Effects of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors on ageing: Molecular mechanisms

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Abstract

Human ageing is determined by degenerative alterations and processes with different manifestations such as gradual organ dysfunction, tissue function loss, increased population of aged (senescent) cells, incapability of maintaining homeostasis, reduced repair capacity, which collectively lead to an increased risk of diseases and death. The inhibitors of HMG-CoA reductase statins are the most widely used lipid-lowering agents which can reduce cardiovascular morbidity and mortality. Accumulating evidence has documented several pleiotropic effects of statins in addition to their lipid-lowering properties. Recently, several studies have highlighted that statins may have the potential to delay the ageing process and inhibit the onset of senescence. In this review, we focused on the statin anti-ageing mechanisms and effects on cardiovascular and non-cardiovascular diseases.

Keywords: RhoA; klotho; Sirtuin-1; senescence-associated secretory phenotype; telomerase

1. Introduction

Human ageing, the process of becoming older after reaching sexual maturity, is due to a complex interplay of degenerative alterations and processes, with different manifestations such as gradual organ dysfunction or tissue function loss, increment of the aged (senescent) cell population, incapability of maintaining homeostasis, reduced repair potency, and increased risk of pathological changes, disease and mortality (Alichniewicz et al., 2012; Marchand et al., 2011).

Inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase known as statins are the most widely used lipid-lowering agents that can reduce cardiovascular morbidity and mortality (Grundy, 1988; Werner et al., 2002). In addition to lipid-reduction properties, statins have shown pleiotropic beneficial properties, such as anti-inflammatory, anti-oxidant benefits, antiproliferative and many other cholesterol-independent effects, thus probably regulating many critical processes involved in vascular biology (Bahrami et al., 2018; Ferretti et al., 2015; Marrone et al., 2013; Owens, 2012; Sahebkar et al., 2015; Serban et al., 2015). Recently, several studies have suggested that statins may have the potential of delaying ageing and inhibiting the onset of senescence. In this review, we have focused on the anti-ageing mechanisms and effects of statins in cardiovascular and non-cardiovascular diseases.

2. Cellular ageing or senescence

Ageing is defined as a reduction in the capability of maintaining homeostasis in several tissues.

In biology, senescence refers to deteriorative processes and irreversible growth arrest that follow development and maturation. Cellular senescence known also as cellular ageing is the hallmark of ageing, i.e. the reduction of the regenerative capacity of cells. In this regard,

cellular senescence seems to be detrimental because it consists in a deficit of tissue renewal and functionality (Campisi and di Fagagna, 2007).

Genetic background of ageing

The main evolutionary conservative pathways known to influence organism longevity are the insulin/insulin-like growth factor (IGF) signaling (IIS) (Kenyon et al., 1993; Tatar et al., 2001), the serine/threonine kinase mechanistic target of rapamycin complex (mTORC) (Kaeberlein et al., 2005; Powers et al., 2006), and the protein kinase A (PKA) pathway (Enns et al., 2009). IIS is a master inducer of cell proliferation and an inhibitor of apoptosis. *In-vivo* experiments have revealed that mutations that interfere with IGF-I/IIS activity are associated with an extended lifespan through several mechanisms such as decrement of insulin concentrations, increased insulin sensitivity, change in carbohydrate/lipid metabolism, reduction in oxidative stress (OS) and in the production of Reactive Oxygen Species (ROS), enhanced resistance to stress, slowdown of the age-associated onset of diseases (Bartke, 2005). Centenarian females when compared to controls showed overrepresentation of heterozygous mutations in the IGF1 receptor (IGF1R) gene, which is related to high IGF1 serum concentrations and lower IGFIR activity (Suh et al., 2008). Therefore, genetic variations in the human IGF1R that lead to alteration of IIS are associated with an increase in human longevity, supporting a role of this axis in the determination of lifespan extension (Suh et al., 2008). Moreover, in laboratory animals, mutations of growth hormone (GH)/GH receptors or GH resistance cause secondary inhibition of circulating IGF1 and insulin levels, a strong extension of lifespan and multiple manifestations of delayed/slower ageing (Bartke, 2011). Possible explanations for this mechanism could be elevated stress resistance and change in insulin/mTOR signaling and metabolic regulations (Bartke, 2011). A major intracellular target of IIS is mTORC, which is activated in response to abundant nutrient

supplies and growth factor signals. It has been shown that suppression of the mTOR pathway through genetic interventions or pharmacological inhibitors is related to longevity among invertebrates and human species (Harrison et al., 2009). PKA is a tetramer, composed of two regulatory (R) and two catalytic (C) subunits, which exist in different isoforms. Functional activation of regulatory subunits, RI and RII, takes place in response to cAMP. cAMP binds to the R subunits, releasing the C subunits that are free to connect to downstream molecules and phosphorylate them (Niswender et al., 2002). Mutant mice for PKA regulatory subunit IIβ (PRKARIIβ) showed extended lifespan and were resistant to age-associated diseases (Enns et al., 2009). Forkhead box O (FOXO) proteins are a group of conserved transcription factors (TFs) that are essential elements upstream of multiple critical cellular pathways (Carter and Brunet, 2007). FOXO transcriptional targets are implicated in the regulation of cell cycle, cell death, oxidative stress resistance, hematopoiesis, metabolism, cell proliferation, life span and tumor suppression (Accili and Arden, 2004). FOXOs are downstream of IIS, while IIS lowers function/expression of FOXOs via activation of phosphoinositide-3-kinase (PI3K)/AKT (PKB). FOXO3 may be crucial in ageing and increasing lifespan since polymorphisms in this gene are closely associated with longevity in numerous human cohorts (Morris et al., 2015).

Inflammatory pathways in ageing

Continuous low-grade inflammatory processes known as “Inflammageing” are a main feature of ageing and possibly causes to some age-related metabolite, genes and pathways. Mild inflammation is closely related to multiple ageing phenotypes such as alterations in body composition, energy balance, metabolic homeostasis, stress tolerance, immune senescence, and neuronal well-being (Biagi et al., 2010; Franceschi and Campisi, 2014). So, targeting inflammation is one of the promising anti-ageing approaches in the near future. Local

expression of inflammatory cytokines e.g. tumor necrosis factor-alpha (TNF-α), interleukin (IL) 1 and IL-6 could activate both local and systemic inflammation, which are known risk factors for age-dependent diseases. Actually, chronic over-expression of pro-inflammatory mediators underlying ageing due to the age-related redox imbalance activates multiple pro-inflammatory pathways, such as NF-κB signaling (Chung et al., 2009). In addition, the induction of NF-κB is a main activator of gene expression of inflammatory proteins, i.e. cytokines, chemokines, growth factors, interleukins and adhesion molecules, all contributing to the spread of the systemic inflammatory process (Makarov, 2000).

