Sestrin family – the stem controlling healthy ageing

Alexander Haidurov a, b, Andrei V. Budanov a, b, * 

a School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Pearse Street, Dublin 2, Ireland 

b Center for Precision Genome Editing and Genetic Technologies for Biomedicine, Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia

ABSTRACT

Sestrins are a family of stress-responsive antioxidant proteins responsible for regulation of cell viability and metabolism. The best known Sestrin targets are mTORC1 and mTORC2 kinases that control different cellular processes including growth, viability, autophagy, and mitochondrial metabolism. Inactivation of the single Sestrin gene in invertebrates has an adverse impact on their healthspan and longevity, whereas each of the three Sestrin genes in mammals and other vertebrate organisms has a different impact on maintenance of a particular tissue, affecting its stress tolerance, function and regenerative capability. As a result, Sestrins attenuate ageing and suppress development of many age-related diseases including myocardial infarction, muscle atrophy, diabetes, and immune dysfunction, but exacerbate development of chronic obstructive pulmonary disease. Moreover, Sestrins play opposite roles in carcinogenesis in different tissues. Stem cells support tissue remodelling that influences ageing, and Sestrins might suppress ageing and age-related pathologies through control of stem cell biology. In this review, we will discuss the potential link between Sestrins, stem cells, and ageing.

ARTICLE INFO

Keywords:
Sestrin
GATOR1/2
mTORC1/2
Mitochondria
Cell death

1. Sestrins – stress-responsive proteins responsible for mTOR regulation

1.1. Sestrins and stress response

Sestrins are a highly evolutionarily-conserved protein family found in most Metazoan species (Budanov et al., 2010, 2002; Parmigiani and Budanov, 2016). While vertebrate genomes encode three Sestrin genes (termed SESN1-3 in humans, Sesn1-3 in other vertebrate species), only one Sestrin gene (Sesn) is found in invertebrate genomes (Budanov et al., 2010). The common feature of all members of the Sestrin family is that they are involved in stress-response, improving the tolerance of organ metabolisms to various stresses including hypoxia, genotoxic stress, oxidative stress, endoplasmic reticulum (ER) stress, and metabolic stress (Dalina et al., 2018). DNA-damage induces transcription of the SESN1 and SESN2 genes through activation of the p53 tumour suppressor protein (Budanov et al., 2004, 2002; Velasco-Miguel et al., 1999). Members of the FOXO family play a key role in activation of the SESN3 gene by oxidative stress (Chen et al., 2010; Hagenbuchner et al., 2012). The p53 and FOXO transcription factors also activate expression of the Sestrin gene (dSesn) in Drosophila melanogaster, indicating that the pathways of Sestrin regulation have been evolutionarily conserved (Lee et al., 2010). Both ER stress, linked with the unfolded protein response (UPR), and metabolic stress, caused by nutrient deficiency and diminished energy production, stimulate SESN2 transcription via the NRF2 and ATF4 transcription factors (Bruning et al., 2013; Ding et al., 2016; Ye et al., 2015). Other transcription factors that contribute to SESN2 expression include the hypoxia regulator HIF-1, the UPR regulators: ATF6 and XBP1, and the regulator of oxidative stress response c-Jun (Dalina et al., 2018). The stress response outcome depends on cell type and cell milieu, determining whether it is more appropriate to support cell viability and protect tissue integrity or stimulate cell death to prevent accumulation of age-related and cancer-supporting damage (Budanov, 2011; Dalina et al., 2018; Ding et al., 2015).

These activities are ensured by several well-defined mechanisms. Sestrins work as antioxidant proteins preventing accumulation of reactive oxygen species (ROS) through their intrinsic antioxidant activity and regeneration of thiol peroxidases – peroxiredoxins, responsible for decomposition of hydrogen peroxide and alkyl peroxides. Sestrins also suppress ROS via stimulation of macroautophagy (herein referred to as autophagy) and its specific form – mitophagy (Budanov et al., 2004; Dalina et al., 2018; Ishihara et al., 2013; Kim et al., 2015a). Autophagy is a process of encapsulation of intracellular content within double-membrane vesicles, called autophagosomes, followed by the degradation of the content in the lysosomes (Galluzzi et al., 2017). Mitochondria are the primary source of ROS in animal cells (Murphy,
Mechanisms of Ageing and Development 192 (2020) 111379

