PROMOTING HEALTHY STEM CELL FUNCTION

Stem cells are undifferentiated cells that have the ability to develop into different types of specialized cells. They are the seeds for regeneration, growth, and repair of the body's cells and tissues throughout life. Aging has profound impacts on stem cell numbers and function, leading to the many and well-known ailments that distinguish old age.[i] Promoting healthy stem cell function is therefore thought to be key to slowing and possibly reversing some of the ravages of aging.[ii]

A growing body of evidence suggests that stem cell rejuvenation may be achievable through altering the stem cell environment to promote more youthful signaling.[iii] Stem cell function is regulated in part by a family of NAD^+-dependent enzymes known as sirtuins, which are identified as SIRT1–7.[iv] [v] Enhancing SIRT expression and activity, such as through long-term calorie restriction, promotes normal stem cell activity and is considered an important anti-aging strategy.[vi] [vii] Inhibitors of age-related signaling by factors such as TNF-alpha, TGF-beta, fibroblast growth factor, mammalian target of rapamycin (mTOR), guanosine triphosphate, and cell division control protein 42, may also contribute to stem cell rejuvenation.[viii]

As researchers continue to explore the possibility of stem cell rejuvenation, questions of whether this can lead to more youthful functioning of cells, tissues, organs, organ systems, and indeed the entire human organism, remain unknown.[ix]

Stem Cell Therapy

Mesenchymal stem cells (MSCs) are multipotent stem cells found in nearly every tissue in the body. As multipotent stem cells, they are not able to differentiate into every cell type in the body (that would make them pluripotent), but they are capable of differentiating into an array of cell types, including fat, bone, and cartilage progenitor cells. Their regenerative capacity, which is subject to SIRT regulation, is finite, diminishing with age.[x]

MSCs that are harvested (generally from bone marrow, adipose, or other connective tissues) and cultured in the laboratory are currently being investigated for their potential usefulness when implanted in damaged or diseased tissues. Much of the research to date has focused on treating degenerative conditions of muscles, bones, and joints. Researchers are also exploring MSCs' immunomodulatory signaling to determine the indirect mechanisms by which they exert their tissue benefits, giving them wider potential applications, such as in autoimmune, inflammatory, cardiovascular, metabolic, and neurodegenerative conditions.[xi] [xii] So far, none of these indications have received FDA approval and the use of MSC therapy remains somewhat controversial.[xiii]

Phytochemicals: Natural Promoters of Stem Cell Activity

Polyphenolic compounds from plants are widely accepted as having broad protective effects on cells and tissues, presumably mediated by antioxidant, anti-inflammatory, and cancer-preventive mechanisms.[xiv] Recent research further suggests that some of these compounds also stimulate normal stem cell function, which may contribute to their benefits in certain diseases of aging.

Resveratrol
Resveratrol is a polyphenol that has attracted attention as a possible stem cell-promoting, anti-aging compound. Clinical trials indicate its potential benefits in numerous disorders associated with inflammation and metabolic disturbance, and resveratrol has been attributed with some of the positive effects of the Mediterranean diet. Resveratrol can activate sirtuin enzymes and, at high concentrations, appears to favorably alter gene expression and slow cellular senescence.[xv]

Numerous studies show that adding resveratrol to stem cell cultures can increase survival, stimulate proliferation and differentiation, and reverse the inhibitory effects of certain toxins. These effects have been seen in MSCs from various sources as well as cardiac and neural stem cells.[xvi]

In a clinical trial, 30 adults aged 65–80 years old participated in a 12-week exercise program while taking either 500 mg/day of resveratrol or placebo. At the end of the trial, those taking resveratrol experienced a 20% greater increase in number of muscle satellite (stem) cells, 45% greater increase in muscle fiber size, and 14% greater increase in average peak muscle torque (rotational force). In addition, density of muscle cell mitochondria increased more in the resveratrol-treated subjects compared with placebo. Because of its apparent anabolic effect on exercising muscles, resveratrol may be useful for preventing or reversing sarcopenia—the age-related loss of muscle mass.[xvii] In another clinical trial, red wine consumption was found to increase the number of endothelial progenitor (stem) cells in circulation more than water, beer, or vodka in healthy young adults, and resveratrol treatment of endothelial progenitor cells in culture increased their activity and prevented senescence.[xviii]

Epigallocatechin gallate
Epigallocatechin gallate (EGCG) is considered to be the most active polyphenol in green tea, responsible for many of its well-known anti-inflammatory and antioxidant properties, as well as its cancer-fighting potential. Numerous reports from animal and laboratory studies suggest it may enhance benefits of MSC therapy in tissues such as bone,[xix] [xx] [xxi] [xxii] [xxiii] [xxiv] cartilage,[xxv] muscle,[xxvi] [xxvii] and skin.[xxviii] It has also been found to increase the number of neurons in brains of adult mice by promoting neuronal stem cell survival and differentiation;[xxix] improve neuronal stem cell function in inner ear tissue harvested from mice; [xxx] and promote growth of new neurons and recovery of function in mice subjected to experimental stroke. [xxxi]

Laboratory research has shown that EGCG can protect cultured MSCs from the damaging effects of oxidative stress,[xxxii] possibly in part by activating the cells' inherent antioxidant production through epigenetic effects.[xxxiii] In animal research, mice given oral EGCG before and after experimental traumatic brain injury experienced reduced trauma-related free radical damage to neuronal stem cells.[xxxiv] Another study in diabetic rats noted that adipose-derived MSCs stimulated cardiac muscle repair and improved cardiac function more effectively in those receiving oral EGCG, and appeared to do so by activating cell-survival signaling.[xxxv]