Cardiac and vascular ageing

Cardiovascular ageing consists in functional and structural modifications of the cardiovascular system leading to cardiac hypertrophy, myocardial fibrosis, decreased ventricular compliance, elevated vascular stiffness and risk of diastolic heart failure (Fowler et al., 2007; Steppan et al., 2012). Among several factors, peroxisome proliferator-activated receptors (PPARs), a class of TFs belonging to the nuclear hormone receptor family, have been recognized as relevant in the heart ageing process (Guellich et al., 2007; Poynter and Daynes, 1998).

Vascular ageing is closely related to the senescence of vascular cells (Minamino et al., 2004), that is the senescence of endothelial cells (ECs) and vascular smooth muscle cells, which are implied in the pathology of vascular dysfunction and atherogenesis (Minamino and Komuro, 2007; Orlandi et al., 2006). Nitric oxide (NO) also plays a key role in the control of vascular cell senescence (Förstermann and Sessa, 2011; Minamino and Komuro, 2007). NO is synthesized from L-arginine by NO synthases (NOSs), which exist in different isoforms: endothelial NOS (eNOS), inducible NOS (iNOS), and neuronal NOS (nNOS) (Förstermann and Sessa, 2011). Remarkable signs of vascular ageing are down-regulation of eNOS,

interruption of eNOS activation, enhanced oxidative stress-induced NO inactivation and finally low NO bioavailability. The integrity and functionality of the endothelial monolayer is fundamental shield from the onset of atherosclerosis (Lusis, 2000). Endothelial progenitor cells (EPCs) might be involved in endothelial integrity maintenance, substitution of apoptotic ECs and neovascularization of ischemic tissue (Asahara et al., 1997; Kawamoto et al., 2001). Furthermore, administration of EPCs in patients with ischemic heart disease promotes neovascularization, and, subsequently, improvement of cardiac function and reduction of myocardial ischemia (Kawamoto et al., 2001).

Telomeres and ageing

Mammalian telomeres are maintained through telomerase that adds polynucleotides repeats at the ends of eukaryotic linear chromosomal DNA (de Lange, 2005). Telomeres are made up of several thousand non-coding, short double-stranded tandem repeats of a guanidine-rich DNA sequence (TTAGGG). The telomere ends in a single-stranded DNA (ssDNA) sequence, known as the G-rich strand overhang, added by telomerase and coated by capping proteins extended over 2–15 kb. The G-overhang folds back onto itself and invade the telomeric ends, making the two higher terminal loop structures, large duplex lariat structure (T-loop) and forming a single-stranded displacement loop (D-loop) (Doksani et al., 2013). Although their function is not fully understood, they possibly contribute to successful DNA replication and chromosomal stability and facilitate the creation of a lariat-like structure to protect the exposed ends of DNA from the genome damage such as degradation, telomere uncapping, unwanted repair, end-to-end fusion, and inappropriate recombination (de Lange, 2005; Greider, 1991). Shelterin or telosome complex, a very dynamic structure, is characterized by six essential proteins including TRF1 (telomeric repeat binding factor 1), TRF2 (TRF1-interaction nuclear factor 2), POT1 (protection of telomeres 1), TIN2 (TRF1 and TRF2

interacting nuclear protein 2 (TIN2), TPP1 (Tripeptidyl-peptidase 1), Rap1 (repressor/activator protein 1), and constitutes a shield cap at the 3'-end of chromosome. TRF1 and TRF2 interact with the double-stranded hexanucleotide telomeric repeats (TTAGGG) and binds to TIN2 and RAP1, respectively. POT1 binds specifically to ssDNA telomeric portion and links to TRF1 and TRF2 via a binding partner, TPP1, forming a complex with TIN2 (Maciejowski and de Lange, 2017).

Telomerase, a ribonucleoprotein (RNP) complex, is a family member of the reverse transcriptases (RTs) and is composed of a telomerase reverse transcriptase (hTERT) protein, a template-containing RNA component (TERC) and dyskerin (Jiang et al., 2018; Musgrove et al., 2018). Since DNA polymerase is not able to complete the replication of the 3′-end of eukaryotic DNA, telomeres become shorter at each round of chromosomal division (Verdun and Karlseder, 2007). Telomerase complex synthetizes and appends multi tandem repeats DNA sequences at the 3′-chromosomal ends (5′→3′) throughout successive cell replication cycles and is responsible for compensating the attrition of the DNA-ends and maintaining telomere length (TL) (Blackburn, 1991; Klegarth and Eisenberg, 2018). Telomerase is not adequate to counteract telomere erosion determined by cell division, therefore telomeres shorten with age (Martínez and Blasco, 2017). The first report of this phenomenon was proposed by Leonard Hayflick and co-researchers who found that isolated human embryo-derived fibroblasts have limited proliferative potential with every subsequent cell division (Hayflick, 1965). This biological clock (also known as Hayflick limit) that is the progressive loss of telomeres after each mitosis, is at present defined as end-replication problem or replicative senescence (Olovnikov, 1973). TL shortening determines a continual DNA damage reaction, which results in replicative cellular senescence and eventually massive cell death (Saliques et al., 2010). In almost all cells, senescence and consequent cell death takes place when the mean TL reaches a critical threshold (Allsopp and Harley, 1995). Therefore,

mean TL provides a potential indicator of cellular biological age, where shorter TL represents increased biological age. However, telomeres can remain genetically stable and intact if the telomere renewal machinery, telomerase, remains entirely functional. Dysfunctional telomere leads to genome fragmentation as a result of the removal of “shelterin protection”(Sfeir and De Lange, 2012). Partial or full loss of telomerase associates with inability of tissues to regenerate and, consequently progressive tissue damage and ultimately reduction of life span (García-Cao et al., 2006; Mitchell et al., 1999).

