (Table 4), indicating that engineering MSCs to acquire specific cytokine secretion phenotypes could be a strategy for enhancing immunomodulatory efficacy.

4. Growth factors

Due to their physiological functions in encouraging cellular growth, proliferation, and differentiation, growth factors have the potential to enhance the efficacy of MSCs-based therapies (Table 5) (Fig. 6). The use of MSCs and growth factors in the treatment of bone fractures reported to be a promising therapeutic method [125]. Among diverse growth factors that may enhance the MSCs activities, here we discuss the following growth factors; suramin, and sphingosine-1-phosphate (SIP), TGF-β, PDGF, IGF1, fibroblast growth factors (FGF), and VEGF.

Scientists succeeded in the production of cardiac tissue in cell culture from human umbilical cord MSCs using growth factors, suramin and SIP by hanging drop technique [126]. Pretreatment of umbilical cord MSCs by TGF-β alter their immunomodulatory functions their extracellular vesicles [127]. Indeed, TGF-β overexpressed MSCs characterized by increased proliferation, normal cell cycle, and no apoptotic or senescence signs [128].

In vivo experiments reported that PDGF increased MSCs' extracellular vesicles protective action via increased expressions of IL-10 and TGF-β in PBMCs from acute muscle ischemia model [129]. IGF1 overexpression stimulated MSCs' immunomodulatory and regenerative potential in Chagas disease [130]. Basic FGF (b-FGF) improved the regenerative effect of MSCs of human amniotic fluid when combined with selenium [131], whereas MSCs which overexpressed FGF-2 were characterized by increased vascular regeneration properties when combined with xenogeneic antigen-extracted cancellous bone (XACB) [132]. In this context, VEGF increased MSCs' neuroprotective efficiency in rat with cerebral ischemia if co-overexpressed with brain-derived neurotrophic factor (BDNF) [133]. To be brief, many growth factors (Table 5) were proved to have a crucial role in enhancement of MSCs' immunomodulatory potency.

