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Review article

Multi-effects of Resveratrol on stem cell characteristics: Effective dose, time, cell culture conditions and cell type-specific responses of stem cells to Resveratrol

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ABSTRACT

Stem cells which defined by dual features of self-renewal and differentiation potential provide a unique source for repairing damaged tissues to treat a wide spectrum of diseases and injuries. Several recent studies suggest that Resveratrol (RSV), a natural polyphenol component, possesses the ability to improve either culture conditions of stem cells or their target differentiation in culture. This review covers the literature that deals with the effects of RSV and its underlying mechanisms on survival, self-renewal and lineage commitment of various stem cells. Concentration of RSV and duration of treatment with this component could exert differential effects on cellular differentiation processes and cell fate. Therefore, RSV could be accounted as an effective small molecule for a variety of cell therapies which should be implemented by a special care considering, effective concentration and duration of exposure.

Contents

1. Introduction 651
2. RSV and pluripotent stem cells (PSCs) 652
 2.1. RSV effect on maintenance of the pluripotent status of PSCs 652
 2.2. Modulation of differentiation rate of PSCs by RSV 652
 2.3. Protecting role of RSV on survival of PSCs 653
3. RSV modulates self-renewal and differentiation of multipotent stem cells 653
 3.1. Cell passage state, time and dose dependent effects of RSV on MSCs 653
 3.2. Culture condition of MSCs regulates the effects of RSV 654
4. Clinical features of RSV on multipotent stem cells 655
5. Conclusion 656
 Conflicts of interest 656
 Acknowledgments 656
 References 656

1. Introduction

Stem cells are undifferentiated cells that distinguished from other cell types by their capacity for unlimited proliferation (self-

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Abbreviations

AMPK	AMP-activated protein kinase
ASCs	adipose-derived MSCs
BDNF	brain-derived neurotrophic factor
BM-MSCs	bone marrow-derived MSCs
CM	cardiomyocyte
CREB	cyclic AMP response element-binding protein
ER	estrogen receptor
ERK	extracellular signal regulated kinase
ESCs	embryonic stem cells
FRE	FOXO response element
GLUT2	glucose transporter 2
GSK-3β	glycogen synthase-3β
ICM	inner cell mass
iPSCs	induced pluripotent stem cells
JAK/STAT3	janus kinase/signal transducers and activators of transcription 3
LAD	left anterior descending
MnSOD	manganese superoxide dismutase
MEK	mitogen-activated protein kinase kinase-1
mTOR	mammalian target of rapamycin
MSCs	mesenchymal stem cells
NFkB	nuclear factor kappa B
NOD-SCID	non-obese diabetic/severe combined immunodeficiency
NPC	neural progenitor cells
Nrf2	nuclear factor erythroid 2–related factor 2
NSE	neuron-specific (γγ) enolasei
PCL	poly-e-caprolactone
PDX1	pancreatic and duodenal homeobox 1
PCNA	proliferating cell nuclear antigen
PI3K	phosphatidylinositol-3-kinase
PKA	protein kinase A
PPARγ	peroxisome proliferator-activated receptor γ
PSCs	pluripotent stem cells
ROS	reactive oxygen species
Ref-1	redox effector factor-1
RSV	Resveratrol
SIRT1	silent information regulator 1
SRF	serum response factor
UC-MSCs	umbilical cord derived mesenchymal stem cells
2D	two dimensional
3D	three-dimensional
Wnt	wingless-type MMTV integration site family member