Quercetin
The polyphenolic compound, quercetin, is considered a senolytic agent—one that helps to clear aged, dysfunctional cells and promotes rejuvenation of the organism. In numerous in vitro

studies, quercetin has been found to stimulate proliferation and differentiation of cultured MSCs through mechanisms including antioxidant, anti-inflammatory, and epigenetic effects.[xxxvi] [xxxvii] [xxxviii] [xxxix] [xl] [xli] It was also reported to reverse senescence in human MSCs in a laboratory model of premature aging.[xlii] A study in rats showed that quercetin enhanced the effectiveness of MSC therapy after loss of blood supply to the brain, reducing markers of inflammation, increasing MSC survival, and improving recovery.[xliii] Other studies in rats have found that quercetin treatment can increase the number and improve the function of neural stem cells, resulting in better cognitive and behavioral performance, after brain injury.[xliv] [xlv]

Curcumin

Curcumin, a carotenoid found in turmeric, has been the subject of numerous clinical trials for a wide range of chronic and age-related conditions. Studies in nematodes and mice suggest curcumin can increase lifespan. Its possible anti-aging effects may be related to its ability to increase sirtuin levels. It has also been shown to induce epigenetic changes that result in reduced cellular free radical production and promote youthful cell function.[xlvi]

Experiments using MSC cultures suggest that curcumin may improve MSC therapy-related cartilage repair by suppressing inflammatory signaling and preventing chondrocyte hypertrophy that can lead to scarring and limit the effectiveness of MSC therapy.[xlvii] [xlviii] Curcumin also appears to stimulate MSC differentiation and function in laboratory models of bone regeneration, even in a high-oxidative stress environment.[xlix] [l]

Curcumin has been found to increase survival and proliferation of adipose-derived[li] and bone marrow-derived MSCs,[lii] as well as neural stem cells,[liii] in the laboratory. In one study, MSCs cultured in a solution containing curcumin showed increased proliferation and production of collagen matrix compared with those cultured without curcumin. When implanted in experimental wounds in mice, the curcumin-treated MSCs promoted faster and more complete skin repair with less scarring.[liv] In rats, treatment with curcumin enhanced neural stem cell activity and lesion healing after spinal cord injury.[lv]

Bitter melon

Bitter melon (*Momordica charantia*) is a phytochemical-rich vegetable cultivated as both food and medicine in many parts of the world. It has demonstrated antioxidant, anti-inflammatory, and anti-cancer properties, and gained popularity for its ability to improve insulin sensitivity and glucose control.[lvi] In mice, bitter melon has been found to reverse the negative impact of a high fat diet on glucose metabolism, insulin sensitivity, and fatty liver,[lvii] as well as sirtuin production and levels of inflammatory cytokines,[lviii] including one linked to healthy stem cell activation and tissue regeneration (IL-17).[lix]

Connective Tissue Components

Glycosaminoglycans (GAGs) are a crucial part of the extracellular matrix of tissues throughout the body. Within the extracellular tissue space, GAGs interact with immune and growth factors to promote stem cell-based tissue regeneration. Hyaluronic acid, glucosamine sulfate, and chondroitin sulfate are well-known GAGs that are widely used as supplements to treat

degenerative joint disease. Their usefulness in promoting tissue repair and regeneration through stem cell activation and immune modulation is drawing the interest of researchers.[lx] [lxi]

In vitro evidence suggests the presence of GAGs in the MSC culture environment can help to promote differentiation into functional chondrocytes (cartilage-producing cells) and increase cartilage production. In particular, chondroitin sulfate,[lxii] [lxiii] [lxiv] glucosamine sulfate,[lxv] [lxvi] [lxvii] and hyaluronic acid plus dermatan sulfate[lxviii] have each been noted to increase stem cell-induced cartilage production. The addition of collagen, a structural extracellular protein, into a GAG-enriched culture medium also appears to encourage proper MSC differentiation and chondrocyte function.[lxix] [lxx] [lxxi] Chondroitin sulfate and other GAGs have also been shown to have stem cell-promoting effects when added to the culture medium for MSCs being primed for bone regeneration[lxxii] [lxxiii] [lxxiv] [lxxv] and skin regeneration.[lxxvi]

Other Natural Compounds

Melatonin

Melatonin is one of the most extensively studied natural promoters of stem cell activation.[lxxvii] [lxxviii] A growing body of research suggests it has anti-aging, regenerative potential via its effects on stem cells in tissues such as cardiac muscle, blood vessel endothelium, brain and peripheral nerves, dental pulp, testes and ovaries, kidney, and liver.[lxxix] [lxxx] [lxxxi] [lxxxii] Numerous in vitro and animal studies suggest melatonin may increase the efficacy of MSC therapy by improving MSC viability, proliferation, and differentiation into cartilage, bone, skin, and muscle cells. [lxxxiii] [lxxxiv] [lxxxv] [lxxxvi] [lxxxvii] [lxxxviii] [lxxxix] [xc] [xci] Melatonin has also been shown to improve the positive effects of MSC therapy on pancreatic beta-cell activity in diabetic rats;[xcii] enhance nerve cell regeneration and functional recovery by stimulating neural stem cells in rats with experimentally-induced spinal cord injury;[xciii] and, increase regenerative capacity of neural stem cells in mice with Alzheimer's and Parkinson's diseases.[xciv]