Cellular senescence is an irreversible growth arrest by which cells cease to replicate; it takes place in somatic cells and restricts their proliferative life span. Cellular senescence is conventionally divided into the following two major forms: intrinsic telomere-dependent (replicative senescence) and extrinsic telomere-independent known as stress-induced premature senescence (SIPS) (Itahana et al., 2004; Wlaschek et al., 2003). When telomeres shorten, the cell goes into replicative senescence, which determines enormous alterations in the cell-cycle gene expression profile resulting in reduced proliferation and ultimately apoptosis (Satyanarayana et al., 2003). SIPS is induced via a variety of external stresses such as OS, oncogenic Ras activation, DNA damage/mutation, mitochondrial injury and chemotherapeutic regimens and radiation. These stresses cause the activation of the premature senescence process independent of TL (Blazkova et al., 2010; Collins, 2000; Jin et al., 2017; Mirzayans et al., 2012). The physiological phenotype of a senescent cell, either reached via an intrinsic or extrinsic path, has been named as the senescence-associated secretory phenotype (SASP) or complex senescence-messaging secretome (Young and Narita, 2009).

Salient features of senescent cells include altered cell size and large flat smoothed shape, emergence of senescence markers such as the senescence-associated β-galactosidase (SA-β-gal)(Dimri et al., 1995), senescence associated heterochromatin foci (SAHF) (Kosar et al.,

2011), lipofuscin accumulation (Georgakopoulou et al., 2013), DNA damage response (DDR) signaling (di Fagagna, 2008), and expression of decoy death receptor 2 (DCR2), embryonic chondrocyte–expressed 1 (DEC1) (Collado et al., 2005), and $p15^{INK4b}$ (Kim and Sharpless, 2006). SA-β-gal, a lysosomal hydrolysis, has an optimal pH 4.0 in young or immortal cells (Lee et al., 2006). However, this activity is increased in senescent cells because of the increments of lysosomal content and hydrolysis degree at suboptimal pH (pH=6) displayed by senescent cells (Lee et al., 2006). In the cellular nucleus during senescence, the epigenetic alterations are related to a global change in heterochromatin via the arrangement of facultative domains heterochromatin termed SAHF (Collado et al., 2005; Lee et al., 2006; Narita et al., 2003). DEC1, as a TF contributes to cell growth, proliferation, differentiation, cell death, and senescence (Boudjelal et al., 1997; Guillaumond et al., 2008; Shen et al., 1997). Indeed, senescent cells are able to secrete different factors for instance growth factors, cytokines, chemokines, some microRNAs (miRNAs) and proteases (Acosta et al., 2013; Collado et al., 2007).

Accumulation of different senescence markers in tissues of aged mammals indicate that senescent cells take part in pathologies (Chkhotua et al., 2003; Dimri et al., 1995; Liu et al., 2009). In addition, development and evolution of age-related diseases could be attributed to the reduction of the regenerative actions of stem cells because of increasing age (Sharpless and DePinho, 2007). Senescent cells can mount up with age along with age-related pathologies, like in osteoarthritis (Martin and Buckwalter, 2002) and atherosclerotic plaques (Gorenne et al., 2006), and can have an effect on the normal function of the tissues, leading to an accelerated degeneration.

Statin drugs

Inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (or statins) represent a family of cholesterol-lowering drugs and are widely used in medical practice for patients at increased risk of atherosclerotic cardiovascular disease (Armitage, 2007; Unit, 2005). Statins influence an essential stage of cholesterol biosynthesis, the "mevalonate pathway". Through inhibiting HMG-CoA reductase and preventing L-mevalonic acid synthesis, statins reduce the generation of subsequent isoprenoid elements of this pathway, i.e. farnesyl pyrophosphate (FPP) as well as geranylgeranylpyrophosphate (GGPP) (Istvan and Deisenhofer, 2001). These intermediates are crucial lipid attachments for post-translational modification of important proteins such as small GTPases of the Ras, Rap, Rab and Rho families (Figure 1) (Van Aelst and D'Souza-Schorey, 1997; Zhang and Casey, 1996). After isoprenylation, RhoA activates its downstream target Rho-kinase (ROCK) and afterwards induces several cellular effects (Yang et al., 2010). FPP and GGPP are essential for Ras farnesylation and Rho geranylgeranylation, respectively (Iguchi et al., 2009). By blocking the mevalonate pathway, statins prevent the biosynthesis of isoprenoid intermediates thus restraining small GTPases isoprenylation that support multiple lipid-independent or "pleiotropic" activities of statins (Laufs and Liao, 1998). Another cholesterol-independent activity of statins is the over-expression and activation of endothelial nitric oxide synthase (eNOS). The inhibition of RhoA geranylgeranylation by statins promotes eNOS expression and NO production so that statins alleviate endothelial dysfunction (Laufs et al., 1998). GGPP, but not FPP, can counteract the eNOS overexpression-induced by statins, indicating an important role of RhoA as a negative regulator of eNOS (Laufs et al., 1998).

Statins are usually prescribed as therapeutic agents as either free acid forms or lactone forms. The prodrug lactone form requires metabolization to the acid form in the liver in order to activated and exert pharmacological activities (Schachter, 2005). Prodrug lactone and active acid forms vary regarding to their pharmacological properties and water solubility, half-life,

and potency. The lactones [i.e. lovastatin (LOV) and simvastatin (SMV)] are lipophilic whereas the acids are hydrophilic [i.e. mevastatin (MEV), pravastatin (PRV), atorvastatin (ATV), fluvastatin (FLV), and rosuvastatin (RSV)] (Corsini et al., 1999). Hydrophilic statins are predominantly targeted to liver tissue, whilst vascular cells are the main target for lipophilic statins (Schachter, 2005).

