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REVIEW

Aging and stem cell therapy: AMPK as an applicable pharmacological target for rejuvenation of aged stem cells and achieving higher efficacy in stem cell therapy

Mohammadhossein Khorraminejad-Shirazi [a,b,*], Mohammad Farahmandnia [a,b], Bahareh Kardeh [a,b], Alireza Estedlal [a,b], Sina Kardeh [a,b], Ahmad Monabati [c,d]

[a] *Cell and Molecular Medicine Student Research Group, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
[b] *Student Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran*
[c] *Department of Pathology, Shiraz University of Medical Sciences, Shiraz, Iran*
[d] *Hematology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*

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Abstract
In recent years, tissue regeneration has become a promising field for developing stem cell-based transplantation therapies for human patients. Adult stem cells are affected by the same aging mechanisms that involve somatic cells. One of the mechanisms involved in cellular aging is hyperactivation of mechanistic target of rapamycin complex 1 (mTORC1) and disruption of 5′ adenosine monophosphate-activated protein kinase (AMPK). Aging of stem cells results in their impaired regenerative capacity and depletion of stem cell pools in adult tissue, which results in lower efficacy of stem cell therapy. By utilizing an effective therapeutic intervention for aged stem cells, stem cell therapy can become more promising for future application. mTORC1 inhibition is a practical approach to preserve the stem cell pool. In this article, we review the dynamic interaction between sirtuin (silent mating type information regulation 2 homolog) 1, AMPK, and mTORC1. We propose that using AMPK activators such as 5-aminoimidazole-4-carboxamide ribonucleotide, A769662, metformin, and oxidized nicotinamide adenine

* Corresponding author at: Student Research Committee, Zand Blvd, PO Box 7134845794 Shiraz, Iran. Tel.: +989379456261.
E-mail address: hkhorraminejad@sums.ac.ir (M. Khorraminejad-Shirazi).

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dinucleotide (NAD^+) are practical ways to be employed for achieving better optimized results in stem cell-based transplantation therapies.

Contents

Introduction

Longevity brings about new challenges regarding aging and degenerative diseases to modern medicine. Cellular senescence is irreversible proliferation arrest and limitation of vitality, induced by a variety of exogenous and endogenous stressors. The accumulative results of aging processes lead to gradual deterioration of structure and decline in function and regenerative potential of organs [1].

Stem cells are affected with the same aging mechanisms that involve somatic cells [2]. In this article, we focus on senescence in stem cells, and explore possible strategies - using small molecules and already Food and Drug Administration (FDA) approved medications - to reverse the aged phenotype in these cells, also known as rejuvenation.

Defining stem cells and their functions

Stem cells are a population of undifferentiated cells capable of self-renewal, as well as differentiating into specialized mature tissue cell types. Adult stem cells are found in mature tissues. The plasticity of these cells allows them to generate various lineages of cells, even different from their organ of origin; hence, these cells can be used for organ regeneration and cellular repair in stem cell therapy [3].

Stem cells and progenitor cells are able to self-renew and differentiate into organ-specific cell types *in vitro*. *In vivo* stem cell transplantation, as shown in animal models of diseases, can reconstitute injured organ systems [4].

The regenerative potential of stem cells has been investigated by many scientists. The promising success of tissue regeneration by utilizing stem cells has given rise to the idea of developing stem cell-based transplantation therapies for human patients. Stem cell therapy proved to be attainable for many patients, like those who suffer from ischemic stroke [5], cardiac diseases [6], neurodegenerative disorders [7], and corneal destruction [8]. Diverse applications for mesenchymal stem cells (MSCs) include regeneration of various connective tissue lineages and parenchymal cells [9–11].

Stem cell senescence negatively affects their quality for cell therapy

As mentioned earlier, stem cells are affected with the same aging mechanisms that involve somatic cells [2]. Although it is believed that the number of stem cells does not decrease with age, their impaired regenerative capacity complicates age-related diseases [12]. Thus, stem cell senescence per se is not directly held responsible for mortality and determination of life span, as age-related diseases are. However, it contributes to worsening of disease conditions; for instance, through poor replacement of damaged somatic cells after myocardial infarction [13].