renewal) and their potential to differentiate into specialized cell types under certain physiologic or experimental conditions (potency). Embryonic and adult (somatic) stem cells are two main types of recognized stem cells that are isolated from the inner cell mass of the blastocyst and specific adult tissues, respectively [1–3]. In 2006, researchers generated another type of stem cells, called induced pluripotent stem cells (iPSCs) by nuclear reprogramming of some specialized somatic cells [4]. Several recent studies suggest that a number of small molecules may play an important role in improving either culture conditions of stem cells or their target differentiation in culture [5–10]. Resveratrol (RSV or 3, 5, 4′-trihydroxystilbene) is non-flavonoid polyphenol phytoalexin with stilbene structure. This natural compound can be found in *cis*- or *trans*-configurations, and the biological effects of *trans*-isoform have been more extensively studied. For the first time, RSV found in root extract of white hellebore (*Veratum grandiflorum*) in 1940. Later, in 1963, RSV was also found in root of *Polygonum cupsidatum* which has been used in traditional Asian medicine for inflammation remedy [11,12]. Significant amount of RSV is also present in other plants including peanuts, eucalyptus, blueberries, cranberries, and grapes [13]. The interest to RSV progressively increased when observed that the rate of cardiovascular disease is inconsiderable in French population in paradox with their high fat diet and moderate red wine consumption, the fact which known as "French Paradox". Later, RSV was suggested to be main agent in red wines responsible for the "French Paradox" [14,15]. Thereafter, a great number of studies reported that RSV possesses various biological and therapeutic potential including anti-oxidant [12], anti-cancer [16] and anti-inflammatory [17,18] properties, protection from cardiovascular [19] and neurodegenerative diseases [20], balancing the effects of high-calorie diet [21] and enhancing longevity of lower organisms [18]. Given that, several studies within the last few years have reported the beneficial effects of RSV on behavior of various stem cells, we were persuaded to report the existing literature in this context. Hence, in present study we describe effects of RSV and its underlying mechanisms on survival, self-renewal and lineage commitment of different types of stem cells.

2. RSV and pluripotent stem cells (PSCs)

PSCs are derived either from inner cell mass of an embryo, termed as embryonic stem cells (ESCs), or from somatic cells that are reprogrammed to a pluripotent state, called iPSCs [2,3]. More recent studies point to the effect of RSV on survival, proliferation, pluripotency maintenance and apoptosis of PSCs which will be discussed in details and summarized in Table 1.

2.1. RSV effect on maintenance of the pluripotent status of PSCs

Researchers found that supplementation of nM concentrations (50 and 500 nM) of RSV to neural differentiated mouse PSCs, restores the stemness characteristics, naive pluripotency and differentiation potential and enhances their proliferation ability through activation of the janus kinase/signal transducers and activators of transcription 3 (JAK/STAT3) and suppression of the mammalian target of rapamycin (mTOR) signaling pathways [22]. Moreover, we recently indicated that RSV (50 μM) could stimulate cell proliferation of human ESCs through cell cycle modulation and enhancement in expression of anti-apoptotic markers without negative effect on stemness characteristics via activation of "silent information regulator-mitogen activated protein kinase kinase-1/extracellular signal regulated kinase" (SIRT1- MEK/ERK) axis [23].

2.2. Modulation of differentiation rate of PSCs by RSV

A number of studies reflected that RSV acts as a pro-differentiating agent for various PSCs.

A recent study by Liu et al. showed that self-renewal of human iPSCs were not affected by different concentrations of RSV (10, 50 and 100 μM) while, 50 μM of RSV could promote mesoderm differentiation of embryoid bodies. RSV at concentration of 50 μM could act via suppressing canonical wingless-type MMTV integration site family member (Wnt) signaling pathway and enhancing serum response factor (SRF)-miR-1 axis to increase cardiomyocyte (CM) differentiation [24]. Ding et al. examined the use of RSV to

Table 1
Effects of RSV on PSCs.

Stem cell type	RSV effective dose	Outcome	Ref.
Mouse ESCs and iPSCs	50–500 nM	Promoting self-renewal through activation of JAK/STAT3 and suppression of mTOR signaling pathways.	[22]
Human ESCs	50 μM	Promoting self-renewal through SIRT1-MEK-ERK axis.	[23]
Human iPSCs	50 μM	CM differentiation via suppressing canonical Wnt signaling pathway and stimulating SRF-miR-1 axis.	[24]
Mouse ESCs	10 μM	CM differentiation.	[25]
Mouse ESCs and iPSCs	20 μmol/L	Osteogenic differentiation and blocking glucocorticoid-induced apoptosis via oxidative stress reduction	[26]
Human ESCs and iPSCs	75 μM	Improved glucose-stimulated insulin secretion in differentiated β-cells and triggered maturation of pancreatic endocrine precursors towards β-cell phenotype through the activation of AMPK and PI3K/AKT signaling pathways.	[27]
Mouse ESCs	10 μM	Inhibiting ethanol-induced apoptosis via oxidative stress reduction.	[28]
Mouse ESCs	10 μM	RSV pretreatment improved survival of mouse ESCs exposed to X-ray radiation via rapid DNA repair.	[29]