Melatonin's well-known anti-inflammatory and antioxidant properties contribute to its protective effects on stem cells.[xcv] [xcvi] Researchers have noted melatonin protects various types of stem cells from toxic effects of pro-oxidants,[xcvii] [xcviii] [xcix] inflammatory cytokines,[c] [ci] [cii] oxygen deprivation,[ciii] [civ] iron-overload,[cv] bacterial toxins,[cvi] and some drugs.[cvii] [cviii]

Melatonin may promote stem cell activation through several mechanisms. It appears to inhibit osteoclast- and induce osteoblast-differentiation in mice in part by acting on melatonin receptors.[cix] For example, in rats with experimentally fractured femurs treated with MSC therapy, melatonin increased MSC differentiation into osteoblasts and encouraged bone healing.[cx] In vitro evidence suggests that interactions with components of the extracellular matrix also contribute to melatonin's ability to stimulate MSCs.[cxi] In addition, new research suggests melatonin can induce positive epigenetic changes that support stem cell activation.[cxii] [cxiii] [cxiv]

Treatment of cultured neural stem cells with melatonin, which is produced not only in the pineal gland but also in mitochondria throughout the body, increased their mitochondrial mass and activity, and appeared to protect the stem cells against elevated oxidative stress produced by mitochondrial activity.[cxv] Melatonin's pro-mitochondrial and other antiaging effects, including

cancer prevention, may be related to its ability to stimulate sirtuin production and activity.[cxvi] [cxvii] [cxviii] [cxix]

NT-020

NT-020 is a proprietary nutrient combination containing blueberry, green tea, vitamin D3, and carnosine. A research team in Tampa, Florida has performed a series of preclinical studies to examine its effects on stem cells. They reported these compounds synergistically stimulate proliferation of several types of human stem cells,[cxx] induce resiliency to oxidative stress, and increase neural stem cell expression of genes associated with nerve cell regeneration.[cxxi] In one report, they noted that NT-020 protected stem cells from oxidative damage both in vitro and in mice given NT-020 orally.[cxxii] Another in vitro study from the same research group suggested that the addition of spirulina to NT-020 treatment of human bone marrow stem cells led to even greater stem cell activation and proliferation.[cxxiii]

This research team has also published animal studies highlighting the anti-aging potential of NT-020. In aged rats, oral supplementation with NT-020 for four weeks led to better cognitive performance, increased neural stem cell proliferation, and greater nerve regeneration compared with placebo (water).[cxxiv] Another study included three groups of rats given identical diets: young rats; aged rats; and aged rats given NT-020 for 28 days. At the end of the study period, cultured rat neural stem cells and MSCs were exposed to serum collected from each group. Serum from the untreated aged rats inhibited stem cell proliferation, but serum from young rats and NT-020-treated aged rats preserved stem cell proliferative capacity.[cxxv]

Conclusions

Stem cells hold the key to rejuvenation and longevity, and it is increasingly clear that factors in the stem cell environment can have a critical impact on stem cell viability and function. There are several recognized mechanisms through which environmental factors can promote stem cells:

- **Reducing oxidative stress**: protecting stem cells from oxidative injury through direct or indirect antioxidant activity
- **Immunomodulation**: balancing immune signaling to stimulate controlled stem cell proliferation and activation without triggering harmful inflammatory processes
- **Epigenetic alteration**: increasing the expression of genes related to youthful cell function, such as those encoding sirtuin enzymes

Certain phytochemicals, extracellular matrix compounds, and melatonin are among the natural anti-aging therapies showing promise in preclinical research as promoters of healthy stem cell function. So far, however, there is little human data on which to draw conclusions. This is important because the complex stem cell environment in living humans includes factors that may interact to impact cell function and senescence in ways that are not yet completely understood. Future controlled trials in which human subjects undergoing MSC therapy are treated with these oral supplements will provide more clues into their usefulness in supporting stem cell-initiated tissue repair and regeneration.

References:

[i] Dang W. The controversial world of sirtuins. *Drug Discov Today*. 2014;12:e9-e17.

[ii] Ullah M, Sun Z. Stem cells and anti-aging genes: double-edged sword-do the same job of life extension. *Stem Cell Res Ther.* 2018;9(1):3.

[iii] Honoki K. Preventing aging with stem cell rejuvenation: Feasible or infeasible? *World J Stem Cells.* 2017;9(1):1-8.

[iv] Dang W. The controversial world of sirtuins. *Drug Discov Today*. 2014;12:e9-e17.

[v] Buler M, Andersson U, Hakkola J. Who watches the watchmen? Regulation of the expression and activity of sirtuins. *FASEB J.* 2016;30(12):3942-60.

[vi] Yu A, Dang W. Regulation of stem cell aging by SIRT1 - Linking metabolic signaling to epigenetic modifications. *Mol Cell Endocrinol.* 2017;455:75-82.

[vii] Zainabadi K. The variable role of SIRT1 in the maintenance and differentiation of mesenchymal stem cells. *Regen Med.* 2018;13(3):343-56.

[viii] Honoki 2017.

[ix] Honoki 2017.

[x] Denu R. SIRT3 Enhances Mesenchymal Stem Cell Longevity and Differentiation. *Oxid Med Cell Longev.* 2017;2017:5841716.

[xi] Ayala-Cuellar A, Kang J, Jeung E, Choi K. Roles of Mesenchymal Stem Cells in Tissue Regeneration and Immunomodulation. *Biomol Ther.* 2018. Epub ahead of print.