Stem cells in aged tissues have been exposed to a relatively greater amount of intrinsic and extrinsic toxic metabolites. In response, various intra- and extracellular pathways are activated, which in turn have undesirable effects on genetic and metabolic characteristics of cells, associated with aging [14]. Genomic alterations include telomere dysfunction, elevation of p53-associated genes, DNA damage, and apoptosis [12]. Moreover, proteostasis and lipid profile do not remain unaffected, and mitochondrial respiratory chain dysfunction, which is mostly believed

to have resulted from mutation accumulation in mitochondrial DNA, ultimately brings about the age-dependent functional decline in cell mitochondria [14].

Various studies have shown that aging reduces the regenerative potential of stem cells. Degenerative changes in tissue-specific stem cells and stem cell niches cues that dysregulation of stem cell activity could be due to adaptive cellular processes attributed to tissue aging changes such as accumulation of toxic metabolites, DNA damage, mitochondrial dysfunction, proliferative exhaustion, extracellular signalling, and epigenetic remodeling [13,14]. Adult stem cells such as aged hematopoietic stem cells (HPSCs) are known to have lost their ability to respond to stress and differentiation [15]. Also, hyperactivation of mechanistic target of rapamycin complex 1 (mTORC1) and disruption of 5′ adenosine monophosphate-activated protein kinase (AMPK) are known to deplete HPSC pools [16], resulting in reduced benefits of cell therapy in elderly patients, the largest target group for regenerative medicine [15,17].

Age-related diseases like diabetes [15] can also negatively impact stem cells by reducing angiogenic capacity and therapeutic potential, leading to age-induced chronic diseases like chronic kidney disease [1]. In addition, stem cell niches lose their ability to maintain stem cells in a quiescent state with aging driving cells to differentiation [18]. Regarding the study of Dexheimer et al [19], no specific parameters other than age could be identified as influencing the quality of stem cells.

Strategies for rejuvenation can yield higher quality stem cells

As reviewed in the previous section, current clinical utilization of stem cells is challenged by multiple factors, such as donor age, degenerative diseases, or identification of senescent cells [20].

There is evidence concluding that modification of nutrients, antioxidants, growth factors, serum depletion and deprivation, and treatment with platelet rich plasma and low oxygen conditions can more or less hinder the aging of cells [20]. Nevertheless, modulation of genetic pathways known to have a pivotal role in the phenomenon of senescence, or making changes in the microenvironment (also known as niche) of the target tissue for stem cells, provides a rather effective mode of therapeutic intervention for stem cell therapy in elderly patients [12].

There are few studies in the literature that investigate the possibility of stem cell rejuvenation by incorporating small molecules or medications. Herein, we review the studies that successfully rejuvenated various lineages of stem cells.

5-Aminoimidazole-4-carboxamide ribonucleotide

Lee et al [16] used 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) to overcome the shortage of available HPSCs/progenitor cells for transplantation. In their study, HPSCs/progenitor cells were preconditioned with AICAR and repopulation capacity of HPSCs after transplantation was assessed. They concluded that preconditioning of HPSC pools with AICAR dramatically improves the long-term repopulation of transplanted HPSCs.

It has been shown that an endogenous pool of multipotential MSCs develops in the territory of infarcted cardiac muscle. Although typically, MSCs differentiate into fibroblasts responsible for collagen secretion and scar tissue formation, this process is impaired in the aging mouse. In fact, fibroblasts derived from aged heart muscle are dysfunctional and produce insufficient collagen and exhibit poor maturation into myofibroblasts. Cieslik et al [21] showed that AICAR can improve this defect *in vitro* via activating AMPK. Introduction of AICAR to aged heart led to mobilization of endogenous MSCs and differentiation towards fibroblasts and myofibroblasts in the infarct and was followed by enhanced collagen deposition and collagen fiber maturation in the scar.