As shown, different concentrations of RSV significantly modified apoptosis, self-renewal and proliferation of stem cells as well as differentiation. AMPK, AMP-activated protein kinase; CM, cardiomyocyte; ESCs, embryonic stem cells; ERK, extracellular signal regulated kinase; iPSCs, induced pluripotent stem cells; JAK/STAT3, janus kinase/signal transducers and activators of transcription 3; mTOR, mammalian target of rapamycin; MEK, mitogen-activated protein kinase kinase-1; PI3K, phosphatidylinositol-3-kinase; RSV, Resveratrol; SIRT1, silent information regulator 1; SRF, serum response factor; Wnt, wingless-type MMTV integration site family member.

improve CM differentiation of mouse ESCs. They found that 10 μM of RSV significantly increased cardiac markers and beating properties of mouse ESCs derived embryoid bodies [25]. Kao and coworkers indicated that addition of 20 μmol/L RSV to osteogenic medium significantly increased osteogenic differentiation of both mouse iPSCs and mouse ESCs. Of note, by transplantation of iPSCs, which has been cultured in osteogenic medium, in to the RSV-fed mice (30 μg/30 g animal/day) they confirmed that RSV could facilitate differentiation of iPSCs into osteocyte-like cells and significantly inhibit tumorigenicity *in vivo* [26]. Pezzolla and coworkers recently evaluated the effect of RSV on differentiation of human ESCs into insulin-producing cells. They showed that RSV treatment can significantly increase insulin content and secretion of human ESCs-derived β-cell-like cells and also facilitate maturation of pancreatic endocrine precursors toward β-cells which are functional when transplanted into non-obese diabetic/severe combined immunodeficiency (NOD-SCID) mice. They also reported that beneficial effects of RSV on differentiation of human ESCs toward β-cell-like cells, mediated by the up-regulation of pancreatic and duodenal homeobox 1 (*PDX1)* and its down-stream target genes, glucose transporter 2 (*GLUT2*), glycerol kinase and insulin, via activation of both AMP-activated protein kinase (AMPK) and phosphatidylinositol-3-kinase/AKT (PI3K/AKT) signaling pathways [27].

2.3. Protecting role of RSV on survival of PSCs

Considering effect of RSV on PSCs, several reports indicated that RSV is capable of protecting PSCs from apoptosis and preserving their survival. Huang and coworkers showed that ethanol-induced apoptosis in mouse ESCs was effectively blocked by 10 μM of RSV pretreatment. Their results indicated that RSV by its antioxidant property protects mouse ESCs from destructive effects of ethanol [28]. Similarly, a recent study on iPSC-derived osteocyte-like cells revealed that RSV pretreatment via up-regulation of the manganese superoxide dismutase (*MnSOD)* and intracellular glutathione level and decreased reactive oxygen species (ROS) production inhibited glucocorticoids, dexamethasone and etoposid-induced apoptosis [26]. Denissova et al. observed that pretreatment of mouse ESCs with 10 μM of RSV improved survival of mouse ESC after exposure to ionizing radiation, 5 Gy of X-rays, through more rapid DNA repair rather than reduced DNA damage or delayed cell cycle progression. Of important, these researchers confirmed that radio-protective effect of RSV on mouse ESCs is independent of its antioxidant properties [29].