[xii] Phelps J, Sanati-Nezhad A, Ungrin M, et al. Bioprocessing of Mesenchymal Stem Cells and Their Derivatives: Toward Cell-Free Therapeutics. *Stem Cell Int.* 2018;2018:9415367.

[xiii] Bauer G, Elsallab M, Abou-El-Enein M. Concise Review: A Comprehensive Analysis of Reported Adverse Events in Patients Receiving Unproven Stem Cell-Based Interventions. *Stem Cells Transl Med.* 2018;7(9):676-85.

[xiv] Attari F, Zahmatkesh M, Aligholi H, et al. Curcumin as a double-edged sword for stem cells: dose, time and cell type-specific responses to curcumin. *Daru.* 2015;23:33.

[xv] McCubrey J, Lertpiriyapong K, Steelman L, et al. Effects of resveratrol, curcumin, berberine and other nutraceuticals on aging, cancer development, cancer stem cells and microRNAs. *Aging.* 2017;9(6):1477-536.

[xvi] Safaeinejad Z, Kazeminasab F, Kiani-Esfahani A, et al. Multi-effects of Resveratrol on stem cell characteristics: Effective dose, time, cell culture conditions and cell type-specific responses of stem cells to Resveratrol. *Eur J Med Chem.* 2018;155:651-7.

[xvii] Alway S, McCrory J, Kearcher K, et al. Resveratrol Enhances Exercise-Induced Cellular and Functional Adaptations of Skeletal Muscle in Older Men and Women. *J Gerontol A Biol Sci Med Sci.* 2017;72(12):1595-606.

[xviii] Huang PH Chen Y, Tsai H, et al. Intake of red wine increases the number and functional capacity of circulating endothelial progenitor cells by enhancing nitric oxide bioavailability. *Arterioscler Thromb Vasc Biol.* 2010;30(4):869-77.

[xix] Liu W, Fan J, Xu D, et al. Epigallocatechin-3-gallate protects against tumor necrosis factor alpha induced inhibition of osteogenesis of mesenchymal stem cells. *Exp Biol Med.* 2016;241(6):658-66.

[xx] Qiu Y, Chen Y, Zeng T, et al. EGCG ameliorates the hypoxia-induced apoptosis and osteogenic differentiation reduction of mesenchymal stem cells via upregulating miR-210. *Mol Biol Rep.* 2016;43(3):183-93.

[xxi] Kaida K, Honda Y, Hashimoto Y, et al. Application of Green Tea Catechin for Inducing the Osteogenic Differentiation of Human Dedifferentiated Fat Cells in Vitro. *Int J Mol Sci.* 2015;16(12):27988-8000.

[xxii] Jin P, Li M, Xu G, et al. Role of (-)-epigallocatechin-3-gallate in the osteogenic differentiation of human bone marrow mesenchymal stem cells: An enhancer or an inducer? *Exp Ther Med.* 2015;10(2):828-34.