NAD^+

In a study conducted by Zhang and colleagues [22], they demonstrated that treatment with the NAD^+ precursor nicotinamide riboside (NR) rejuvenated muscle stem cells (MuSCs) in aged mice. They showed that NR inhibits MuSCs senescence and improves MuSCs function by enhancing mitochondrial function in a sirtuin 1 (SIRT1)-dependent manner. Furthermore, they showed that NR decreases senescence of neural and melanocyte stem cells.

Metformin

As reviewed by Hickson et al [1], several drugs are shown to affect endothelial progenitor cells, among which are biguanides and namely, metformin. As a result of their study, they reported that administration of metformin leads to enhancement of endothelial progenitor cell function and mobilization.

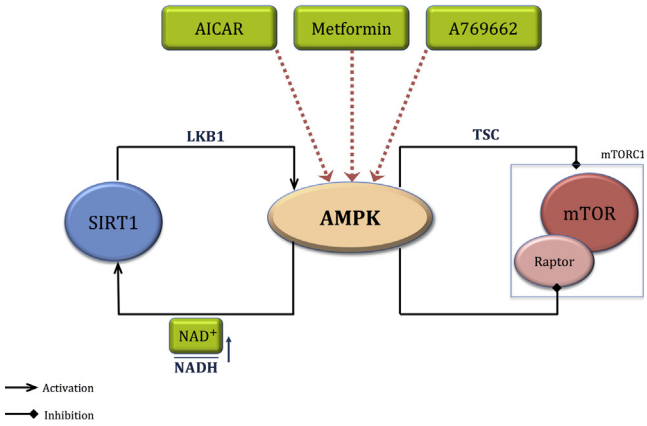

Fig. 1 5′ Adenosine monophosphate-activated protein kinase (AMPK) as an applicable drugs/small molecules target and its interaction with mechanistic target of rapamycin complex 1 (mTORC1).

Table 1 Direct and indirect Modes of 5′ adenosine monophosphate-activated protein kinase (AMPK) activation by different drugs and small molecules.

Drug/small molecule	Mode of AMPK activation
NAD^+	Indirect: Increased NAD^+ to NADH ratio leads to activation of SIRT1, which in turn activates LKB1 by deacetylation. LKB1 then proceeds to upregulate AMPK activity by phosphorylation of T172 [29]
Metformin	Controversy: Recent research points to an indirect mechanism mediated through metformin inhibition of AMP deaminase, resulting in an increase in cellular AMP levels, which in turn activates AMPK [36]
AICAR	Direct: Upon introduction into cells, AICAR is converted to ZMP, a nucleotide that mimics the effect of AMP, ergo activating AMPK [35]
A769662	Direct: A769662 mimics the effect of AMP by binding and activating AMPK. Furthermore, it inhibits dephosphorylation of T172 on AMPK, keeping this protein in active state [39]

Interaction between SIRT1, AMPK, and mTORC1 explains a mechanism for rejuvenation

SIRT1 is a member of mammalian sirtuins (SIRT1—SIRT7), a conserved family of NAD^+-dependent deacetylases that are required for many chief cellular processes including metabolic regulation [23,24]. SIRT1 improves mitochondrial biogenesis through deacetylation and activation of peroxisome proliferator-activated receptor gamma coactivator 1-alpha [25].

It has been difficult to unwind the epistasis of SIRT1 and AMPK, a strictly conserved heterotrimeric kinase complex constituted of a catalytic (a) subunit and two regulatory (b and g) subunits, activated under conditions of energy stress, as occurs in nutrient deprivation or hypoxia when intracellular adenosine triphosphate decreases and intracellular adenosine monophosphate (AMP) rises [26]. AMPK has a dynamic interaction with SIRT1; it has been shown that AMPK activates SIRT1, probably through an indirect increase in cellular NAD^+ levels [27], whilst SIRT1 deacetylates the AMPK kinase, liver kinase B1 (LKB1), which leads to increased phosphorylation and activation of AMPK [28,29].