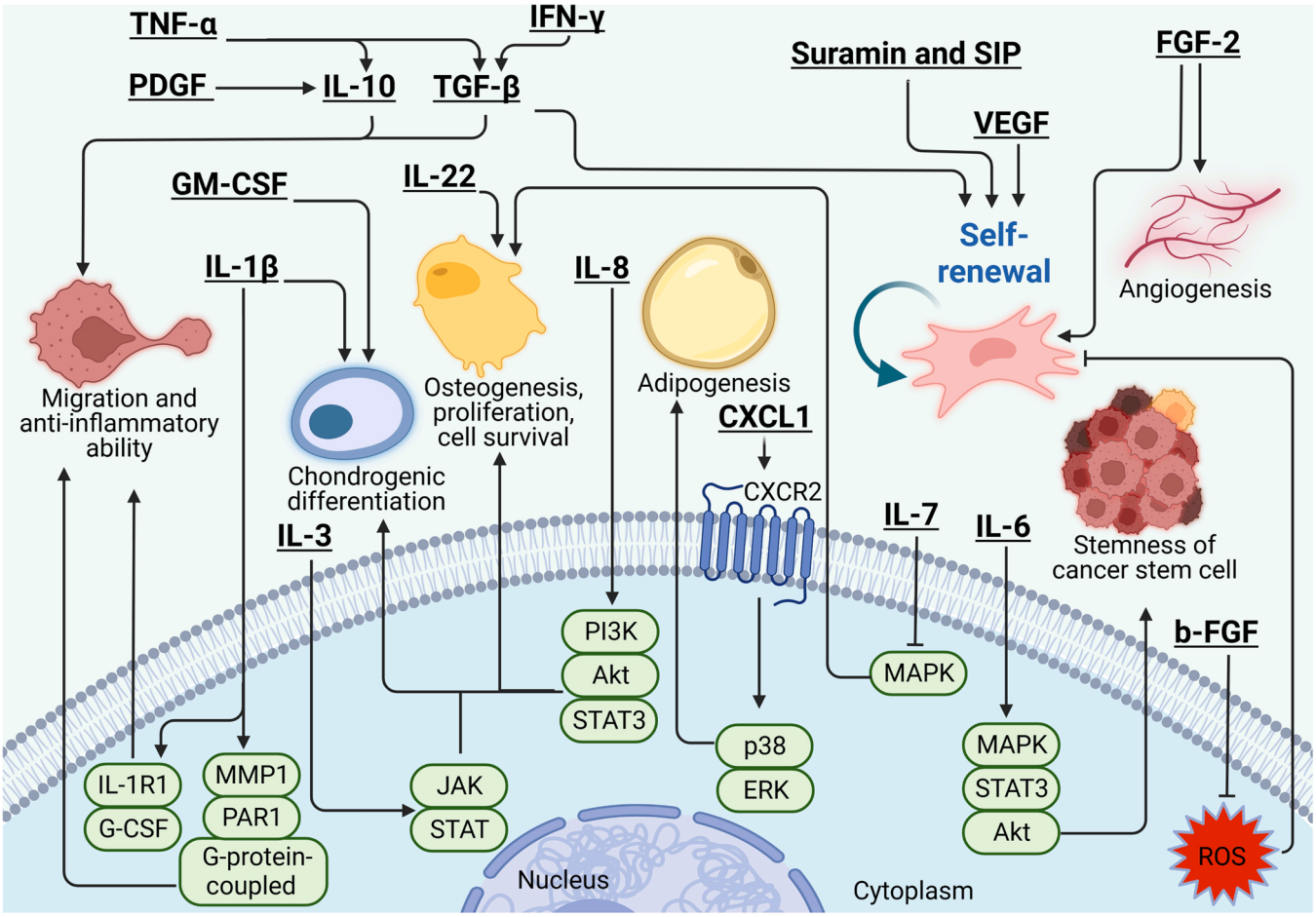

Fig. 6. Cytokines and growth factors enhance MSCs' stemness. TNF-α, IFN-γ, and PDGF regulate IL-10 and TGF-β to induce migration and anti-inflammatory ability of MSCs. IL-1β, IL-3, IL-8, IL-22, CXCL1, and GM-CSF activate osteogenesis, adipogenesis, chondrogenesis, proliferation, and cell survival through regulation of indicated signaling pathways. TGF-β, suramin, SIP, VEGF, FGF-2, and b-FGF contribute to MSCs' rejuvenation. IL-7 inhibits osteogenic differentiation through inhibiting MAPK pathway, while IL-6 induces stemness of cancer stem cell via MAPK/STAT3/Akt pathway. Activate or regulate (⟶), Inhibit (⟞), cytokine or growth factor (**bold** and underlined).

5. Hormones

In addition to their well-known functions in human physiology and endocrinology, hormones may potentially affect MSCs' therapeutic efficacy (Table 5) (Fig. 7). For example, leptin, a hormone generated from adipose tissue, inhibited the development of MSCs in rabbits by regulating ERK1/2 pathways [134], but melatonin enhanced their therapeutic effectiveness in diabetic rats [135]. In the same context, melatonin corrects iron overload-mediated biased differentiation and senescence of MSCs, and inhibits P53 and ROS [136].

Growth hormone increased osteogenesis at the expense of adipogenesis in MSCs through Wnt signaling [137] but inhibited adipogenesis by increased myogenesis [138]. Ghrelin, a ligand for the growth hormone, also triggered chondrogenic differentiation of MSCs through ERK1/2 [139]. Notably, higher circulating MSCs in postmenopausal osteoporosis patients following injection of the parathyroid hormone 1–34 (PTH 1–34) have been described. Also, after PTH administration, MSCs' osteogenesis was accelerated in vitro [140]. The glycoprotein hormone stanniocalcin 2 improved the immunoregulatory capabilities of MSCs in inhibiting activated T cells [141]. Erythropoietin hormone enhanced MSCs' regenerative potential by increasing their differentiation toward osteocytes using p38/MAPK pathway [142]. Another hormone, β-estradiol, was reported to protect MSCs from the damaging effects of oxidants via Nrf2/Sirt3/MnSOD pathway [143] as well as from caffeine through ER β/cAMP pathway [144].