[xxiii] Jin P, Wu H, Xu G, et al. Epigallocatechin-3-gallate (EGCG) as a pro-osteogenic agent to enhance osteogenic differentiation of mesenchymal stem cells from human bone marrow: an in vitro study. *Cell Tissue Res.* 2014;356(2):381-90.
[xxiv] Chen C, Ho M, Chang J, et al. Green tea catechin enhances osteogenesis in a bone marrow mesenchymal stem cell line. *Osteoporos Int.* 2005;16(12):2039-45.
[xxv] Sato K, Mera H, Wakitani S, Takagi M. Effect of epigallocatechin-3-gallate on the increase in type II collagen accumulation in cartilage-like MSC sheets. *Bioscience, biotechnology, and biochemistry.* Jun 2017;81(6):1241-1245.
[xxvi] Kim A, Kim K, Byun M, et al. Catechins activate muscle stem cells by Myf5 induction and stimulate muscle regeneration. *Biochem Biophys Res Commun.* 2017;489(2):142-8.
[xxvii] Kim A, Kim K, Byun M, et al. (-)-Epigallocatechin-3-gallate stimulates myogenic differentiation through TAZ activation. *Biochem Biophys Res Commun.* 2017;486(2):378-84.
[xxviii] Li M, Xu J, Shi T, et al. Epigallocatechin-3-gallate augments therapeutic effects of mesenchymal stem cells in skin wound healing. *Clin Exp Pharmacol Physiol.* 2016;43(11):1115-24.
[xxix] Ortiz-Lopez L, Marquez-Valadez B, Gomez-Sanchez A, et al. Green tea compound epigallo-catechin-3-gallate (EGCG) increases neuronal survival in adult hippocampal neurogenesis in vivo and in vitro. *Neurosci.* 2016;322:208-20.
[xxx] Zhang Y, He Q, Dong J, et al. Effects of epigallocatechin-3-gallate on proliferation and differentiation of mouse cochlear neural stem cells: Involvement of PI3K/Akt signaling pathway. *Eur J Pharm Sci.* 2016;88:267-73.
[xxxi] Zhang J, Xu H, Yuan Y, et al. Delayed Treatment with Green Tea Polyphenol EGCG Promotes Neurogenesis After Ischemic Stroke in Adult Mice. *Mol Neurobiol.* 2017;54(5):3652-64.
[xxxii] Wang D, Wang Y, Xu S, et al. Epigallocatechin-3-gallate Protects against Hydrogen Peroxide-Induced Inhibition of Osteogenic Differentiation of Human Bone Marrow-Derived Mesenchymal Stem Cells. *Stem Cells Int.* 2016;2016:7532798.
[xxxiii] Shin J, Jeon H, Park J, Chang M. Epigallocatechin-3-gallate prevents oxidative stress-induced cellular senescence in human mesenchymal stem cells via Nrf2. *Int J Mol Med.* 2016;38(4):1075-82.
[xxxiv] Itoh T, Imano M, Nishida S, et al. (-)-Epigallocatechin-3-gallate increases the number of neural stem cells around the damaged area after rat traumatic brain injury. *J Neural Transm (Vienna).* 2012;119(8):877-90.
[xxxv] Chen T, Liou S, Kuo C, et al. Green tea epigallocatechin gallate enhances cardiac function restoration through survival signaling expression in diabetes mellitus rats with autologous adipose tissue-derived stem cells. *J Appl Physiol (Bethesda).* 2017;123(5):1081-91.
[xxxvi] Pang X, Cong Y, Bao N, et al. Quercetin Stimulates Bone Marrow Mesenchymal Stem Cell Differentiation through an Estrogen Receptor-Mediated Pathway. *BioMed Res Int.* 2018;2018:4178021.
[xxxvii] Miladpour B, Rasti M, Owji A, et al. Quercetin potentiates transdifferentiation of bone marrow mesenchymal stem cells into the beta cells in vitro. *J Endocrinol Invest.* 2017;40(5):513-21.
[xxxviii] Pinchuk S, Vasilevich I, Kvacheva Z, Volotovski I. The influence of quercetin on the hepatic differentiation of human adipose-derived mesenchymal stem cells. *Tsitologiia.* 2016;58(7):517-25.
[xxxix] Zhou Y, Wu Y, Jiang X, et al. The Effect of Quercetin on the Osteogenesic Differentiation and Angiogenic Factor Expression of Bone Marrow-Derived Mesenchymal Stem Cells. *PloS One.* 2015;10(6):e0129605.
[xl] Li Y, Wang J, Chen G, et al. Quercetin promotes the osteogenic differentiation of rat mesenchymal stem cells via mitogen-activated protein kinase signaling. *Exp Ther Med.* 2015;9(6):2072-80.
[xli] Wu X, Qu X, Zhang Q, et al. Quercetin promotes proliferation and differentiation of oligodendrocyte precursor cells after oxygen/glucose deprivation-induced injury. *Cell Mol Neurobiol.* 2014;34(3):463-71.
[xlii] Geng L, Liu Z, Zhang W, et al. Chemical screen identifies a geroprotective role of quercetin in premature aging. *Protein Cell.* 2018. Epub ahead of print.
[xliii] Zhang L, Zhang H, Cai Y, et al. Anti-inflammatory Effect of Mesenchymal Stromal Cell Transplantation and Quercetin Treatment in a Rat Model of Experimental Cerebral Ischemia. *Cell Mol Neurobiol.* 2016;36(7):1023-34.