3. RSV modulates self-renewal and differentiation of multipotent stem cells

Multipotent stem cells, which found in most tissue of adult body, so called adult stem cell, produce a limited number of cell types compare to PSCs [2]. Mesenchymal stem cells (MSCs) are well-recognized multipotent stem cells which differentiate into diverse cell types, including bone, cartilage, fat, tendon, skeletal and cardiac muscle, epithelial and nerve cells. MSCs can be found in many tissues like bone marrow, fat, muscle, placenta and umbilical cord [30]. There is an increasing interest in RSV implementation for *in vitro* growth and differentiation of MSCs to improve their application in cell therapy (Table 2):

3.1. Cell passage state, time and dose dependent effects of RSV on MSCs

Peltz et al. examined the effects of RSV on self-renewal and differentiation of human adipose-derived MSCs (human ASCs) at concentrations ranging from 0.1 to 10 μM after both short-(<2 weeks) and long-term (4–10 weeks) exposure. Their results implicated that 0.1 μM of RSV is beneficial for stem cell development which promoted both self-renewal, by inhibiting cellular senescence, and osteogenesis over a short- and long-term exposure and it promoted adipogenesis only over a long-term exposure. Also, at 1 μM, RSV blocked self-renewal of human ASCs after a long term exposure via enhancing senescence rate and at 5 and 10 μM, RSV inhibited self-renewal by increasing senescence rate, cell doubling time and S-phase cell cycle arrest. Meanwhile, Peltz et al. showed that 1, 5 and 10 μM of RSV regulated osteogenesis and adipogenesis similarly as 0.1 μM. Collectively, their results suggested that RSV possessed the biphasic effect, termed hormesis, on self-renewal of human MSCs while promoted osteogenic and adipogenic differentiation depending on dosage and duration of RSV treatment [31].

Yoon and colleagues showed that RSV (0.1 and 1 μM)-mediated SIRT1 activation by suppressing the acetylation of SOX2 and therefore its stabilizing in the nucleus, maintained self-renewal, colony-forming ability, multipotency and differentiation potential to osteogenic/adipogenic lineages of human bone marrow-derived MSCs (human BM-MSCs) [32]. Recently, Yoon et al. elucidated the effects of 1 μM of RSV on self-renewal, multipotency and cellular senescence in early passage (P1-3) and late-passage (P9-10) human BM-MSCs. Data demonstrated that RSV enhanced the colony formation ability and osteogenic/adipogenic differentiation potential of early passage-MSCs, but accelerated cellular senescence of late-

Table 2
Effects of RSV on multipotent stem cells.

Stem cell type	RSV effective dose/s	Outcome	Ref.
Human ASCs	0.1 μM	Promoting self-renewal and osteogenesis after short- and long- term exposure and promoting adipogenesis after long term exposure.	[31]
Human BM-MSCs	0.1 and 1 μM	Regulating self-Renewal and multipotency through SIRT1-SOX2 axis.	[32]
Human BM-MSCs	1 μM	Enhanced self-renewal and multipotency of early passage MSCs, but accelerated cellular senescence of late passage MSCs through modulating ERK/GSK3β/β-Catenin axis.	[33]
Mouse mesenchymal cell line C3H10T1/2 and primary rat BM-MSCs	50 μM	Promoting osteogenesis and inhibiting adipogenesis through SIRT1 activation.	[34]
Mouse BM-MSCs	10^{-8}-10^{-6} M	Reversing the inhibitory effect of Cyclosporin A on BM-MSCs proliferation and osteoblastic differentiation via ER/NO/cGMP pathway.	[35]
Human BM-MSCs	10^{-8}-10^{-6} M	Promoting proliferation and osteoblastic differentiation via ER/ERK activation.	[36]
Mouse mesenchymal cell line ST2	1–50 μM	Promoting osteoblastic differentiation via canonical Wnt signaling pathway.	[37]
Human embryonic mesenchymal progenitors	2–10 μM	Promoting osteoblastic differentiation via activation of SIRT1/FOXO3A complex.	[38]
Rat ASCs	25 μM	Promoting osteogenic differentiation of rat ASCs in 2- and 3-D cultures via enrichment of the cell source for osteoprogenitor cells.	[39]
Human and rat ASCs	≤25 μM	Dose dependent differences in the mineralization response of human and rat ASCs to RSV on 3D cultures.	[40]
Rat cardiac stem cells	2.5 μM and 2.5 mg/kg	Enhanced stem cell survival and proliferation to improve cardiac function of infarcted myocardium via enhancing the redox potential of the myocardium.	[41,42]
Human UC-MSCs	≤10 μM	Promoting self-renewal and neural differentiation in SIRT1 dependent manner.	[43]
Human UC-MSCs	30 mg/L	Promoting neural differentiation.	[44]
Human UC-MSCs	10 μM	Promoting neural differentiation through PKA-GSK3β-β-Catenin and PKA-ERK1/2 signaling pathways.	[45]
Human BM-MSCs	1 μM	Promoting neural differentiation through SIRT1 activation.	[48]
Rat NPCs	10 μM and 20 mg/kg	Promoting proliferation and survival through activation of ERKs and p38 kinases.	[49]
Mice NPCs	20 and 50 μM and 1–10 mg/kg	Reduction of proliferation through AMPK activation.	[50]