Interestingly, pre-processing to enhance insulin activity half-life by incorporation of insulin-loaded poly lactic-co-glycolic-acid (PLGA) nanospheres with nono-hydroxyapatite/collagen (nHAC) scaffolds was reported to support proliferation, adhesion, differentiation of MSCs in vitro and induce rabbit bone regeneration in vivo [145]. In order to get insulin-producing cells from MSCs, scientists recommended stimulation by the gut hormone, obestatin [146].

MSCs pre-treatment with dihydrotestosterone also promoted their regeneration ability in regeneration of cardiac tissue through activating pro-angiogenic factors [147]. Oxytocin has also been used to recruit MSCs for therapeutic usage [148]. Finally, a peptide hormone, angiotensin II was recommended to enhance MSCs' adipogenic differentiation due to its activity mediated by angiotensin type 2 receptor [149]. To put it concisely, considering intrinsic or supplementary hormones in inducing MSCs' stemness is a promising strategy (Table 5).

6. Vitamins

Vitamins are essential co-factors in eukaryotes' metabolism because they induce anti-oxidants mechanisms. Recent research investigated the relevance of vitamins to increase MSCs' therapeutic efficacy (Table 5) (Fig. 7). For instance, in vitro and in vivo treatment of MSCs with ascorbic acid 2-glucoside enhanced proliferation, migration, and angiogenesis. These effects were attributed to demethylation process by regulating TET2 and VEGF expression through inducing PI3K/Akt

Table 5
Growth factors, hormones, and vitamins used in research to modulate the MSCs' stemness.

Enhancer	Mode of action	Output	Ref.
Growth factors			
Suramin and SIP	Inducing differentiation ability of MSCs	Promote MSCs' regenerative potency	[126]
TGF-β	Modulation of MSCs' immunomodulatory functions	Induces cell survival and rejuvenation	[127], [128]
PDGF	Activation of anti-inflammatory cytokine, IL-10	Induces MSCs' therapeutic potency	[129]
IGF1	Modulation of MSCs' immunomodulatory functions	Induces MSCs' therapeutic potency	[130]
b-FGF	Inhibiting ROS accumulation	Promotes MSCs' regenerative potency	[131]
FGF-2	Inducing angiogenesis and osteogenesis	Promotes MSCs' regenerative potency	[132]
VEGF	Increasing MSCs' neuroprotective efficiency in rat.	Promotes MSCs' regenerative potency	[133]
Hormones			
Leptin	Regulation ERK1/2 pathways	Inhibits MSCs growth	[134]
Melatonin	Activates antioxidants' mechanisms	Induces MSCs stemness	[135]
Growth hormone	Through Wnt pathway	Corrects biased MSCs' differentiation	[137]
Ghrelin	Via ERK1/2 pathway	Stimulates chondrogenesis of MSCs	[139]
PTH 1–34	Through inducing ALP activity and mineralization	Promotes MSCs' osteogenesis	[140]
Stanniocalcin 2	By regulating oxygenase 1 (HO-1)	Improves MSCs' immunoregulatory ability	[141]
Erythropoietin	Through p38/MAPK pathway	Induces MSCs' osteogenesis	[142]
β-estradiol	By Nrf2/Sirt3/MnSOD pathway	Exerts antioxidant effects	[143]
Insulin	Through increasing MSCs' proliferation, adhesion, and differentiation using PLGA-nHAC scaffold	Enhances bone regeneration	[145]
Obestatin	-	Promotes differentiation to β-cells	[146]
Dihydrotestosterone	By upregulation of androgen receptors	Induces MSCs' therapeutic potency	[147]
Oxytocin	Inducing cell survival and antioxidants' mechanisms	Induces MSCs' therapeutic potency	[148]
Angiotensin II	Through angiotensin type 2 receptor	Enhances MSCs' adipogenesis	[149]
Vitamins			
Ascorbic acid	Increasing expression of decorin	Promotes MSCs' differentiation	[151]
Retinoic acid	Inducing MSCs' differentiation into neuroretinal cells in presence of taurine	Promotes MSCs' differentiation	[158]
Vitamin K2	Activating Runx2 / Wnt/β-catenin pathway using DKMF	Promotes MSCs' osteogenesis	[157]
Vitamin D3	Activating expression of integrin, and TGF-β by ERK/JUNK pathway using DKMF [157]	Improves MSCs' osteogenesis and chondrogenesis	[154,155]
Ado B12	Using 3D cell culture and photoresponsive protein hydrogels	Maintains MSCs' viability in vitro	[159]
Vitamin E	Maintains MSCs's viability and proliferation	Induces MSCs' therapeutic potency	[160]