[xliv] Qu X, Qi D, Dong F, et al. Quercetin improves hypoxia-ischemia induced cognitive deficits via promoting remyelination in neonatal rat. *Brain Res.* 2014;1553:31-40.
[xlv] Zhang L, Cao Q, Hu Z, et al. [Effect of quercetin on neural stem cell proliferation in the subventricular zone of rats after focal cerebral ischemia-reperfusion injury]. *Nan Fang Yi Ke Da Xue Xue Bao.* 2011;31(7):1200-3.
[xlvi] McCubrey J, Lertpiriyapong K, Steelman L, et al. Effects of resveratrol, curcumin, berberine and other nutraceuticals on aging, cancer development, cancer stem cells and microRNAs. *Aging (Albany NY).* 2017;9(6):1477-536.
[xlvii] Buhrmann C, Mobasheri A, Matis U, Shakibaei M. Curcumin mediated suppression of nuclear factor-kappaB promotes chondrogenic differentiation of mesenchymal stem cells in a high-density co-culture microenvironment. *Arthritis Res Ther.* 2010;12(4):R127.
[xlviii] Cao Z, Dou C, Dong S. Curcumin Inhibits Chondrocyte Hypertrophy of Mesenchymal Stem Cells through IHH and Notch Signaling Pathways. *Chem Pharm Bull.* 2017;65(8):762-7.
[xlix] Gu Q, Cai Y, Huang C, et al. Curcumin increases rat mesenchymal stem cell osteoblast differentiation but inhibits adipocyte differentiation. *Pharmacogn Mag.* 2012;8(31):202-8.
[l] Wang N, Wang F, Gao Y, et al. Curcumin protects human adipose-derived mesenchymal stem cells against oxidative stress-induced inhibition of osteogenesis. *J Pharmacol Sci.* Nov 2016;132(3):192-200.
[li] Pirmoradi S, Fathi E, Farahzadi R, et al. Curcumin Affects Adipose Tissue-Derived Mesenchymal Stem Cell Aging Through TERT Gene Expression. *Drug Res.* 2018;68(4):213-21.
[lii] Attari 2015.
[liii] Ma X, Liu J, Wang C, et al. Low-dose curcumin stimulates proliferation of rat embryonic neural stem cells through glucocorticoid receptor and STAT3. *CNS Neurosci Ther.* 2018;24(10):940-6.
[liv] Yang Z, He C, He J, et al. Curcumin-mediated bone marrow mesenchymal stem cell sheets create a favorable immune microenvironment for adult full-thickness cutaneous wound healing. *Stem Cell Res Ther.* 2018;9(1):21.
[lv] Bang W, Kim K, Seo Y, et al. Curcumin Increase the Expression of Neural Stem/Progenitor Cells and Improves Functional Recovery after Spinal Cord Injury. *JKorean Neurosurg Soc.* 2018;61(1):10-8.
[lvi] Dandawate P, Subramaniam D, Padhye S, Anant S. Bitter melon: a panacea for inflammation and cancer. *Chin J Nat Med.* 2016;14(2):81-100.
[lvii] Yu Y, Zhang X, Ebersole B, et al. Bitter melon extract attenuating hepatic steatosis may be mediated by FGF21 and AMPK/Sirt1 signaling in mice. *Sci Rep.* 2013;3:3142.
[lviii] Nerurkar P, Johns L, Buesa L, et al. Momordica charantia (bitter melon) attenuates high-fat diet-associated oxidative stress and neuroinflammation. *J Neuroinflammation.* 2011;8:64.
[lix] Cao W, Cao K, Cao J, et al. Mesenchymal stem cells and adaptive immune responses. *Immunol Lett.* 2015;168(2):147-53.
[lx] Ayerst B, Merry C, Day A. The Good the Bad and the Ugly of Glycosaminoglycans in Tissue Engineering Applications. *Pharmaceuticals (Basel).* 2017;10(2).
[lxi] Scharnweber D, Hubner L, Rother S, et al. Glycosaminoglycan derivatives: promising candidates for the design of functional biomaterials. *J Mater Sci Mater Med.* 2015;26(9):232.
[lxii] Agrawal P, Pramanik K, Vishwanath V, et al. Enhanced chondrogenesis of mesenchymal stem cells over silk fibroin/chitosan-chondroitin sulfate three dimensional scaffold in dynamic culture condition. *J Biomed Mater Res B Appl Biomater.* 2018;106(7):2576-87.
[lxiii] Wang T, Yang F. A comparative study of chondroitin sulfate and heparan sulfate for directing three-dimensional chondrogenesis of mesenchymal stem cells. *Stem Cell Res Ther.* 2017;8(1):284.
[lxiv] Gupta V, Tenny K, Barragan M, et al. Microsphere-based scaffolds encapsulating chondroitin sulfate or decellularized cartilage. *J Biomater Appl.* 2016;31(3):328-43.
[lxv] Yao H, Xue J, Wang Q, et al. Glucosamine-modified polyethylene glycol hydrogel-mediated chondrogenic differentiation of human mesenchymal stem cells. *Mater Sci Eng C Mater Biol Appl.* 2017;79:661-70.
[lxvi] Agrawal P, Pramanik K, Biswas A. Chondrogenic differentiation of mesenchymal stem cells on silk fibroin:chitosan-glucosamine scaffold in dynamic culture. *Regen Med.* 2018;13(5):545-58.

[lxvii] Derfoul A, Miyoshi A, Freeman D, Tuan R. Glucosamine promotes chondrogenic phenotype in both chondrocytes and mesenchymal stem cells and inhibits MMP-13 expression and matrix degradation. *Osteoarthritis Cartilage.* 2007;15(6):646-55.
[lxviii] Petrov P, Granados N, Chetrit C, et al. Synergistic Effects of a Mixture of Glycosaminoglycans to Inhibit Adipogenesis and Enhance Chondrocyte Features in Multipotent Cells. *Cell Physiol Biochem.* 2015;37(5):1792-806.
[lxix] Zhu M, Feng Q, Sun Y, et al. Effect of cartilaginous matrix components on the chondrogenesis and hypertrophy of mesenchymal stem cells in hyaluronic acid hydrogels. *J Biomed Mater Res B Appl Biomater.* 2017;105(8):2292-300.
[lxx] Tamaddon M, Burrows M, Ferreira S, et al. Monomeric, porous type II collagen scaffolds promote chondrogenic differentiation of human bone marrow mesenchymal stem cells in vitro. *Sci Rep.* 2017;7:43519.
[lxxi] Sanjurjo-Rodriguez C, Martinez-Sanchez A, Hermida-Gomez T, et al. Differentiation of human mesenchymal stromal cells cultured on collagen sponges for cartilage repair. *Histol Histopathol.* 2016;31(11):1221-39.
[lxxii] Kim H, Lee E, An Y, et al. Chondroitin Sulfate-Based Biomineralizing Surface Hydrogels for Bone Tissue Engineering. *ACS Appl Mater Interfaces.* 2017;9(26):21639-50.
[lxxiii] Lee J, Pereira C, Ren X, et al. Optimizing Collagen Scaffolds for Bone Engineering: Effects of Cross-linking and Mineral Content on Structural Contraction and Osteogenesis. *J Craniofac Surg.* 2015;26(6):1992-6.
[lxxiv] Salbach-Hirsch J, Ziegler N, Thiele S, et al. Sulfated glycosaminoglycans support osteoblast functions and concurrently suppress osteoclasts. *J Cell Biochem.* 2014;115(6):1101-11.
[lxxv] Mathews S, Mathew S, Gupta P, et al. Glycosaminoglycans enhance osteoblast differentiation of bone marrow derived human mesenchymal stem cells. *J Tissue Eng Regen Med.* 2014;8(2):143-52.
[lxxvi] Bhowmick S, Rother S, Zimmermann H, et al. Biomimetic electrospun scaffolds from main extracellular matrix components for skin tissue engineering application - The role of chondroitin sulfate and sulfated hyaluronan. *Mater Sci Eng C Mater Biol Appl.* 2017;79:15-22.
[lxxvii] Wang B, Wen H, Smith W, et al. Regulation effects of melatonin on bone marrow mesenchymal stem cell differentiation. *J Cell Physiol.* 2018. Epub ahead of print.
[lxxviii] Zhang S, Chen S, Li Y, Liu Y. Melatonin as a promising agent of regulating stem cell biology and its application in disease therapy. *Pharmacol Res.* 2017;117:252-60.
[lxxix] Majidinia M, Reiter R, Shakouri S, et al. The multiple functions of melatonin in regenerative medicine. *Ageing Res Rev.* 2018;45:33-52.
[lxxx] Zhang 2017.
[lxxxi] Lee R, Lee M, Wu C, et al. Cerebral ischemia and neuroregeneration. *Neural Regen Res.* 2018;13(3):373-85.
[lxxxii] Yu X, Li Z, Zheng H, et al. Protective roles of melatonin in central nervous system diseases by regulation of neural stem cells. *Cell Prolif.* 2017;50(2).
[lxxxiii] Shuai Y, Liao L, Su X, et al. Melatonin Treatment Improves Mesenchymal Stem Cells Therapy by Preserving Stemness during Long-term In Vitro Expansion. *Theranostics.* 2016;6(11):1899-917.
[lxxxiv] Gao W, Lin M, Liang A, et al. Melatonin enhances chondrogenic differentiation of human mesenchymal stem cells. *J Pineal Res.* 2014;56(1):62-70.
[lxxxv] Chu Z, Li H, Sun S, et al. Melatonin promotes osteoblast differentiation of bone marrow mesenchymal stem cells in aged rats. *Eur Rev Med Pharmacol Sci.* 2017;21(19):4446-56.
[lxxxvi] Zhang L, Su P, Xu C, et al. Melatonin inhibits adipogenesis and enhances osteogenesis of human mesenchymal stem cells by suppressing PPARgamma expression and enhancing Runx2 expression. *J Pineal Res.* 2010;49(4):364-72.
[lxxxvii] Radio N, Doctor J, Witt-Enderby P. Melatonin enhances alkaline phosphatase activity in differentiating human adult mesenchymal stem cells grown in osteogenic medium via MT2 melatonin receptors and the MEK/ERK (1/2) signaling cascade. *J Pineal Res.* 2006;40(4):332-42.