2009), and mitochondrial malfunction causes oxidative stress associated with extensive damage of different intracellular macromolecules, thereby leading to mutagenesis and senescence (Cui et al., 2012). Mitophagy participates in protection of mitochondrial function through elimination of dysfunctional mitochondria, and this process is also responsible for removal of abundant mitochondria to negate or limit mitochondrial activity in certain cell types (Palikaras et al., 2018). One study demonstrates that SESN2 regulates mitophagy in macrophages by interacting with p62/SQSTM1 and facilitating perinuclear clustering of damaged mitochondria that prime mitophagy process (Kim et al., 2016). Our recent work has shown that a significant portion of the SESN2 protein is located on the outer membrane of the mitochondria, suggesting that Sestrins may also support mitochondrial respiration through mitochondrial integrity surveillance and mitophagy activation (Ding et al., 2016; Kovaleva et al., 2020) (Fig. 1A).

1.2. Regulation of mTORC1 and mTORC2

The most characterized activity of Sestrins is regulation of the mechanistic target of rapamycin (mTOR) kinase presented in cells in two complexes, namely mTORC1 and mTORC2 (Budanov and Karin, 2008; Lee et al., 2013, 2010; Lee et al., 2012). Both complexes are involved in regulation of metabolism and cell growth (Liu and Sabatini, 2020). Nutrients, such as glucose and amino acids, and growth factors activate mTORC1 that in turn stimulates protein, DNA, and lipid biosynthesis. As a result, mTORC1 activates cell growth and proliferation and suppresses autophagy (Liu and Sabatini, 2020). On the contrary, different stress factors inhibit mTORC1 causing a cellular switch from growth/proliferation mode towards survival/repair mode, therefore supporting homeostasis in adverse environmental conditions (Budanov, 2011; Liu and Sabatini, 2020). The major regulator of mTORC1 is the small GTPase Rheb that in its GTP-bound form directly binds and activates mTORC1 on the lysosomes. Collaterally, mTORC1 is regulated by a group of the small GTPases RagA-D that form RagA/B:RagC/D heterodimers. RagA/B:RagC/D in their active form (RagA/B is bound to GTP and RagC/D – with GDP) recruit mTORC1 to the lysosomes propping up its activation by Rheb (Liu and Sabatini, 2020). Rheb activity is suppressed by the tuberous sclerosis complex (TSC), which works as a GTPase-activating protein (GAP), and growth factors stimulate GTP-loading of Rheb through TSC inhibition (Wullschleger et al., 2006). RagA/B is activated by the Regulator protein complex that works as a GAP/GTP exchange factor and is inhibited by the GATOR1 protein complex that acts as GAP for RagA/B. GATOR1 in turn is negatively regulated by the GATOR2 protein complex (Liu and Sabatini, 2020). GATOR2 is responsible for mTORC1 activation in response to leucine, arginine, and potentially some other amino acids (Meng et al., 2020; Wolfson and Sabatini, 2017). The mechanisms of mTORC2 activation are not well-known, however, mTORC2 was recently described to be activated by phosphatidylinositol 3-phosphate (PI3P) in response to insulin receptor activation via mTORC2 recruitment to the cytoplasmic membrane (Liu et al., 2015a). The AMPK protein kinase, the major energy sensor in the cell, inhibits the activity of mTORC1 through phosphorylation of the TSC2 and Raptor proteins, the components of TSC and mTORC1, respectively (Gwinn et al., 2008; Inoki et al., 2003). At the same time, AMPK can activate mTORC2 and its target – the AKT protein kinase through direct phosphorylation of several mTORC2 proteins (Kazken et al., 2019; Lee et al., 2012).