pathway [150]. Ascorbic acid and b-FGF contribute to the tenogenic differentiation of MSCs from adipose tissue and bone marrow, and tendon cells [151]. Indeed, ascorbic acid and iron induce MSCs potency in minipigs knee joints chondrogenesis [152]. Consistently, L- ascorbic acid involved in adipose tissue MSCs chondrogenic differentiation combined with platelets rich plasma on silk fibroin scaffold [153].

Vitamin D was reported as an activator for osteogenesis ability of MSCs through expression of $\alpha_v\beta3$ integrin modulation [154], and chondrogenesis through TGF-β/ERK/JUNK pathway regulation [155]. Recently, it has been reported that vitamin D_3 can partially modulate Sirt1 and then promote stemness and osteogenesis of MSCs from human bone marrow [156]. Vitamin D_3, K2, and magnesium loaded in composites nanofibers (DKMF) promote the osteogenesis of MSCs, and the underline mechanism is upregulation of Runx2 and downregulation of PPARγ through activation of Wnt/β-catenin pathway [157].

Retinoic acid with taurine promotes the differentiation of human bone marrow-derived MSCs into neuroretinal or photoreceptor-like cells in vitro [158]. Moreover, adenosylcobalamin B12 (Ado B12)-dependent photoresponsive protein hydrogels may promote MSCs' viability in 3D culture [159]. Furthermore, vitamin E treated Wharton's jelly MSCs displayed more efficient therapeutic output in the treatment of breast cancer [160] and liver fibrosis [161]. Concisely, vitamins, A, C, E, D3, B12, and K3 can modulate the therapeutic potency of MSCs through different mechanisms (Table 5).

7. Enhancing MSCs' stemness in human body

Based on the above enhancers, it is becoming possible that clinically approved modulators could be used to improve the stemness of MSCs in vivo as a way to prevent aging [14] and treat diseases related to aging. Finding fully clinical proof for the biomolecules discussed above is an urgent need in order to introduce novel strategies in fighting diseases such as autoimmune diseases, cardiovascular diseases, and cancer. More basic details about this section were explained in the previous parts of this review.

More important, following the protocols to modify lifestyle and improve the quality of life may naturally incite our body MSCs' stemness without pharmacological intervention. This can contribute to avoid suffering from a wide variety of diseases, including aging-related diseases. Lifestyle modifications include, exercise on a regular basis, appropriate nutritious diet, adequate and high-quality sleep, quitting smoking, and fighting pollution (Fig. 8). For example, it is has been reported that obesity can cause disruption in the bone marrow microenvironment through regulating many molecular modulators, and this has an effect on bone marrow MSCs [162]. Obesity was identified as a major factor that could lead to global genomic epigenetic alterations [163], induce inflammation and adipogenesis, and suppress proliferation and osteogenesis of MSCs [164]. In obese children, MSCs favored differentiation toward adipocytes which in turn promote pro-inflammatory cytokines to activate osteoclast, thereby promoting bone fragility [165]. Although the injection of human MSCs in mice has anti-obesity effects by inducing upregulation of uncoupling protein-1 [166], the markers of cancer stem cells were promoted by colonic MSCs through regulation of Bas-FXR pathway in mice with a high-fat diet [167]. High-fat diet in rats may also predispose MSCs' dysfunction characterized by CXCL2/ROS activation and senescence [168]. In addition, high glucose microenvironment containing GSK3β suppressed MSCs' stemness by inhibiting osteogenesis in diabetic mice by inactivation of β-catenin/Tcf7/Ccn4 pathway [169]. However, combining a fasting-mimicking diet and MSCs in mice boosts the therapeutic output in treatment of type 2 diabetes by decreasing hyperglycemia and inducing the metabolism of fat [170]. Thus, keeping a lower body mass index by following the different clinically approved and optimized dietary systems may improve the body MSCs' stemness, thereby having healthier body away from obesity-related diseases.