Statins reduce blood cholesterol levels and thereby delay the onset of atherosclerosis, lower the risk of cardiovascular diseases (CVDs), particularly ischemic heart disease, strokes, and reduce CVD-associated morbidity and mortality (Mehta et al., 2006; Nakamura et al., 2006). Statins are also potentially therapeutic in hypertension (Strazzullo et al., 2007) and several malignancies (Gronich and Rennert, 2013). Statins decrease the production of ROS and expression of angiotensin II (AngII)–type 1 receptor in vessels determining vasodilation. Shang *et al* claimed that SMV inhibits lipopolysaccharide-induced TNF-α expression in rat cardiomyocytes via suppression of NADPH oxidase activation and following ROS production (Shang et al., 2006). Statins can decrease ROS through activating NF-E2-related factor 2 (Nrf2) via the PI3K/Akt pathway. Nrf2 as a TFs mediate the transcriptional cells response to OS and electrophilic xenobiotics (Chartoumpekis et al., 2010). Furthermore, statins can reduce large artery stiffness and enhance arterial wall compliance (Strazzullo et al., 2007). Moreover, statins have anti-tumor properties through the reduction of p-MEK1/MEK2 protein levels, which are associated to the Ras/Raf/MEK/ERK signaling pathway responsible for cell proliferation. After statin treatment, malignant cells had a proapoptotic alterations in the Bcl-2/Bax protein ratio, and a translation of their potential from high-invasive to low-invasive (Gronich and Rennert, 2013).

Anti-ageing effect of statins

Accumulating evidences indicate that the pleiotropic effects of statins involve restoring endothelial function, stabilization of the atherosclerotic plaques, remodeling of myocardial

interstitial collagen, modifying apoptosis in vascular ECs and reducing oxidative stress and vascular inflammation, plus anti-neoplastic, immune-modulative, and reno-protective properties (Choi et al., 2010; Kureishi et al., 2000; Sverdrup et al., 2010). Recently, the anti-ageing effects of statins have attracted the attention of researchers (Table 1).

The *klotho* gene, originally discovered through insertional mutagenesis in mouse, is currently described as an anti-ageing gene (Kuro-o et al., 1997). Homozygous *klotho* gene knock-out mice (*klotho* mice) show reduced ageing-related morbidity. Deregulation of *klotho* is associated with ageing-related manifestations like infertility, arteriosclerosis, soft tissues calcification, neural degeneration, osteoporosis, skin atrophy, pulmonary emphysema and lower lifespan, while high-expression of *klotho* suppresses the ageing process (Kuro-o et al., 1997; Nagai et al., 2003). *Klotho* transcript and protein are mostly found in the kidney and play a key role in contrasting endothelial dysfunction, enhancing vascular action (Saito et al., 2000), protecting from AngII-stimulated renal damage and ameliorating kidney function (Mitani et al., 2002). Several studies demonstrated that statins can increase the renal transcription of *klotho* mRNA in a dose-dependent way (Figure 2) (Kuwahara et al., 2008; Yoon et al., 2012). Actually, inhibition of RhoA via statins is necessary for *klotho* amplification. ATV appears to be more potent on RhoA/ROCK pathway inhibition and *klotho* mRNA expression induction when compared to PTV. Controversially, AngII activates RhoA and concurrently hampers *klotho* transcription. However, ATV pretreatment reduces AngII-induced response without any tubular damage. Altogether, these findings reveal that the Rho/ROCK pathway suppression is involved in *klotho* mRNA transcription induction through statins (Narumiya et al., 2004). In another study, the effects of NOS inhibition on *klotho* protein expression as well as the anti-arteriosclerotic effects of statin were explored (Kuwahara et al., 2008). Chronic NOS blockade significantly reduces kidney *klotho* protein expression, whereas administration of ATV or PTV entirely counteracts the NOS-induced

decrement in *klotho* expression in rats (Kuwahara et al., 2008). Besides, statin therapy attenuates the downregulation of renal *klotho* mRNA in cyclosporine A (CsA)-induced nephropathy. CsA prescription promotes phosphorylated FOXO1 (p-FOXO1) expression and reduces p-FOXO3a expression, while statin administration reverses those alterations by up-regulating antioxidant enzymes and inhibiting the transcription of pro-apoptotic proteins (Yoon et al., 2012).

Sabbatini and colleagues examined the effects of ATV on ischemic acute renal failure (ARF) in aged rats with a higher risk of developing ischemia. ATV pretreatment attenuates kidney vasoconstriction in aged rats and restores GFR levels to those of young rats. ATV does not influence the Ras pathway in aged rats but can partially block Rho activation. Low-dose and short-term ATV therapy increases NO availability in old rats, ameliorating renal dynamics and providing a specific tubular protection post-ischemia. Altogether, ATV decreases NO deficiency in old rats via a remarkable impact on eNOS and RhoA proteins. These favorable effects of statins in aged animals may also be mediated by alterations in lipid oxidation and by reduced ROS generation and cell turnover (Sabbatini et al., 2004). The ability of statins in reducing ROS in stressed cells may be due to reduction of oxidized-LDL, down-expression of vascular AngII (AT_1) receptor (Wassmann et al., 2001), suppression of small G-proteins (i.e. Rho) expression (16) and inactivation of the mitogen activated protein kinase (MAPK)-extracellular signal-regulated kinase1/2 (ERK1/2) (Gueler et al., 2002).

It has been suggested that statins may have a potential role in delaying cardiac ageing. The cardioprotective mechanism of statins has been investigated in several reports (Han et al., 2013; Han et al., 2012; Takemoto and Liao, 2001; Thunyakitpisal and Chaisuparat, 2004; Wilson et al., 2005). For instance, during ATV treatment, myocardial lipofuscin amounts, an index of oxidative stress and cardiac ageing status, are remarkably decreased in the aged rats. Moreover, long-term ATV treatment can delay or rather reverse many ageing-associated

pathophysiological alterations, such as cardiac hypertrophy, myocardial apoptosis, and myocardial oxidative stress in a dose-dependent manner. Furthermore, owing to their anti-inflammatory effects, statins are able to delay the heart ageing process (Han et al., 2013).

Statin therapy leads to remarkable reduction of cardiomyocytes diameter, left ventricular wall thickness, collagen condensation, type I/III collagen ratio, heart β-galactosidase levels, heart tissues malondialdehyde activity and to over-activation of superoxide dismutase, catalase and NOS in Wistar rats (Han et al., 2012). Statin administration could reduce expression of several ageing-associated inflammatory cytokines (i.e. IL-1 and TNF-α) on both the transcriptional and translational levels, and enhances the expression of PPARs (Han et al., 2012). Statin may also reduce the expression and secretion of a family of proteolytic enzymes and matrix metalloproteinases (MMPs), thanks to the blockade of mevalonate synthesis (Takemoto and Liao, 2001; Thunyakitpisal and Chaisuparat, 2004; Wilson et al., 2005). In atherogenesis, MMPs may take part in plaque rupture and thereby treatment with statins may relieve the symptoms of cardiac ageing (Davies, 1995; Richardson et al., 1989).