Both mTOR-containing complexes tightly control each other. Thus, mTORC2 activates AKT that in turn stimulates mTORC1 through phosphorylation of the proteins responsible for mTORC1 inhibition, such as PRAS40, the mTORC1 partner, and TSC2 (Manning and Toker, 2017). In contrast, mTORC1 inhibits the mTORC2-AKT axis through phosphorylation of the IRS1 and GRB10 proteins, the regulators of the PI3K-AKT pathway, and through inhibitory phosphorylation of Rictor, a

Fig. 1. The functions of Sestrins. A) The role of Sestrins in regulation of biological processes. Expression of Sestrins is induced by various stress insults through activation of stress-responsive transcription factors, and Sestrins control cell viability and functionality in response to stress. B) Regulation of cellular signalling by Sestrins. Sestrins are key regulators of the mTORC1 and mTORC2 kinases that play a critical role in many physiological and pathophysiological processes including cell growth, stress response, and tissue remodelling.
component of mTORC2 (Dalina et al., 2018; Hsu et al., 2011; Julien et al., 2010; Shah and Hunter, 2006; Yu et al., 2011) (Fig. 1B).

1.3. Sestrins are inhibitors of mTORC1

Sestrins inhibit mTORC1 and stimulate mTORC2 through two parallel mechanisms: AMPK activation and GATOR2 inhibition (Budanov and Karin, 2008; Chantranupong et al., 2014; Kowalsky et al., 2020; Lee et al., 2012; Parmigiani et al., 2014). To activate AMPK, Sestrins work as scaffold proteins facilitating the interaction between AMPK and its upstream LKB1 kinase resulting in AMPK phosphorylation (Budanov and Karin, 2008; Morrison et al., 2015). Sestrins also directly bind GATOR2 and relieve inhibition of GATOR1 by GATOR2, resulting in mTORC1 inhibition (Budanov, 2015; Chantranupong et al., 2014; Parmigiani et al., 2014). Interestingly, SESN1 and SESN2 work as leucine sensors, and leucine binds these proteins via a particular region within the protein called the leucine-binding pocket (Saxton et al., 2016; Wolfson et al., 2016). However, whether Sestrins play a general role in leucine sensing or their effects are context-dependent is not well-understood as several other potent leucine sensors were described (Kim et al., 2012; Son et al., 2019). Moreover, Sestrins are still capable of interacting with GATOR2 when abundant leucine is present in the medium (Parmigiani et al., 2014) (Fig. 1B).

1.4. Sestrins are mTORC2 activators

Besides their role in mTORC1 suppression, Sestrins also activate AKT through mTORC1 inhibition, exerting a negative feedback loop (Lee et al., 2012). Recent studies also demonstrated that Sestrins might activate the mTORC2-AKT pathway through mTORC1-independent mechanisms such as direct protein-protein interaction with Rictor or through association with GATOR2 (Kowalsky et al., 2020; Tao et al., 2015). Another AKT activation mechanism, found in melanoma cells and mouse embryonic fibroblasts, operates via suppression of translocation of the PI3P phosphatase PTEN to the cytoplasmic membrane where PTEN inhibits the pathways responsible for AKT phosphorylation (Zhao et al., 2014) (Fig. 1B).

2. Sestrins and ageing

2.1. Sestrin in Caenorhabditis elegans

mTORC1 is one of the most critical regulators of ageing in a diverse range of eukaryotic organisms – from yeast and worms to flies and mice. Suppression of mTORC1 by specific inhibitors or through genetic approaches leads to lifespan extension in the majority of eukaryotic species, including yeast and mice (Johnson et al., 2013). Through mTORC1 inhibition, Sestrins might regulate lifespan in various Metazoan species (Dalina et al., 2018). Inactivation of C. elegans cSesn shortens, while cSesn overexpression extends the nematode worm’s lifespan (Yang et al., 2013; Zeltukhin et al., 2018). Additionally, cSesn protects worms against various stress insults, such as treatment with hydrogen peroxide, Cu²⁺ ions, and heat (Yang et al., 2013). Longevity is linked with stress resistance (Johnson et al., 2001), and Sestrins are known to provide tolerance to inward and outward stresses. Moreover, cSesn-deficient animals display reduced locomotor activity and pharyngeal contraction that are the features of a muscle degenerative phenotype (Yang et al., 2013; Zeltukhin et al., 2018).