Beside diet systems, the knowledge about nutritional composition of daily food may introduce some key points about the most important bioactive compounds that can induce MSCs' biological activities. In the

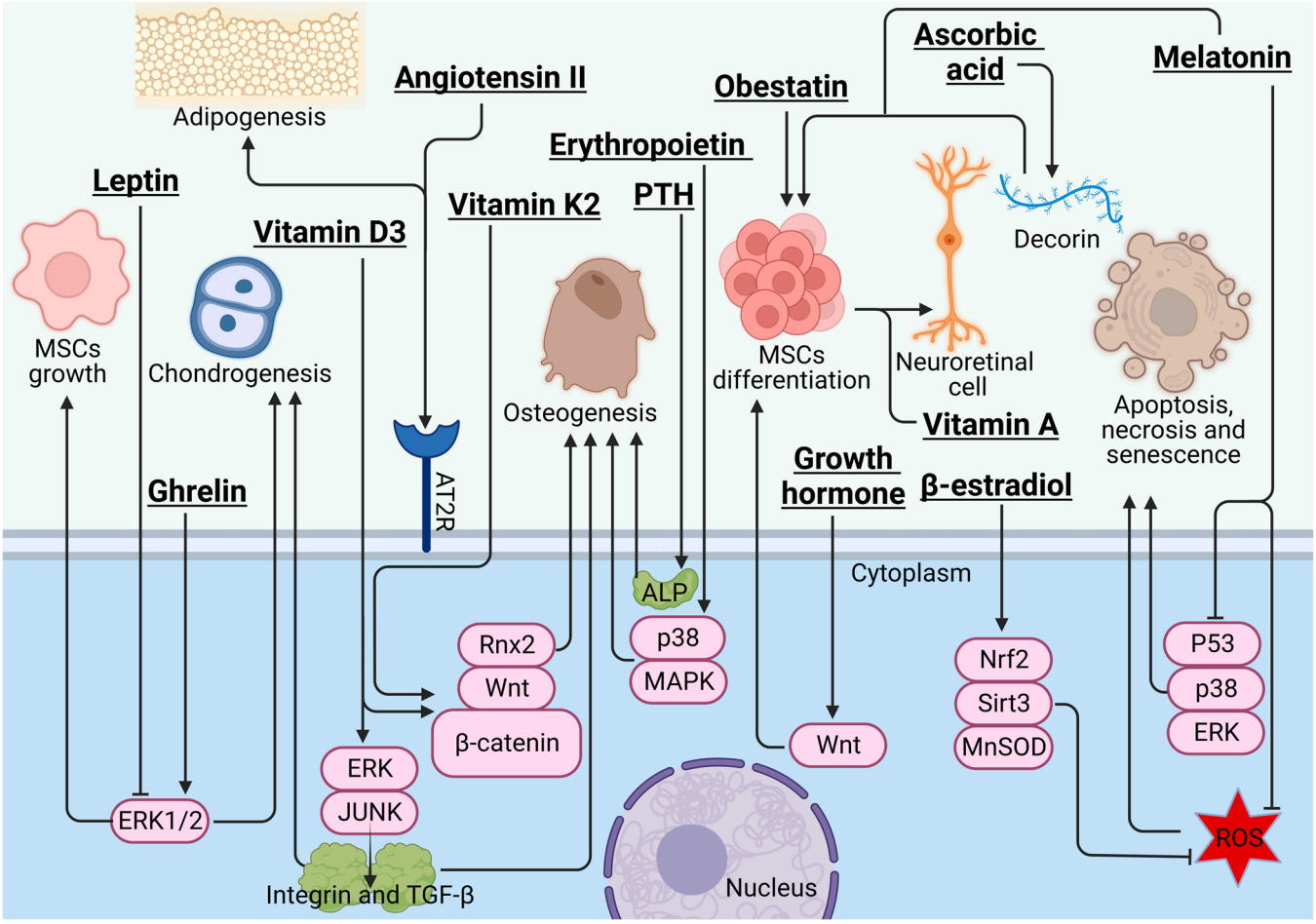

Fig. 7. Hormones and vitamins enhance MSCs' stemness. Leptin and ghrelin regulate MSCs' growth and differentiation through ERK pathway. Vitamin D_3, Vitamin K_2, PTH, Erythropoietin, and angiotensin II induce osteogenesis, adipogenesis, and chondrogenesis of MSCs by regulating indicated pathways. Vitamin D_3 promotes expression of integrin and TGF- β through ERK/JUNK pathway. Vitamin K_2 increased expression of Runx2 by activating Wnt/β-catenin pathway, while PTH induce expression of ALP. Growth hormone, obestatin, ascorbic acid, and tetinoic acid improve MSCs' differentiation. Growth hormone activates Wnt signaling, ascorbic acid induce decorin expression, and retinoic acidpromotes MSCs' differentiation toward neuroretinal cell. Melatonin maintains MSCs survival and reverses senescence through inhibiting oxidative stress, and regulating ERK/P38 pathway and p53. β-estradiol has antioxidant role in MSCs via activating Nrf2/Sirt3/MnSOD pathway. Activate or regulate (⟶), Inhibit (⟞), hormone or vitamin (**bold** and underlined).

literature, there are plenty of articles that explain this issue, and we regret that we cannot include them all here. As a representative example, the bioactive compounds found in olive oil were considered to have positive effects on MSCs' proliferation, differentiation, viability and regenerative capacity, the detailed aspects of olive oil outputs were reviewed in this study [171]. Inducing MSCs' osteogenesis at the cost of adipogenesis