Long-term treatment with ATV lessens the SASP, enhances endothelium relaxation, reduces malondialdehyde levels, increases superoxide dismutase, eNOS, and SIRT1 regulation and translation, and normalizes eNOS/iNOS imbalance in aortas of ageing-rat in comparison with non-treated controls (Gong et al., 2014). These data suggest the efficacy of long-term ATV treatment in improving age-associated endothelial dysfunction, representing a promising approach for atherosclerosis prevention (Figure 2).

Sirtuin-1 (SIRT1; silent information regulator 1), belongs to the Sir2 family proteins whose expression falls with age (Hall et al., 2013). SIRT1 is a NAD+-dependent deacetylase that targets both histone and non-histone proteins, like TFs (Zhang and Kraus, 2010) in order to preserve chromatin silencing, genomic stability, cell differentiation, and is a regulator of metabolism and endothelial function (Menghini et al., 2009). SIRT1 can suppress

atherosclerosis, endothelial dysfunction and vascular cell senescence via interaction with and stimulation of eNOS (Potente and Dimmeler, 2008a, b). Recently, accumulating evidences showed that mammalian SIRT1 activity increases longevity in animal models (Guarente, 2013; Imai and Guarente, 2016; Mercken et al., 2014), and statin administration counteracts endothelial senescence in human umbilical vein ECs through SIRT1 increment (Ota et al., 2010).

MicroRNAs (miRs) belong to a family of short non-coding RNAs that control mRNA expression of target genes through the cleavage or repression of translation (Amerizadeh et al., 2018; Bahreyni et al., 2019). It has been shown that SIRT1-related miRs, such as *miR-34a*, can target SIRT1 causing endothelial senescence. Ota *et al* studied the expression pattern of SIRT1-related miR profiles, including *miR-9*, *-34a*, *-132*, *-181a*, *-195*, *-199a*, *-199b* and *-204*, SIRT1 in EPCs of CAD patients, and the statin influence on these patterns. Among all the analyzed miRs, only *miR-34a* levels were increased and SIRT1 protein levels lower in CAD patients when compared to non-CAD controls. Values of *miR-34a* were slightly inversely correlated with SIRT1 protein amounts. After ATV supplementation, *miR-34a* significantly decreased and SIRT1 increased, while after RSV treatment no change was observed (Ota et al., 2010). Concentrations of other miRs did not differ before and after treatment with ATV or RSV. Conclusively, *miR-34a* may modulate SIRT1 transcription in EPCs and the ATV-induced over-expression of SIRT1 through suppression of *miR-34a* might concur to the beneficial effects of ATV on endothelial function among CAD patients (Ota et al., 2010).

Progeroid syndromes are heritable human diseases characterized by the very early occurrence of signs/symptoms of premature ageing. Hutchinson-Gilford progeria syndrome (HGPS), a rare childhood premature ageing syndrome, caused by a spontaneous point mutation in *lamin A* (*LMNA*) gene, leads to an aberrant alternative splicing of prelamin A towards the synthesis

of a truncated nuclear protein named progerin (Gordon et al., 2014). Accumulation of farnesylated forms of progerin at the nuclear membrane distorts nuclear architecture and affects chromatin stability determining many severe damages. There is an association between HGPS and progressive TL shortening (Burla et al., 2016; Chojnowski et al., 2015; McCord et al., 2013) that causes DNA damage. Combined treatment with statins and aminobisphosphonates (aBP) effectively blocks farnesylation and geranylgeranylation of prelamin A and progerin and significantly reduces the ageing-related phenotypes of mice mutant for the Zmpste24-MMP. Differently from statins, aBP act on the last enzymes of the mevalonate synthetic pathway, blocking FPP synthase and isopentenyl pyrophosphate isomerase (IPP) (Konstantinopoulos and Papavassiliou, 2007). The additive/synergistic effects of statins and aBP on Zmpste24$^{-/-}$ ageing-related phenotypes may be due to the sequential actions on different steps of the mevalonate axis, thereby inhibiting both protein farnesylation and geranylgeranylation (Varela et al., 2008).

Statins were found to be able to effectively prevent inflammatory arthritis (Leung et al., 2003). In recent reports, chondrocyte ageing was reported to be linked to the progression of cartilage damages (Yudoh and Karasawa, 2010). Yoduh and co-researchers demonstrated that the OA-associated catabolic factor IL-1β induces down-expression of cellular function, over-activation of the senescent marker β-galactosidase and reduces the cellular lifetime of chondrocytes *in vitro*. Statin administration in chondrocytes suppresses the IL-1β-triggered synthesis of cartilage matrix degenerating enzymes (MMP-1 and MMP-13), reduces premature senescence, and promotes the biosynthesis of cartilage matrix proteoglycans. In an animal model of OA, statin therapy remarkably decreased the degradation of articular cartilage, whereas the control group displayed progressive knee joints cartilage destruction over time. Statin may have the great advantage to counteract the catabolic stress-stimulated

chondrocyte dysfunction found in the articular cartilages of the elderly (Yudoh and Karasawa, 2010).

Effect of statins on cellular senescence and Telomere

Cellular senescence interferes with tumor development by inhibiting the proliferation of damaged cells and enforcing oncogenic cells to enter cell cycle arrest, though senescent cells are able to induce malignancy by the pro-inflammatory SASP (Figure 2) (Campisi, 2001). It has been shown that SMV attenuates the SASP and its tumor-inducing potential in senescent human fibroblasts by hindering protein prenylation and inactivation of Rho family GTPases Rac1 and Cdc42. IL-6 is one of the SASP factors that can trigger proliferation of breast tumor cells by activation of the MEK-ERK1/2 pathway, but SMV impedes this SASP-induction proliferation (Liu et al., 2015).