[lxxxviii] Lee S, Jung Y, Oh S, et al. Melatonin enhances the human mesenchymal stem cells motility via melatonin receptor 2 coupling with Galphaq in skin wound healing. *J Pineal Res.* 2014;57(4):393-407.
[lxxxix] Lee M, Yin T, Sung P, et al. Melatonin enhances survival and preserves functional integrity of stem cells: A review. *J Pineal Res.* 2017;62(2).
[xc] Luchetti F, Canonico B, Bartolini D, et al. Melatonin regulates mesenchymal stem cell differentiation: a review. *J Pineal Res.* 2014;56(4):382-97.
[xci] Wu Z, Qiu X, Gao B, et al. Melatonin-mediated miR-526b-3p and miR-590-5p upregulation promotes chondrogenic differentiation of human mesenchymal stem cells. *J Pineal Res.* 2018;65(1):e12483.
[xcii] Kadry S, El-Dakdoky M, Haggag N, et al. Melatonin improves the therapeutic role of mesenchymal stem cells in diabetic rats. *Toxicol Mech Meth.* 2018;28(7):529-38.
[xciii] Zheng B, Hao D, Guo H, He B. Melatonin alleviates acute spinal cord injury in rats through promoting on progenitor cells proliferation. *Saudi Pharm J.* 2017;25(4):570-4.
[xciv] Mendivil-Perez M, Soto-Mercado V, Guerra-Librero A, et al. Melatonin enhances neural stem cell differentiation and engraftment by increasing mitochondrial function. *Pineal Res.* 2017;63(2).
[xcv] Lee S, Le N, Kang D. Melatonin alleviates oxidative stress-inhibited osteogenesis of human bone marrow-derived mesenchymal stem cells through AMPK activation. *Int J Med Sci.* 2018;15(10):1083-91.
[xcvi] Gao 2018
[xcvii] Shu T, Fan L, Wu T, et al. Melatonin promotes neuroprotection of induced pluripotent stem cells-derived neural stem cells subjected to H2O2-induced injury in vitro. *Eur J Pharmacol.* 2018;825:143-50.
[xcviii] Ma W, He F, Ding F, et al. Pre-Treatment with Melatonin Enhances Therapeutic Efficacy of Cardiac Progenitor Cells for Myocardial Infarction. *Cell Physiol Biochem.* 2018;47(3):1287-98.
[xcix] Mehrzadi S, Safa M, Kamrava S, et al. Protective mechanisms of melatonin against hydrogen-peroxide-induced toxicity in human bone-marrow-derived mesenchymal stem cells. *Can J Physiol Pharmacol.* 2017;95(7):773-86.
[c] Tan S, Zhan W, Poon C, et al. Melatonin promotes survival of nonvascularized fat grafts and enhances the viability and migration of human adipose-derived stem cells via down-regulation of acute inflammatory cytokines. *J Tissue Eng Regen Med.* 2018;12(2):382-92.
[ci] Gao B, Gao W, Wu Z, et al. Melatonin rescued interleukin 1beta-impaired chondrogenesis of human mesenchymal stem cells. *Stem Cell Res Ther.* 2018;9(1):162.
[cii] Li Z, Li X, Chan M, et al. Melatonin antagonizes interleukin-18-mediated inhibition on neural stem cell proliferation and differentiation. *J Cell Mol Med.* 2017;21(9):2163-71.
[ciii] Fu J, Zhao S, Liu H, et al. Melatonin promotes proliferation and differentiation of neural stem cells subjected to hypoxia in vitro. *J Pineal Res.* 2011;51(1):104-12.
[civ] Wang F, Zhou H, Du Z, et al. Cytoprotective effect of melatonin against hypoxia/serum deprivation-induced cell death of bone marrow mesenchymal stem cells in vitro. *Eur J Pharmacol.* 2015;748:157-65.
[cv] Yang F, Li Y, Yan G, et al. Inhibition of iron overload-induced apoptosis and necrosis of bone marrow mesenchymal stem cells by melatonin. *Oncotarget.* 2017;8(19):31626-37.
[cvi] Song J, Kang S, Lee K, Lee J. The protective effect of melatonin on neural stem cell against LPS-induced inflammation. *BioMed Res Int.* 2015;2015:854359.
[cvii] Rodriguez-Lozano F, Garcia-Bernal D, Ros-Roca Mde L, et al. Cytoprotective effects of melatonin on zoledronic acid-treated human mesenchymal stem cells in vitro. *J Maxofac Surg.* 2015;43(6):855-62.
[cviii] Ekthuwapranee K, Sotthibundhu A, Govitrapong P. Melatonin attenuates methamphetamine-induced inhibition of proliferation of adult rat hippocampal progenitor cells in vitro. *J Pineal Res.* 2015;58(4):418-28.
[cix] Maria S, Samsonraj R, Munmun F, et al. Biological effects of melatonin on osteoblast/osteoclast cocultures, bone, and quality of life: Implications of a role for MT2 melatonin receptors, MEK1/2, and MEK5 in melatonin-mediated osteoblastogenesis. *J Pineal Res.* 2018;64(3).
[cx] Dong P, Gu X, Zhu G, et al. Melatonin Induces Osteoblastic Differentiation of Mesenchymal Stem Cells and Promotes Fracture Healing in a Rat Model of Femoral Fracture via Neuropeptide Y/Neuropeptide Y Receptor Y1 Signaling. *Pharmacol.* 2018;102(5-6):272-80.