is exerted by one polyphenol found in olive tree products, oleuropein. This effect was attributed to decreasing expression of PPARγ, suggesting olive tree products to be used in treatment of bone loss and osteoporosis [172]. Moreover, EGCG, a polyphenol derived from green tea inhibited the expression of IL1β, IL-6, CCL2, and CCL5 in MSCs treated by a conditioned medium of triple-negative breast cancer cell [173]. In sum, optimizing diet is more than just a diet; it should also include nutrients that have been proven to have clinical significance.

Physical exercise, and enough and high-quality sleep are significant determinants of our life quality; both have a favorable impact on the general health, and the health of MSCs is not far behind. For instance, MSCs' therapeutic potency in animal models was improved due to the physical exercises [174,175]. The training exercise can change in the mechanisms of amino acid metabolism in adipocytes derived from MSCs [176]. More interesting, aerobic exercises for pregnant women may improve the biological activities of MSCs isolated from their neonates [177]. In addition, it is reviewed that exercise can reduce MSCs' SASP, thereby decreasing MSCs' exhaustion and inducing osteogenesis [178]. Indeed, the combination of exercises and MSCs was proposed as a promised strategy in treatment of multiple sclerosis [179]. Collectively, regular exercise could contribute positively to the MSCs' stemness in vivo. In relation to sleep, engineering MSCs to produce sleep-related circRNA3503-loaded exosomes induced the therapeutic outputs in preventing osteoarthritis progression [180]. This may indicate the importance of high-quality sleep in improving the stemness of MSCs in vivo.