Cellular OS can determine a unique phenotype characterized by telomeres shortening, DNA/protein/lipid peroxidation, and cellular senescence-induced by p38-MAPK activation (Behnia et al., 2016; Behnia et al., 2015). p38MAPK protein kinases are a family of intracellular acute stress response proteins that contribute to inflammation, cell death and senescence (Coulthard et al., 2009). p38MAPK is important for the senescence because of its capability to induce the p53 and pRb/p16 growth arrest pathways (Iwasa et al., 2003). Thus, regulating p38MAPK activation and diminishing premature senescence activation could reduce the incidence of OS-associated disorders. Ayad *et al* reported that both SMV and RSV potentially down-express OS-stimulated p38MAPK activation, premature senescence and SASP, whereas RSV exhibits a promoting effect (Ayad et al., 2018).

Inflammation and OS are two major factors that induce age-associated telomere shortening, suggesting that telomere erosion could be considered a promising marker of OS and inflammation (Babizhayev et al., 2011; Houben et al., 2008). Recent evidences pointed out

that statins can affect cell senescence, suggesting a connection between statins and telomere/telomerase system (Nielsen et al., 2012). The TL of EPCs has poor inverse correlation with oxidative DNA stress (Satoh et al., 2008). It has been demonstrated that EPCs obtained from CAD patients present shorter TL and higher telomere shortening rate and oxidative damage to DNA than those from control subjects (Satoh et al., 2008). Evidence supported that EPC telomere shortening due to the oxidative DNA lesions may contribute to the development of CAD. Other supporting evidence comes from a study which assessed both the association between history of statin treatment and leukocyte telomere length (LTL) and its connection with plausible novel indicators of oxidative DNA damages and ROS-induced inflammation. As telomere shortening is nearly equal in various human tissues, blood circulating mononuclear cells can be used as an easily applicable surrogate tissue for TL assessment. Results demonstrated that patients undergoing statin treatment have longer mean LTL when compared with non-treated patients. Indeed, the expression levels of two potent biomarkers of oxidative damage and inflammation, leukocyte Finkel-Biskis-Jinkins osteosarcoma (FOS) and 8-oxoguanine DNA glycosylase (OGG1), were not different between two groups. This finding showed that statin treatment was correlated with longer LTL. Notably, higher FOS and OGG1 can be considered as a novel pertinent biomarkers of LTL (Saliques et al., 2011). A randomized controlled trial compared the effect of intensive cholesterol-reduction treatment (ATV; 10 mg per day) and moderate cholesterol-reduction treatment (PTV; 10 mg per day) on EPC telomere biology (Satoh et al., 2009). Intensive cholesterol-reduction treatment enhanced EPC frequencies and limited EPC telomere shortening, but moderate cholesterol-reduction treatment did not affect EPC populations or withhold EPC telomere shortening (Satoh et al., 2009).

Boccardi and co-workers investigated the effect of statin administration on peripheral blood leukocyte telomerase activity (TA), leukocyte telomere length (LTL) instability, and the

relationship with telomere shortening rate through ageing (Boccardi et al., 2013). In this cross-sectional survey, individuals receiving statin showed significantly increased TA, longer LTL and less telomere erosion *versus* the control group, after adjustment for potential confounders including age, sex, smoking, blood pressure and blood levels of glucose, lipids, and inflammatory parameters. LTL reduction was 0.03 Kb per each year of age in statin receiving group, and 0.06 Kb in controls, respectively. Interestingly, a major difference in telomere erosion between the two groups was found in older subjects (65 year) (Boccardi et al., 2013). Several studies demonstrated that low TA and subsequent weaker telomere maintenance potency are connected to a higher risk for CVD, independent of chronological age (Serrano and Andrés, 2004), whereas over-expression of telomerase without net telomere lengthening possibly enhances cellular longevity and genome stability *in vitro* (Zhu et al., 1999). In healthy women, TA rather than TL is negatively related with main CVD risk factors (Satoh et al., 2009).

Another study recruited 484 cases at high risk for coronary heart disease (CHD) events and 1058 controls to evaluate the relationship between TL and observed clinical advantage of statin therapy. Results showed that mean LTL declined with age by 9% and 5.9% per decade in controls and cases, respectively. Subjects in the middle and in the lowest tertiles of LTL were at significantly greater risk of developing a CHD event when compared to subjects in the highest tertile (odds ratio [OR] for CHD: 1.5; 95% confidence interval [CI]:1.1–2.0 in the middle tertile; 1.4; 1.1–1.9 in the lowest). In the placebo group, risk of CHD was approximately two-fold in individuals in the lower two tertiles of LTL when compared to individuals in the highest tertile (1.9; 1.3–2.8, $p<0.001$ and 1.9; 1.3–2.85, $p<0.001$ in the middle and lowest tertiles, respectively). In patients treated with PRV, the elevated risk due to shorter telomeres, was remarkably mitigated (1.1; 0.7–1.7, $p=0.58$ and 1.02; 0.7–1.5, $p=0.94$ in the middle and lowest tertiles, respectively). Mean LTL seems to be a predictor of

CHD events. It seems that patients with higher risk according to mean LTL did greatly benefit from statin treatment in term of CHD prevention (Brouilette et al., 2007).

Low-dose FLV or valsartan or their combination significantly raised TA by 106.9%, 59.5%, and 228.0% respectively when compared to paired controls who received placebo. Remarkably, increased TA from the combination arm was significantly related to the improvement of arterial function as evaluated by flow-mediated dilation as well as inflammation/OS reduction as assessed by C-reactive protein and total antioxidant capacity. The authors recommended the introduction of a combination of low-dose FLV and valsartan as a novel innovative approach for "arterial rejuvenation" (Janić et al., 2016).

In a prospective randomized trial, 100 hypercholesterolemic patients in primary prevention were randomized to receive either ATV (20 mg daily) or placebo for one year. At the trial end, the log-value of TA changed from 0.46 to 0.68 (p = 0.004) and from 0.67 to 0.60 (p = 0.48) in the ATV and placebo arms, respectively. ATV treatment was the only significant predictor of TA alterations, independently of inflammatory and oxidative biomarkers levels (Strazhesko et al., 2016).