AMPK, AMP-activated protein kinase; ASCs, adipose-derived mesenchymal stem cells; BM-MSCs, bone marrow-derived mesenchymal stem cells; ER, estrogen receptor; ERK, extracellular signal regulated kinase; GSK-3β, glycogen synthase-3β; MSCs, mesenchymal stem cells; NPCs, neural progenitor cells; PKA, protein kinase A; RSV, Resveratrol; SIRT1, silent information regulator 1; UC-MSCs, umbilical cord-derived mesenchymal stem cells; 2D, two dimensional; 3D, three-dimensional; Wnt, wingless-type MMTV integration site family member.

passage -MSCs and SIRT1-knockdown early passage-MSCs. Mechanistic investigation revealed that in early passage-MSCs, which express SIRT1, RSV prohibits nuclear translocation of β-catenin via ERK leading glycogen synthase-3β (GSK-3β) inactivation while in the late passage-MSCs, which lacks of SIRT1expression, RSV acted through ERK inactivation leading to GSK-3β activation and promoted nuclear localization and activity of β-catenin. This mechanism was similar to what observed in SIRT1-knockdown early passage-MSCs. Their data also showed that MSCs grown in RSV-containing medium maintained their self-renewal and multipotency up to 10 passages. Overall, their data suggested that the effects of RSV on the cellular senescence and stemness of MSCs depend on cell passage and SIRT1 expression [33]. Bäckesjö et al. studied the effect of RSV on differentiation of MSCs. They cultured mouse mesenchymal cell line C3H10T1/2 and primary rat BM-MSCs in osteoblast differentiation medium supplemented with RSV and isonicotinamide, SIRT1 activators, or SIRT1 inhibitor, nicotinamide. Their data revealed that treatment with either 50 μM of RSV or 25 mM of isonicotinamide, blocked adipocyte development and increased the expression of osteoblast markers, while nicotinamide treatment increased adipocyte number and increased expression of adipocyte markers. Additionally as RSV totally blocked the effect of peroxisome proliferator-activated receptor γ (PPARγ)-agonist troglitazone on adipocyte formation, they concluded that RSV inhibited PPARγ to block adipogenesis. Positive effect of RSV on osteogenesis was not mediated by estrogenic character of RSV as estrogen receptor (ER)-ligand was not able to affect adipocyte or osteoblast differentiation of C3H10T1/2 cells [34]. Research by Song et al. revealed that RSV (10^{-8}-10^{-6} M) stimulated proliferation and osteoblastic differentiation of mouse BM-MSCs in a dose dependent manner and reversed the inhibitory effects of Cyclosporin A, a potent immunosuppressive agent, on BM-MSCs cultures via enhancing of NO production and cGMP content. As ER antagonist, ICI182780, abolished both NO production and the anabolic effect of RSV (10^{-6} M) on mouse BM-MSCs, RSV is suggested to exert its function through ERs and that the NO–cGMP pathway may act downstream of ERs [35]. Similarly, Dai and coworkers reported that a range of RSV dose (10^{-8}-10^{-6} M) induced proliferation and osteoblastic differentiation of human BM-MSCs through ER-mediated ERK1/2 activation [36]. RSV in murine multipotent mesenchymal ST2 cell line stabilized β-Catenin and promoted its nuclear accumulation, resulting in up-regulation of the genes which are critical for osteoblastic differentiation. The increase level of β-Catenin in response to RSV is mediated by phosphorylation and inactivation of GSK-3β, an inhibitor of β-Catenin, via ERK1/2 activation [37]. Tseng et al. described that in human MSCs, RSV acts through SIRT1-dependent and ER-independent pathway to promote osteogenesis while via PPARγ2 inhibition attenuated adipogensis. These authors reported that RSV administration activated SIRT1, enhanced FOXO3A protein and elevated activity of SIRT1-FOXO3A transcriptional complex. As a consequence, SIRT1-FOXO3A bound to distal FOXO response element (FRE) of the RUNX2 promoter which up regulated expression of this key osteogenic transcription factor and its downstream target genes [38].