Mechanistic target of rapamycin (mTOR) is a prime determinant of lifespan in numerous organisms such as yeast, worms, flies, and mice. mTOR constitutes two complexes in cells: mTORC1 and mTORC2, each having their unique structure. mTOR and approximately all of the mTOR-regulated processes are fundamental in cellular and organismal aging. By the same token, mTORC1 inhibition may represent a viable approach to preserve the stem cell pool, therefore, maintaining functionality of tissues and organs over time [30]. Moreover, mTORC1 is an inhibitor of the process of autophagy, which has a key role in stem cell rejuvenation, and inhibition of mTORC1 activity is an important step for autophagy initiation [31].

Physiologically, glucose and oxygen control mTORC1 activation. Furthermore, one mechanism through which intracellular energy level affects mTORC1 activation is through AMPK kinase activity upon tuberous sclerosis complex (TSC) 2 tumor suppressor upstream of mTORC1, on conserved serine sites distinct from those targeted by other kinases, hence indirectly inhibiting mTORC1 [32,33]. Over and above that, regulatory-associated protein of mTOR has been identified as a direct substrate of the AMPK. Phosphorylation of regulatory-associated protein of mTOR by AMPK on Ser722/Ser792 is essential for inhibition of mTORC1 [34].

The research by Hickson et al [1], Lee et al [16], and Zhang et al [22], mentioned in the previous section, does not explain the detailed mechanism through which rejuvenation of aged stem cells was achieved; this mechanism can be explained in light of SIRT1-AMPK-mTORC1 interaction. AICAR and metformin can directly upregulate AMPK activity [35,36], resulting in enhanced inhibition of mTORC1 [32—34]. When inhibited, mTORC1 can no longer mediate cellular aging, and autophagy pathway will be unblocked, which eventually leads to stem cell rejuvenation [31].

NAD^+ mediates its role in stem cell rejuvenation through upregulation of SIRT1 activity. SIRT1 proceeds to activate LKB1 by means of deacetylation, and LKB1 then directly interacts with AMPK and leads to its activation. Thus, NAD^+ has an indirect effect of AMPK activation [29] (Fig. 1).

Recent studies indicate that metformin might mediate its AMPK-activating role indirectly. A study conducted by Ouyang et al [36] points out metformin inhibition of AMP deaminase that results in an increase in cellular AMP level, which then activates AMPK. Furthermore, metformin effect on AMPK activation is well established both *in vitro* and *in vivo* [37].

The modes of AMPK activation for the mentioned compounds are summarized in Table 1.

Discussion

As mentioned earlier, senescent adult stem cells have reduced quality and potential for proliferation and differentiation [2,12—16]. Pretreatments that induce rejuvenation of these cells can increase their efficacy in cell therapy and will be highly favored in clinical application and regenerative medicine. As an example in the field of hematopoietic cell transplantation, current data show that mTOR pathway is hyperactive in aged mice HSCs. The imbalance caused by aging induced impairment of HSC development in the elderly contributes to impaired therapeutic responses more than in the young and lowers the success rate of HSC

transplantation from aged donors. Inhibition of mTOR via pharmaceutical agents enhances the regenerative capacity of aged mice HSCs and reveals the conservation mechanisms for functional restoration of HSCs [31]. We suggest that AICAR and NAD^+ are such compounds, and they can improve stem cell function and enhance the efficacy of stem cell therapy. Metformin, an already FDA-approved, widely utilized medication, is another feasible option for induction of stem cell rejuvenation in adult stem cell therapy [38]. The mechanism by which NAD^+ repletion refines mitochondrial and stem cell function and promotes lifespan acts mainly through the AMPK-mTORC1 pathway. Also, since AICAR, metformin, and NAD^+ derivatives mediate their rejuvenating role through AMPK activation, we suggest that other novel AMPK activators, such as A769662 [39], are practical compounds to be employed in stem cell therapy methods to achieve higher efficacy and better results. The application of the mentioned compounds *in vitro*, *in vivo*, and in clinical research could have a beneficial effect on stem cells technology and cell therapy using aged adult stem cells, in autologous and allogeneic cell transplantation, and rejuvenation of damaged tissues *in vivo*.

Conflicts of interest

The authors declare no conflict of interest.

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