EPCs play a significant role in neo-angiogenesis after ischemia (Zhang et al., 2002). ATV and MEV hamper the onset of EPC senescence in culture in a dose-dependent way. In addition, ATV accelerates proliferation of EPCs, while FPP or GGPP attenuate the anti-ageing effect of ATV. On the other hand, NOS withholding, antioxidants, or Rho kinase inhibitors do not affect ATV effects. ATV regulates several cell cycle genes transcription, for instance inducing the expression of cyclins and reducing expression of p27Kip1, the cyclin dependent kinase–G1 phase inhibitor. Accordingly, statins prevented EPCs senescence independently of NO, ROS, and Rho kinase, but dependently on GGPP. ATV-induced inhibition of EPCs senescence seems to be mediated by the regulation of different cell cycle proteins via the PI3K pathway. The increase of cell cycle related proteins together with a

decrement of the p27 may contribute to cell cycle progression and thereby inhibit the initiation of replicative senescence. Therefore, activation of the PI3K/Akt axis via statins may have several protective consequences on EPCs such as the expansion of EPCs frequency and ultimately suppression of apoptosis and senescence (Assmus et al., 2003).

Opposing evidences about anti-ageing effects of statin

Suppression of HMG-CoA reductase leads to a reduced production of cholesterol and other metabolites downstream of mevalonate, a precursor of the coenzyme Q10 (CoQ10, ubiquinone) synthesis (Mabuchi et al., 2007). CoQ10 is a unique lipid soluble benzoquinone and a major component of the respiratory chain that is located in the hydrophobic sections of cellular membranes and involved in oxidative phosphorylation for adenosine triphosphate (ATP) biosynthesis (Langsjoen, 1994).

The cellular roles of CoQ10 in humans include transport of electrons from mitochondrial respiratory complexes I and II to complex III, production of superoxide anion radicals via autoxidation ubisemiquinone, as well as anti-oxidant scavenging of free radicals (Kishimoto et al., 2003; Sun et al., 1992).

The antioxidant activity of CoQ is particularly important in inhibiting ceramide-associated apoptosis (Navas et al., 2007), a crucial regulator of longevity concerning of normal aging (Martin-Montalvo et al., 2016). Aging seems to be contribute in lowering the concentrations of CoQ10 (Littarru and Langsjoen, 2007). A remarkable decrement in the speed of CoQ production has been found during the aging process and aging-related disorders (Alleva et al., 1995; Kalén et al., 1989). It was suggested that aging-associated elevation in mitochondrial OS may be due to CoQ depletion (Miles et al., 2004). On the other hand, Ishii *et al.* demonstrated that CoQ10 extends the life span of wild-type *Caenorhabditis elegans*, the most widely used organism as a model for ageing in longevity researches. In fact, CoQ10

supplementation can significantly increase *C. elegans* longevity possibly by lowering the superoxide anion levels and subsequently decreasing OS in mitochondria (Ishii et al., 2004). However, there are several inconsistencies about the association between CoQ concentrations and the aging process. Mice deficient of one of the alleles of the *COQ7* gene present extended lifespan even though their CoQ concentrations are similar to those of wild-type mice, indicating that factors different from CoQ could be implicated in life time extension (Lapointe and Hekimi, 2008). However, a direct association between lifespan and mitochondrial CoQ contents has been documented in a senescence-accelerated mice model (Tian et al., 2014). Furthermore, CoQ treatment delays senescence (Tian et al., 2014).

Since cholesterol and coQ10 share a similar biosynthetic pathway, a statin-induced decrement in cholesterol may also lead to a block of CoQ10 generation. A recent systematic review and meta-analysis of eight placebo-controlled trials reported a significant reduction in plasma CoQ10 levels after statins therapy (weighted mean difference=−0.44 mol/L, 95%CI: −0.52 to −0.37, $p<0.001$) (Banach et al., 2015b).

Adverse side effects related to statins, known as statin-associated muscle symptoms (SAMS), are mainly attributed to the reduction of CoQ10 in muscle tissue and subsequent mitochondrial dysfunction (Banach et al., 2015a; Deichmann et al., 2010). However, not all studies support the possible mitochondrial dysfunction induced by statins. In humans, Laaksonen and coworkers found no alterations in CoQ10 levels in muscle biopsies from subjects before and after SMV therapy (Laaksonen et al., 1996).

We previously mentioned that longevity is closely related to decrement in circulating insulin concentrations and increased insulin sensitivity. However, whether statins by decreasing insulin sensitivity and secretion triggers the progression of diabetes is an issue of concern. Mechanisms behind the relationship of statins with diabetes mellitus remain unknown. Researches about the effects of statin therapy on insulin sensitivity are contradicting. Some

studies reported detrimental or neutral effects of statins on insulin sensitivity and secretion (Cederberg et al., 2015; Gannagé-Yared et al., 2005; Moutzouri et al., 2011; Puurunen et al., 2013; Szendroedi et al., 2009), whereas others have demonstrated beneficial effects (Naples et al., 2008; Paolisso et al., 2000; Sugiyama et al., 2007). In a systematic review and meta-analysis of clinical trials, PRV and SMV were reported to respectively improve and worsen significantly insulin sensitivity (Baker et al., 2010). It may be possible that lipophilic and hydrophilic statins have various effect on glucose tolerance and thereby differential metabolic effects (Axsom et al., 2013). Therefore, statins have unique affinities to cell membranes and thus subjects competencies to influence cell functions. Future researches should focus not only on shedding light on the impact of the different statins on the glycemic control, but also on their association with the ageing processes.

Conclusion

Cellular ageing or senescence is a complex response to different stimuli that lead to a progressive loss of tissue and organ function. Statin therapy improves cell functionality, delays senescence, and suppresses telomere shortening and apoptosis. Since senescence is an irreversible process, slowing its progression through statins could offer new opportunities and targets for preventive approaches. However, the interplay between TL and statin therapy has not been specifically studied in humans; maintaining the shelterin complex stability, a high TA or a beneficial effect on telomere function are the potential effects of statins in humans. Finally, statins display several benefits on cardiac/vascular cells and endothelial function, including inflammation and oxidative stress reduction, which could participate to the pleiotropic lipid-lowering independent effects of statins. Statin administration could be considered as a promising strategy for the treatment of age-related pathologies.

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Figure legends:

Figure 1. Effects of statins on the cholesterol biosynthesis pathway. **Abbreviations**: eNOS, endothelial nitric oxide synthases; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; FPP, farnesyl pyrophosphate; GGPP, geranylgeranylpyrophosphate; ROCK, Rho-kinase; ROS, Reactive Oxygen Species.