3.2. Culture condition of MSCs regulates the effects of RSV

Erdman et al. used RSV to enrich the yield of ASCs and osteoprogenitor cells from rat inguinal fat and to increase osteogenic differentiation of the enriched cell population in two- and three-dimensional (2D and 3D) cultures. They cultured adherent cells in growth medium or osteogenic medium supplemented with RSV (0, 12.5, and 25 μM) for 7 days and evaluated osteogenic differentiation in 2D cultures by assessing the expression of osteogenic

biochemical markers and in 3D cultures, PCL (poly-e-caprolactone)-collagen scaffolds, via calculating the amount of mineralization after 4, 8 and 12 weeks. Their data are as a follows: 1) RSV increased the population (population%) and number of ASCs in both growth medium and osteogenic medium dose dependently while at concentration of 25 μM increased only the number of osteoprogenitor cells in growth medium. 2) In both media, 25 μM of RSV enhanced alkaline phosphatase activity and osteocalcin levels. Additionally, the levels both of these osteogenic differentiation markers were higher in osteogenic medium than in growth medium. 3) On the PCL/collagen scaffold, 25 μM RSV-pretreated cells significantly generated greater amount of mineralized matrix than untreated cells at early steps. Interestingly, the pretreated group showed levels of mineralization similar to that of the continuously treated group, demonstrating that short exposure to RSV is sufficient to obtain a beneficial effect on osteogenic differentiation. Moreover, PCL/collagen scaffold implementing in media without osteogenic supplements showed little or no mineralization for both treated and untreated cells at all-time points [39]. Further, they showed that in human ASCs doses below 25 μM caused significantly more mineralization than 0 (untreated) and 25 μM treated cells in a 3D culture environment. They also found that rat ASCs produced a larger quantity and more mature mineralized matrix compared to the human cell constructs [40].

4. Clinical features of RSV on multipotent stem cells

Through the aforementioned reports, RSV could be considered as a suitable candidate for prevention of postmenopausal osteoporosis and development of bone tissue engineering (Fig. 1). Furthermore, Gurusamy et al. examined effects of RSV on adult cardiac stem cell therapy. They induced heart attack by occlusion of left anterior descending (LAD), in RSV (2.5 mg/kg/day for 2 weeks) fed rats and injected adult cardiac stem cells which stably expressing EGFP into the myocardium of those rats. Their results showed that 28 days after LAD occlusion, RSV enhanced the redox potential in cardiac environment via overexpression of the nuclear factor erythroid 2–related factor 2 (Nrf2) and redox effector factor-1 (Ref-1) which enhanced proliferation, myocardial regeneration and cardiac function [41]. Later, they also demonstrated that RSV (2.5 μM for 2 weeks)-pretreated cardiac stem cells could survive up to 120 days, and were able to proliferate and promote cardiac function after transplantation to myocardium subjected to LAD occlusion via prolonged induction of Nrf2 and Ref-1 and nuclear factor kappa B (NF_kB) [42]. Several research teams with different experimental settings demonstrated that RSV mediated neuronal differentiation of human multipotent stem cells. Accordingly, RSV pretreatment of human MSCs might be beneficial for stem cell based therapy of neurodegenerative and neural injury disorders. In