Environmental factors such as smoking and pollution can contribute to disruptions in MSCs' physiological activities. Here we show findings from five recently published articles. However, there are many more reports in the same issue that we can't show because of space constraints. It is reported that the regenerative potential of MSCs was compromised by smoking and nicotine use. These habits mediate unease proliferation, differentiation, and migration of MSCs [181]. A comparative review study between MSCs and dental stem cells showed that nicotine can violate the main biological activities of both of them [182]. It is reviewed that inducing oxidative stress because of smoking and nicotine use is one of the major mechanisms which interrupt the therapeutic potency of MSCs [183]. On the basis of these findings, we may conclude that quitting smoking and nicotine usage can increase stemness in MSCs in vivo, hence preventing a wide range of disorders caused by compromised MSCs. Concerning pollution, the mixture containing

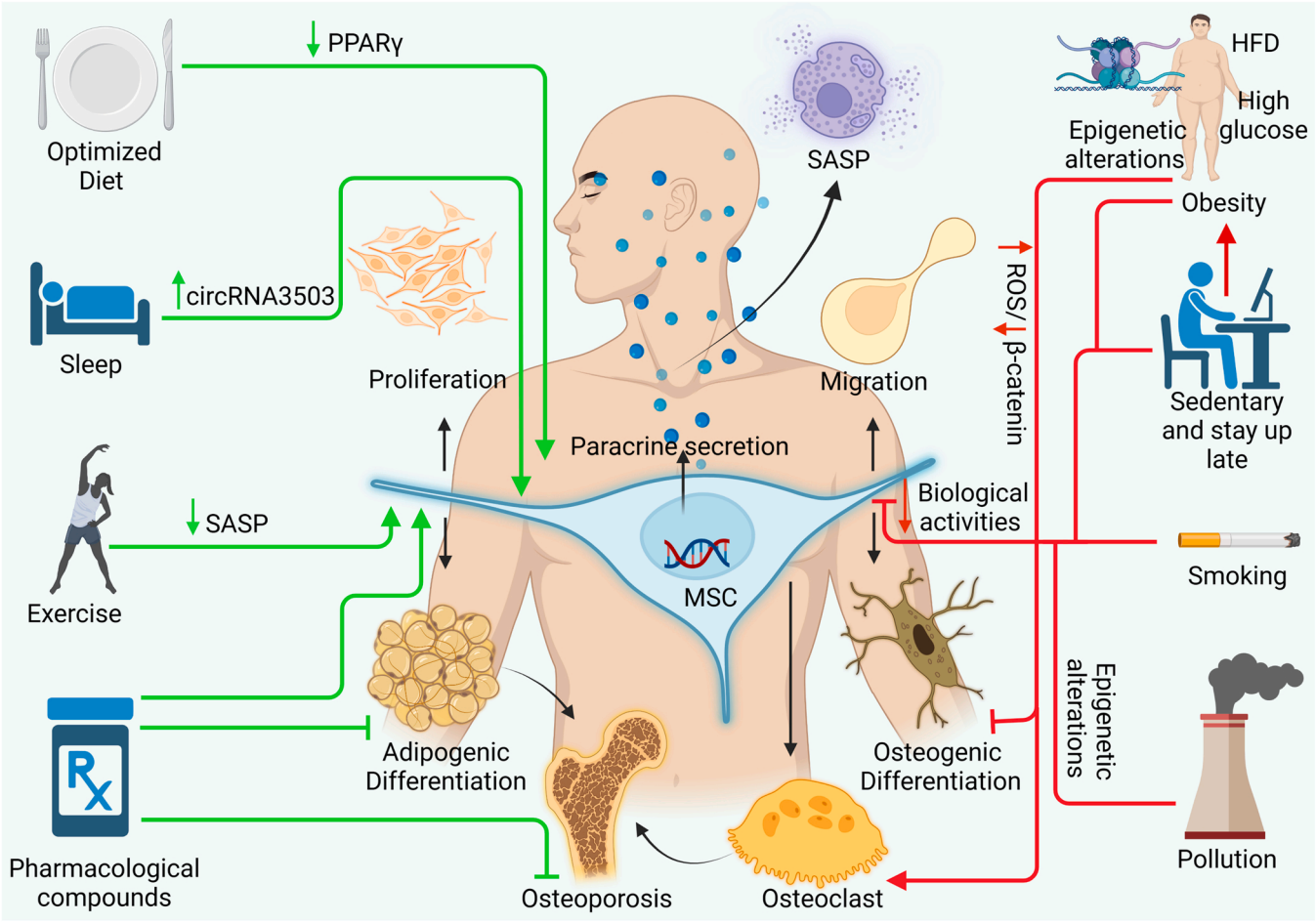

Fig. 8. Enhancers of human MSCs' stemness in vivo. Proper diet with healthy nutritious contents, sleep, exercise, and clinically approved medications may maintain the biological activities of human body' MSCs in vivo through regulating PPAR-γ, circRNA3503-loaded exosomes, SASP, and skewed differentiation respectively. Conversely, obesity, sedentary, disturbed sleep, smoking, and pollution can downregulate MSCs' biological activities in vivo by alternating epigenetics and inducing oxidative stress. Activate or regulate stemness (⟶, green), Inhibit stemness (⟙, red).

many pollutants, endocrine disrupting chemicals induced MSCs' adipogenesis and epigenetic alteration [184]. Another pollutant, cadmium, also affected the physiological activity of MSCs-derived adipocytes by inducing expression of proinflammatory cytokines, IL-1β, IL-6, and CCL2 [185]. To preserve the stemness of in vivo MSCs, we must avoid polluting resources and maintain a pollution-free environment.

8. Conclusion remarks and future prospects

Recent and current research has explored a variety of facts for improving the therapeutic efficacy of MSCs-based therapies, each with its own set of advantages and disadvantages. Methodologies targeted at increasing potency by increasing stemness factors expression showed promising outputs. Clinical trials assessing some of the MSCs-based product advancements should begin in the next years. The exact enhancements examined will be chosen based on clinical or/and commercial issues such as safety, the convenience of use, potential toxicity, and cost, as well as their performance in preclinical models.