2.2. Sestrin in Drosophila melanogaster

Sestrin plays a critical role in the regulation of healthspan but not lifespan in D. melanogaster. dSesn is barely expressed during the larvae stage, but its expression becomes evident when drosophila reaches the adult stage. dSesn inactivation in fruit flies leads to metabolic changes associated with lipid and trehalose accumulation in the animals (Lee et al., 2010, 2012). Similar to the phenotypes observed in C. elegans, dSesn knockout in D. melanogaster causes muscle dysfunction that is manifested by observable deterioration in the structure of thoracic muscle with disturbed Z discs and M bands, scrambled actomyosin arrays, and fuzzed sarcomere boundaries, resulting in poor physical performance (Kim et al., 2020; Lee et al., 2010). dSesn+/− flies are also characterised by a low heart rate and arrhythmia (Lee et al., 2010). The phenotypes observed in relatively young 2–3-week old animals were similar to the phenotypes of old wild-type flies, indicating that dSesn inactivation accelerates D. melanogaster ageing (Lee et al., 2010).

2.3. Sestrins expression in mammalian tissues

To understand Sestrin role in regulation of physiological functions in mammals, the expression of Sestrins was analysed in different tissues and, according to several studies, Sestrins are expressed in most human tissues (Fig. 2A-B). SESN1 is expressed in two major mRNA forms: 4.4 kb and 2.6 kb. The 2.6 kb mRNA is the main form that is observed in every tissue. SESN1 is excessively expressed in skeletal muscle. SESN1 is also highly expressed in pancreas, kidney, lung, placenta, brain, and ovary, but its expression is low in leukocytes, colon, small intestine, and thymus (Fig. 2B). In skeletal muscle and kidney, both mRNA forms are expressed at similarly high levels, while in other tissues the 4.4 kb form is expressed at much lower levels compared to the 2.6 kb form (Velasco-Miguel et al., 1999). SESN2 is moderately expressed in skeletal muscle and extensively expressed in brain, placenta, lung, liver, kidney, pancreas, testis, colon, and peripheral blood leukocytes. However, its expression is low in spleen, thymus, prostate, and small intestine (Fig. 2A-B). Interestingly, SESN2 in skeletal muscle and pancreas expresses additional transcripts with the sizes significantly smaller than the typical 3.9 kb mRNA form, found in all tissues, and the products of these smaller transcripts may play some regulatory role, however, it is yet to be determined in which types of cells they are expressed in these tissues. SESN3 encodes two mRNA forms expressed at moderate levels in most tissues except for lung and thymus (Budanov et al., 2002; Peeters et al., 2003; Velasco-Miguel et al., 1999). Therefore, each of the Sestrin genes probably predominates in the regulation of homeostasis in a particular tissue with the most prominent SESN1 role in muscle and SESN2 – in kidney and testis (Fig. 2A-B).

2.4. Sestrins – are regulators of muscle function in vertebrates

The ability of Sestrins to protect muscle function is conserved between invertebrates and vertebrates. SESN1 is strongly expressed in human skeletal muscle (Velasco-Miguel et al., 1999), while the expression of SESN2 and SESN3 is also notable in this tissue (Peeters et al., 2003). The considerable expression of Sestrins in muscle implies that they play an indispensable role in regulation of muscle function. Accordingly, inactivation of all three Sestrin genes in mice strongly impedes voluntary and forced running of the animals and extends the period of recovery after forced running (Kim et al., 2020). Sestrins support muscle function through activation of mitochondrial biogenesis and mitochondrial respiration that are critically important for muscle endurance (Kim et al., 2020). SESN1 also prevents disuse-induced and ageing-associated muscle atrophy. These effects are mediated by suppression of mTORC1 activity and ensuing activation of autophagy that is responsible for quality control of muscle structure and function (Segales et al., 2020). SESN1 also activates AKT in muscles, and this kinase phosphorylates the FOXO family transcriptional factors causing their detention in the cytoplasm and preventing activation of muscle atrogenes, the genes responsible for atrophy in muscle tissue. The protein products of atrogenes stimulate proteolysis of muscle proteins and contribute to muscle wasting in Sesn1-null animals (Segales et al., 2020). Both SESN1 and SESN2 are activated in response to acute exercise in mouse skeletal muscle (Crisol et al., 2018). However, temporal inactivation of muscle SESN1 by leucine leads to mTORC1 activation and may
support muscle growth during certain intervals of nutrient supply after feeding (Xu et al., 2019). Accordingly, transcriptional upregulation of SESN2 and other Sestrin genes by exercise leads to AMPK activation and mTORC1 suppression, provoking autophagy and other catabolic pathways to increase energy production and prevent accumulation of tissue damage (Crisol et al., 2018; Lenhare et al., 2017; Liu et al., 2015b). The importance of Sestrins in control of muscle function is underpinned by their downregulation in muscle tissue of elderly humans and mice, therefore, compromised Sestrin activity might lead to development of muscle atrophy and frailty (Lenhare et al., 2017; Segales et al., 2020; Zeng et al., 2018).