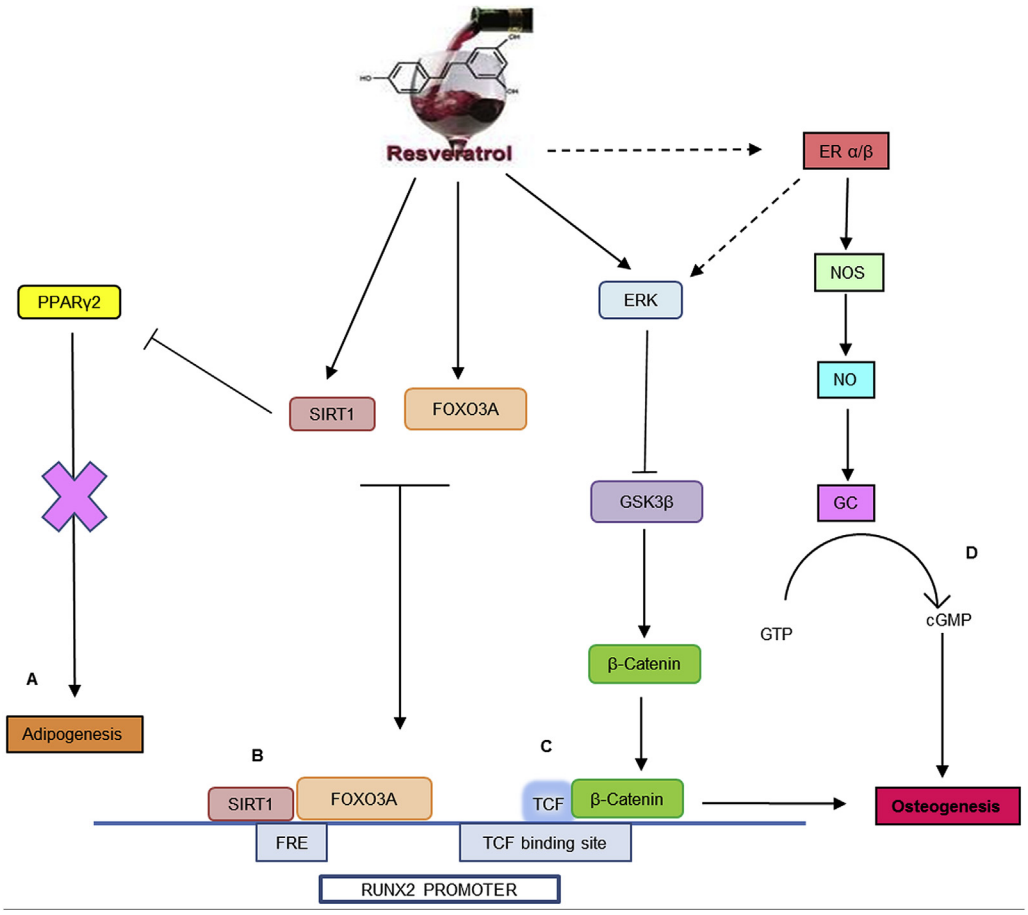

Fig. 1. Schematic signaling pathways indicate modulating by RSV which leads to adipogenesis inhibition while promotes osteogenesis in MSCs. A) Activation of SIRT1 by RSV leads to a decrease in adipogenesis via repressing PPARγ2 [34,38]. B) RSV activates SIRT1 and enhances FOXO3A expression, thereby triggers activity increment of SIRT1-FOXO3A transcriptional complex. Consequently, SIRT1-FOXO3A complex binds to distal FRE of the RUNX2 promoter to stimulate osteogenesis [38]. C) RSV activated ERK1/2 signaling directly or indirectly, through ERs, stimulates osteogenesis through stabilizing of β-Catenin and promoting its nuclear accumulation. β-Catenin-TCF/LEF complex directly stimulates transcription of *Runx2* by an attachment to its TCF-binding site [36,37]. D) Besides to aforementioned pathways, RSV may involve in osteoblastic differentiation via ER/NO/cGMP pathway [35]. FRE: FOXO response element, ERs: estrogen receptors, NO: NO synthase, GC: guanylate cyclase.