Figure 2. Mechanisms by which statins influence cellular ageing and senescence. Statins inhibit telomerase shortening, *miR-34a*, eNOS down-regulation, eNOS inactivation, and OS-stimulated p38MAPK activation; statins induce SIRT1, FoxO3 and klotho mRNA over-expression. Statins also minimize the SASP and its ageing-enhancing consequences in senescent cells. **Abbreviations**: FoxO, Forkhead box O; SASP, senescence-associated secretory phenotype; SA-β-gal, senescence-associated β-galactosidase; SAHF, senescence associated heterochromatin foci; DDR, DNA damage response; DCR2, decoy death receptor 2; DEC1, embryonic chondrocyte–expressed 1.

Table 1. Effect of statin treatments on ageing and longevity.				
Condition	Arm	Experiment model	Outcome	Ref.
Ageing	ATV (1 μmol/l)	-mIMCD3 tubular cell line	-RhoA/ROCK pathway inhibition -klotho mRNA expression induction	(Narumiya et al., 2004)
CsA-nephropathy	PRV	- normal mice - chronic CsA nephropathy-induced mice	-attenuation of the down-regulation of renal klotho mRNA in CsA-nephropathy induced by oxidative stress -reduction of p-FoxO1 expression and increase of p-FoxO3a expression	(Yoon et al., 2012)
ARF	ATV (12 mg/kg/d for 14 d)	-young (3 mo old) rats -old (18 mo old) rats	-block the Rho activation -enhancement of NO availability	(Sabbatini et al., 2004)
Cardiac ageing	ATV (10 or 1mg/kg/day); 4 months -saline	Aged rats	-reversion of the ageing-associated pathophysiological alterations (i.e. cardiac hypertrophy, myocardial apoptosis, myocardial oxidative stress) -decrement of myocardial lipofuscin	(Han et al., 2013)
Cardiac ageing	-AVT (10 or 1 mg/kg/ day); 4 months -saline	-old Wistar rats	-decrement of the transcription and translation of several ageing-associated inflammatory cytokines (i.e. IL-1 and TNF-α) -overexpression of PPARs -inhibition of the expression and secretion MMPs	(Han et al., 2012)
Endothelial dysfunction	-ATV (5 mg/kg/day); 8 months	-Male old Wistar rats	-reduction of the SASP -enhancement of the relaxation of endothelium -decrement of MDA -increase of SOD, eNOS, and SIRT1 regulation -normalization of eNOS/iNOS imbalance	(Gong et al., 2014)
CAD	- ATV(10 mg/day) ; 8 months -RSV (2.5 mg/day); 8 months	-cultured EPCs from 70 patients with CAD and 48 subjects without CAD	-ATV leads to over-expression of SIRT1 through suppression of *miR-34a*	(Ota et al., 2010)
Premature ageing	-PRV(100 mg/kg/d) -amino-bisphosphonates	-Zmpste24$^{-/-}$mice	-blocking of farnesylation and geranylgeranylation of prelamin A and progerin -reduction of the ageing-related phenotypes	(Varela et al., 2008)
Osteoarthritis	-statin (1.0 or 10.0 μM)	-human articular cartilage samples from OA patients -STR/OrtCrlj mice	-suppression of the IL-1β-triggered generation of cartilage matrix degenerating enzymes -increase of the biosynthesis of cartilage matrix proteoglycan -stress-stimulated chondrocyte dysfunction	(Yudoh and Karasawa, 2010)
Breast cancer -induced by senescent cells	-SMV	- HCA2 human fibroblast cells - MCF7 and ZR-75 human breast cancer cells	-minimizing the SASP and its tumor-enhancing consequences in senescent human fibroblasts through hindering protein prenylation and inactivation of Rho family GTPases Rac1 and Cdc42	(Liu et al., 2015)
Premature	-SMV (100 and 200	-fetal membranes	-down-expression of OS-stimulated	(Ayad et

senescence	ng/mL) -RSV (100 and 200 ng/mL) - progesterone (10−6 mol/L)		p38MAPK activation, premature senescence and SASP	al., 2018)
Arteriosclerosis	-ATV (10 mg per day) -PTV (10 mg per day)	-Wistar rats	-ATV enhances EPC frequencies and barricaded EPC telomere shortening	(Satoh et al., 2009)
Ageing	-RSV -ATV -SMV	-230 Caucasians	-increment in TA, LTL levels and reduction in telomere erosion	(Boccardi et al., 2013)
CHD	- PRV (40 mg daily); 4.9 years -placebo	-6595 statin-naive men	-mitigation of elevated risk with shorter telomeres	(Brouilette et al., 2007)
Arterial wall rejuvenation	-FLV (10 mg daily); 30 days -valsartan (20 mg daily); 30 days -combination (10 mg daily); 30 days - placebo	-130 middle-aged, apparently healthy	-low-dose FLV or valsartan or combination significantly elevate TA by 106.9%, 59.5%, and 228.0% when compared to controls -improvement of arterial function and reduction of inflammation/oxidative stress	(Janić et al., 2016)
CVD	- ATV (20 mg/day); 12 months -placebo)	-100 hypercholesterolemic patients	-increment in TA	(Strazhesko et al., 2016)
Ischemic tissue	-ATV -MEV	-Mononuclear cells	-acceleration of the proliferation of EPCs -modification of mRNA transcription of several cell cycle genes -prevention of the GGPP-dependent senescence in EPCs	(Boccardi et al., 2013)

ARF, ischemic acute renal failure;ATV, atorvastatin; CAD, coronary artery disease; CHD, coronary heart disease; CsA; cyclosporine; EPCs: Endothelial progenitor cells; FLV, fluvastatin; GGPP: geranylgeranylpyrophosphate; IL, interleukin 1; LTL: leukocyte telomere length; MAPK, mitogen activated protein kinase; MDA, Malonyldialdehyde; MEV, mevastatin; miR, microRNA; MMPs, matrix metalloproteinases; NO, nitric oxide; NOS, nitric oxide synthases; iNOS: inducible NOS; OA: osteoarthritis; OS, oxidative stress; p-FoxO1, phosphorylated Forkhead box O; PRV, pravastatin; ROCK, Rho-kinase; PPARs, peroxisome proliferator-activated receptors; RSV, rosuvastatin; SASP, senescence-associated secretory phenotype; SIRT1, Sirtuin-1 ; SMV, simvastatin ; SOD, superoxide dismutase; TA, telomerase activity; TNF-α, tumor necrosis factor;

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