### 2.5. Sestrins in heart

SESN2 expression is increased in mouse heart in response to ischemia in a model of myocardial infarction and this protein suppresses ischemia-reperfusion induced necrotic cell death through AMPK activation (Morrison et al., 2015; Quan et al., 2017). Similar to the phenotype observed in skeletal muscle, Sestrin expression is declined in cardiac cells of aged mice, and these cells become more susceptible to ischemia-reperfusion induced cell death (Quan et al., 2017). The protective effects of Sestrins are mediated by increased translocation of glucose transporter GLUT4, which augments glucose influx in the affected cells. Additionally, Sestrins support mitochondrial biogenesis through activation of PGC1α and its downstream targets: TFAM and UCP2 in an AMPK-dependent manner (Quan et al., 2017, 2018). On the contrary, Sestrins were accumulated in the plasma of patients with coronary artery disease (CAD), and Sestrin levels correlated with the disease severity (Ye et al., 2017). It will be important to determine if Sestrin accumulation in plasma of CAD patients is an indicator of increased Sestrin expression in heart where these proteins might play a protective role against the disease, or that excessive Sestrin expression aggravates the phenotype of this disease.

### 2.6. Sestrins in neuronal tissue

Being important mediators of stress response, Sestrins play an important role in protection of neuronal cells against stress. In D. melanogaster, dSesn defends larvae ganglia cells against apoptotic cell death induced by Cr(IV) (K2Cr2O7) (Singh and Chowdhuri, 2018). Mammalian SESN2 might also protect against Alzheimer’s disease (AD) and some other neurodegenerative age-related pathologies. Earlier studies reported that SESN2 is upregulated in cortical neuronal cell culture treated with β-amyloids, the peptides found accumulated in the brain of AD patients, and in the AD model based on the expression of the APPsw/PSEN1dE9 transgene (Chen et al., 2014; Kim et al., 2003). The importance of SESN2 activation for neuronal protection is substantiated by increased vulnerability of SESN2-deprived neurons to cell death associated with attenuated autophagy (Chen et al., 2014). In patients with AD, SESN2 is accumulated in the sites with elevated expression of phospho-Tau protein that is built up in the brain of AD patients, especially in the areas of neurofibrillary lesions. Increased SESN2 levels were also found in neuronal soma in response to HIV infection (Soon-torniyomkij et al., 2012). Another study demonstrated that deficiency of the PSEN1 gene, mutated in the patients with inherited predisposition to AD, causes SESN2 downregulation and consequent mTORC1 activation. mTORC1 overactivation inhibits nuclear translocation of the TFEB transcription factor, hence impairing transcription of the CLEAR gene network, which is responsible for autolysosomal degradation of protein aggregates (Reddy et al., 2016). SESN2 also suppresses ROS production in neurons in response to NMDA activation (Papadia et al., 2008). Therefore, Sestrins might be responsible for the regulation of metabolism and protein proteostasis in neuronal tissue preventing accumulation of protein aggregates, oxidative stress, and cell death. SESN3 also mediates the protective effects of the FOXO proteins against axonal degeneration in ageing brain through mTORC1 suppression, autophagy stimulation, and ROS downregulation (Hwang et al., 2018). However, another study indicates that SESN3 expression is activated in the hippocampus of epileptic patients. As a result, SESN3 promotes the expression of pro-convulsant and pro-inflammatory genes, such as IL-1β and TNF, in mammalian macrophages, BV2 microglia, and primary neuronal cells in vitro and in a zebrafish model in vivo. SESN3 potentially contributes to epileptic seizures through activation of a pro-convulsant transcriptional programme in epilepsy. Accordingly, inhibition of SESN3 expression in zebrafish reduces the severity of behaviour seizures induced by pentylenetetrazole (PTZ) (Johnson et al., 2015). Therefore, Sestrins may be involved in different aspects of brain physiology protecting neurons from stress and ageing but also exacerbating some brain pathologies under certain conditions.
2.7. Sestrins in liver