this regard, Wang et al. examined the effect of different concentrations of RSV on self-renewal or neural differentiation of human umbilical cord-derived mesenchymal stem cells (human UC-MSCs). Their study revealed that incubation of hUC-MSCs with 0.1, 1 and 2.5 μM RSV for 6 days enhanced cell viability and proliferation and suppressed senescence by stimulating SIRT1 and proliferating cell nuclear antigen (PCNA), the S-phase cell marker, and inhibiting p53 and p16. Whereas, 5 and 10 μM RSV reduced cell viability and proliferation and enhanced senescence and apoptosis which leaded to cell cycle arrest in S and/or G2/M phase by inhibiting SIRT1 and PCNA and stimulating of p53 and p16. In addition, they found that RSV, dose dependently, could facilitate neuronal differentiation of human UC-MSCs by regulating of *Nestin*, tubulin-βIII, neuron-specific (γγ) enolasei (*NSE*), neurogenin 1 and 2 as well as *Mash1* [43]. Guo and colleagues described that high concentration of RSV (30 mg/L) without any negative effect on GFAP, glial marker, significantly increased corresponding neural markers, nestin and NSE, in human UC-MSCs. These researchers suggested that RSV can stimulate differentiation of human UC-MSCs into neuron-like cells [44]. Jahan et al. examined the molecular mechanisms of RSV-mediated neuronal differentiation of human UC-MSCs. Their results showed that 10 μM of RSV alone or in combination with 50 μM of nerve growth factor increased intracellular cAMP and Ca^{2+} to enhance protein kinase A (PKA) activity. They also confirmed that PKA activation results in stimulation of cyclic AMP response element-binding protein (CREB) phosphorylation and finally neural markers expression through induction of GSK3β and ERK1/2 phosphorylation [45]. Joe and colleagues showed that treatment of human BM-MSCs with 1 μM of RSV, prior to neuronal induction, effectively enhanced neurite lengths and expression of neuroprogenitor markers, *Nestin*, *Musashi*, *CD133*, as well as neuronal specific markers *MAP-2*, *NF-M* and *KCNH1*. They also showed that these effects were abolished by a SIRT1 inhibitor EX527 and concluded that RSV treatment effectively stimulated neuronal differentiation of human BM-MSCs through SIRT1 activation [46]. Neural progenitor cells (NPCs) are considered as another type of multipotent cells that generate neurons, astrocytes, and oligodendrocytes in the nervous system [47,48]. The beneficial effect RSV on NPCs remained controversial. Kumar et al. showed that RSV displays hormesis on the proliferation and survival of cultured primary rat fetuses NPCs. On the other words, low concentrations of RSV (10 μM) stimulated NPC proliferation and survival through activation of ERKs and p38 kinases, whereas high concentrations (>20 μM) exhibited inhibitory effects. Other data also revealed that administration of RSV (20 mg/kg for 45 days) to elderly (15 month-old) male Wistar rats, effectively stimulated neurogenesis in hippocampus by up-regulation of CREB and SIRT1 protein levels [49]. In contrast, Park and coworkers reported that 20 and 50 μM RSV decreased the proliferation of cultured mouse multipotent NPCs via AMP-activated protein kinase (AMPK) activation. Administration of RSV (1–10 mg/kg for 14 days) to young (5-week-old) C57BL/6 mice reduced the proliferation and survival of NPCs in the dentate gyrus of the hippocampus with up regulation of AMPK and down regulation of CREB and brain-derived neurotrophic factor (BDNF) of the hippocampus [50]. Utilization of different experimental settings including RSV dosage, organism spices and age as well as study duration presumably vindicated such discrepancies.

5. Conclusion

In summary, RSV is a suitable natural agent for regulating survival, self-renewal and differentiation of various stem cells (Tables 1 and 2). Accordingly, RSV can facilitate cell generation and maintenance *in vitro* and enhance their differentiation prior to stem cell therapy. This contradictory and somewhat confusing effect of RSV was explained by various factors like its concentration, time and duration of treatment, culture conditions and the type of stem cell or organism being studied. Taken together, these aspects should be considered for the treatment or prevention of aged-related disorders such as osteoporosis as well as cardiovascular and neurodegenerative diseases. Furthermore, a variety of laboratory and clinical researches indicated that in contrast to high oral absorbance of RSV, this small substance has low bioavailability due to its rapid metabolism [51–55]. Therefore, various natural and synthetic RSV derivatives such as methoxylated-, hydroxylated- and halogenated-form of this compound were studied recently for diverse therapeutic applications [56–58]. It is worth noting, although different investigators focus on therapeutic ability of these derivatives [56–60], effects of these products on different types of stem cells remain as an open question.

Conflicts of interest

The authors declare that they have no conflicts of interest to declare.

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