MSCs' therapy is entering a new era, with the focus shifting from initial feasibility studies to improvements in therapeutic regimen and treatment potency. In this context, the current research knowledge suggested many compounds to improve the therapeutic potency and stemness of MSCs. Among these are metformin, resveratrol, antioxidants, mTOR inhibitors, cytokines, hormones, growth factors, and vitamins. In addition, improving in vivo viability and stemness of MSCs by lifestyle modifications or clinically approved pharmacological interventions may contribute effectively to combat aging-related diseases and improving general health quality. Complete identification and understanding of the molecular mechanisms associated with these compounds and activities could lead to the development of effective mixtures of cell-free trophic factors that can be used to treat tissue damage and degeneration, thereby eliminating the risk of MSC transformation. However, major difficulties such as poor stem cell potency and age/disease-related host tissue deterioration may temper enthusiasm for stem cell translation in general. The methodologies discussed in this review provide a testable foundation for launching innovative clinical trials based on MSCs treatment rational design. Engineered or enhanced MSCs have been used to treat a variety of diseases and conditions in animal models with great effectiveness. However, it is crucial to note that animal models may not be comparable to human diseases and conditions. As a result, the findings should be regarded with caution, as a translational trial in humans has yet to yield a positive result. Nonetheless, MSCs-based therapy remains a promising novel therapeutic approach for a variety of conditions, and it is obvious that inducing the expression of favorable factors can improve its therapeutic effects. More research is needed to accelerate the translation of preclinical discoveries into clinical practice.

Funding

This work was funded by Guangzhou Women and Children Medical Center, Guangzhou Institute of Pediatrics.

CRediT authorship contribution statement

MA designed the review. MA, EI, MS assisted language. MA, MS, EI, KH aided in literature analysis. MA, EI drawing the figures. MA, MS drafted the manuscript. MA supervised the review. All authors read and approved the manuscript.

Acknowledgments

The figures created with BioRender.com. We appreciate the help of Marwan Almoiliqy, Salah Adlat, Fadhl Al-Shaebi, and Mohammed Alradhi in topical discussions about this review.

Conflict of interest

The authors declare none.

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