Liver is the organ involved in various physiological processes including regulation of metabolism and detoxication of poisonous chemicals. While liver is susceptible to oxidative damage and cell death, this organ has a high regenerating capacity. Although hepatocytes are capable of re-entering the cell cycle and normally participate in liver regeneration, liver remodelling also involves stem-like hepatic stellate cells and liver progenitor oval cells (LPCs) that can differentiate into hepatocytes and cholangiocytes (Miyajima et al., 2014; Yin et al., 2013). Being important metabolism and antioxidant response regulators, Sestrins protect liver function. As demonstrated in several mouse models of obesity, SESN2 is activated in the liver of obese animals, and Sesn2 inactivation in mice leads to insulin resistance and glucose intolerance due to impaired suppression of glucose production in response to insulin. Also, Sesn2-null animals exhibit increased amounts of liver fat deposits, the feature of non-alcoholic steatohepatitis (NASH). Therefore, SESN2 plays a critical role in regulation of liver metabolism in the conditions of stress caused by obesity (Lee et al., 2012). Insulin resistance and NASH in Sesn2-null obese animals were linked with AMPK and AKT inhibition and mTORC1 activation, and dysregulation of these kinases compromises liver functions due to impaired autophagy, mitochondrial homeostasis, and fatty acid oxidation (Lee et al., 2012). Sesn3 inactivation also causes insulin resistance due to attenuated AKT activation in liver and muscle (Lee et al., 2012; Tao et al., 2015). SESN2 expression is also increased in liver in response to starvation, and this protein protects liver from oxidative stress and cell death associated with refeeding (Ba et al., 2013). Particularly, SESN2 suppresses liver damage via activation of NRF2, the major activator of transcription of antioxidant and pro-survival genes (Cuadrado et al., 2019). According to these data, SESN2 interacts with the SQSTM1/p62 autophagy receptor protein and the RBX ubiquitin ligase, and the SESN2-p62-RBX complex stimulates degradation of the KEAP1 protein, the major partner and inhibitor of NRF2 (Ba et al., 2013). SESN2 also protects from hepatotoxicity induced by chronic lipid overload in the NASH model or caused by consumption of acetaminophen, the widely-used analgesic/antipyretic non-prescription drug (Kim et al., 2017; Park et al., 2014). Similar to SESN2, SESN3 suppresses NASH development by reducing liver inflammation (Huang et al., 2020).

Both SESN2 and SESN3 also protect against liver fibrosis. Liver fibrosis is the process of accumulation of scar tissue in liver that in the long run leads to cirrhosis, liver dysfunction, and hepatocarcinogenesis (Tsichina and Friedman, 2017). Liver fibrosis is driven by constant cycles of injury, inflammation, and regeneration in this tissue. Liver damage caused by NASH, hepatotoxic exposure, or viral infection leads to activation of quiescent hepatic stellate cells and their trans-differentiation from vitamin A-storing cells to myofibroblasts that are responsible for production of extracellular matrix proteins, such as type I collagen and α-SMA. This transdifferentiation is driven by activation of the TGFβ-SMAD signalling pathway (Tsichina and Friedman, 2017). Sestrin3 inactivation cause accumulation of cellular deposits, composed of type I collagen and other matrix proteins, in the liver of mice with NASH, and SESN3 overexpression inhibits this process (Huang et al., 2020). Similarly, SESN2 overexpression protects mouse liver from CCL4-induced damage by suppressing liver inflammation and liver fibrosis (Hu et al., 2018; Yang et al., 2019). Both SESN2 and SESN3 attenuate TGFβ signalling, therefore preventing activation and trans-differentiation of hepatic stellate cells and accumulation of matrix deposits (Huang et al., 2020; Yang et al., 2019).