[cxi] He F, Liu X, Xiong K, et al. Extracellular matrix modulates the biological effects of melatonin in mesenchymal stem cells. *J Endocrinol.* 2014;223(2):167-80.
[cxii] Lee J, Han Y, Lee S. Potentiation of biological effects of mesenchymal stem cells in ischemic conditions by melatonin via upregulation of cellular prion protein expression. *J Pineal Res.* 2017;62(2).
[cxiii] Chu 2017.
[cxiv] Shu T, Wu T, Pang M, et al. Effects and mechanisms of melatonin on neural differentiation of induced pluripotent stem cells. *Biochem Biophys Res Comm.* 2016;474(3):566-71.
[cxv] Mendivil-Perez 2017.
[cxvi] Reiter R, Tan D, Rosales-Corral S, et al. Melatonin Mitigates Mitochondrial Meltdown: Interactions with SIRT3. *Int J Med Sci.* 2018;19(8).
[cxvii] Hardeland R. Recent Findings in Melatonin Research and Their Relevance to the CNS. *Cent Nerv Syst Agents Med Chem.* 2018;18(2):102-14.
[cxviii] Jenwitheesuk A, Park S, Wongchitrat P, et al. Comparing the Effects of Melatonin with Caloric Restriction in the Hippocampus of Aging Mice: Involvement of Sirtuin1 and the FOXOs Pathway. *Neurochem Res.* 2018;43(1):144-52.
[cxix] Jung-Hynes B, Reiter R, Ahmad N. Sirtuins, melatonin and circadian rhythms: building a bridge between aging and cancer. *J Pineal Res.* 2010;48(1):9-19.
[cxx] Bickford P, Tan J, Shytle R, et al. Nutraceuticals synergistically promote proliferation of human stem cells. *Stem Cells Dev.* 2006;15(1):118-23.
[cxxi] Flowers A, Lee J, Acosta S, et al. NT-020 treatment reduces inflammation and augments Nrf-2 and Wnt signaling in aged rats. *J Neuroinflamm.* 2015;12:174.
[cxxii] Shytle R, Ehrhart J, Tan J, et al. Oxidative stress of neural, hematopoietic, and stem cells: protection by natural compounds. *Rejuvenation Res.* 2007;10(2):173-8.
[cxxiii] Bachstetter A, Jernberg J, Schlunk A, et al. Spirulina promotes stem cell genesis and protects against LPS induced declines in neural stem cell proliferation. *PloS One.* 2010;5(5):e10496.
[cxxiv] Acosta S, Jernberg J, Sanberg C, et al. NT-020, a natural therapeutic approach to optimize spatial memory performance and increase neural progenitor cell proliferation and decrease inflammation in the aged rat. *Rejuvenation Res.* 2010;13(5):581-8.
[cxxv] Bickford P, Kaneko Y, Grimmig B, et al. Nutraceutical intervention reverses the negative effects of blood from aged rats on stem cells. *Age (Dordr).* 2015;37(5):103.