2.8. Sestrins and immune system

Ageing is characterised by chronic inflammation in different tissues, and dysregulated inflammatory process contributes to tissue degeneration and dysfunction (Ferrucci and Fabbri, 2018; Furman et al., 2019). Functional decline in different tissues is triggered by accumulation of malfunctioned senescent cells that stimulate pro-inflammatory process through production of cytokines, metalloproteinases, and other factors responsible for the so-called senescence-associated secretory phenotype (SASP) (Campisi et al., 2019). The experiments based on depletion of senescent cells from tissues demonstrate that senescent cells may contribute to development of a variety of age-related diseases including cardiovascular diseases, AD, Parkinson’s disease, atherosclerosis, NASH, osteoarthritis, pulmonary fibrosis, and cancer. Senescent cells also compromise muscle and haematopoietic stem cells functions (Campisi et al., 2019). Sestrins suppress cellular senescence in fibroblasts and may protect from ageing and age-related diseases by suppressing SASP in different tissues (Budanov et al., 2004). Additionally, Sestrins play a critical role in regulation of inflammation. SESN2 inhibits the release of nitric oxide (NO) and pro-inflammatory cytokines in the murine macrophage RAW264.7 cell line (Yang et al., 2015). Another study demonstrates that SESN2 inhibits activation of the NFκB inflammasome and sepsis through mitophagy stimulation in macrophages (Kim et al., 2016). SESN2 also suppresses the inflammatory process during development of atherosclerosis in aortic tissue (Hwang et al., 2017).

In contrast to the protective role of Sestrins against ageing in multiple tissues, a recent study demonstrated that Sestrins expression is elevated in CD4+ T-cells in old mice and is linked with senescence (Budanov et al., 2004). Interestingly, in our analysis of SESN2-containing complexes from mammary epithelia MCF10 cells none of the MAPK proteins were found among SESN2 interactors (Kim et al., 2015b; Parmigiani et al., 2014). Moreover, it was shown that SESN2 suppresses JNK and p38 activation in RAW264.7 cells in response to LPS (Yang et al., 2015). The potential explanation of the discrepancy in MAPK regulation by Sestrins might be a unique role of Sestrins in regulation of pro-inflammatory signalling in a particular cell type.

2.9. Sestrins and lung tissue

Another unexpected SESN2 feature is its adverse role in development of chronic obstructive pulmonary disease (COPD), a slow-developing inflammatory pathology attributed to lung ageing (Ito and Barnes, 2009). As demonstrated in mouse models of COPD based on inactivation of the short splice variant of the Ltbp4 protein (Ltbp4S), an activator of TGFβ signalling, simultaneous knockout of Sesn2 suppresses development of the emphysema phenotype in the Ltbp4 KO animals (Wemme et al., 2010). Moreover, Sesn2-null animals are less susceptible to COPD development caused by tobacco smoke exposure. These data also cohere with the observations that SESN2 is accumulated in smokers suffering from COPD (Heidler et al., 2013), indicating that excessive SESN2 expression may predispose smokers to COPD.

Therefore, Sestrins play a controversial role in regulation of ageing in different tissues that has to be better understood (Fig. 2C). Future studies should apply different animal models of ageing and various environmental conditions to address the precise impact of Sestrins on ageing in different tissues focusing on particular cell types including parenchymal cells, progenitor cells, and stem cells.
3. Sestrins, stem cells, and progenitor cells

3.1. Role of Sestrins in stem cell biology and differentiation

Stem cells are undifferentiated cells responsible for production of many different cell types within a tissue while maintaining their undifferentiated state, known as stemness. Cell differentiation is mediated by production of partially differentiated progenitor cells that eventually give rise to highly-specialised cells in a tissue (Morrison and Spradling, 2008). Stem cells were described in different tissues and include the following cell types: germline stem cells in seminiferous tubules, epithelial stem cells in the bulge of hair follicles (HFSC), neural stem cells (NSC) in the subventricular zone of the lateral ventricles and sub-granular zone of the hippocampal dentate gyrus of the brain, muscle stem cells (also called satellite cells) under the basal lamina of myofibers, intestinal stem cells in intestinal crypts, and hematopoietic stem cells (HSC) in bone marrow (Morrison and Spradling, 2008). Stem cells occupy a certain place within a tissue, called the stem cell niche, and play a critical role in maintenance of tissue homeostasis by replacing deteriorated and dysfunctional cells (Morrison and Spradling, 2008).

Sestrin-regulated mTORC1 and mTORC2 play an important role in control of ageing and stem cell biology (Meng et al., 2018). mTORC1 activity is required for differentiation programmes, such as neurogenesis, spermatogenesis, adipogenesis, and embryogenesis, but this kinase also supports HSC maintenance (Meng et al., 2018). In contrast, excessive mTORC1 activation in HSC stimulates cell proliferation and trigger ROS accumulation in these cells causing their exhaustion (Chen et al., 2008). Similarly, increased mTORC1 activity leads to depletion of mammary stem cells (Meng et al., 2018). Therefore, tight mTORC1 control in stem cells is important for maintenance of proliferative potential and stemness, as either upregulation or downregulation of mTORC1 activity might result in deterioration of stem cell function. One of the most critical functions of mTORC1 is autophagy inhibition. Autophagy supports functionality of parenchymal cells as well as stem cells. For example, autophagy is critical for HSC maintenance, as inactivation of the autophagy gene ATG7 in HSC leads to accumulation of dysfunctional mitochondria, oxidative stress, and DNA-damage (Mortensen et al., 2011). Autophagy presumably supports homeostasis of quiescent NSC. NSC from aged mice accumulate protein aggregates and have fewer lysosomes than NSC from young animals. Stimulation of lysosomal proteolysis in the cells from aged animals causes quiescent NSC the ability to activate (Leeman et al., 2018). Autophagy also maintains functionality of mesenchymal stem cells (MSC) supporting their quiescent state and preventing accumulation of age-related damage (Garcia-Prat et al., 2016).

The critical activator of autophagy in stem cells is the FOXO3A transcription factor. FOXO3A is highly expressed in HSC and its inactivation in this cell type compromises the maintenance of quiescent state, self-renewal, and haematopoiesis (Miyamoto et al., 2007; Warr et al., 2013). FOXO3A also plays a key role in autophagy regulation in NSC, as FOXO3A deficiency leads to the accumulation of protein aggregates in NSC (Audesse et al., 2019). FOXO3A deficiency in MSC compromises the quiescent state, impairs self-renewal, and facilitates differentiation in these cells (Gopinath et al., 2014). Although this study attributed the FOXO3A function in MSC maintenance to activation of the Notch signalling pathway, autophagy activation by FOXO3A might also be important for this process.

Another critical regulator of stem cell biology is the tumour suppressor p53 that supports the quiescent state in NSC and HSC, suppressing their self-renewal and proliferation (Liu et al., 2009). However, p53 also facilitates differentiation in embryonic stem cells (Akdemir et al., 2014; Jain et al., 2012). p53 is a critical intracellular stress sensor activated by DNA-damage, ROS accumulation, and metabolic deregements. This protein controls cell fate through antioxidant response, regulation of autophagy and metabolism, cell cycle inhibition, and cell death activation (Budanov, 2014; Sablina et al., 2005). p53 potentially supports stem cell homeostasis by adjusting metabolism and preventing excessive ROS accumulation and DNA-damage under low-stress conditions. p53 might also eliminate stem cells with unreparable DNA-damage through induction of cell death or senescence (Kruiswijk et al., 2015), preventing carcinogenesis.

Sestrins are FOXO and p53 targets, therefore, Sestrins might mediate the effects of these transcription factors on stem cell functions. While Sestrins are not critical for cell differentiation during embryogenesis (Budanov et al., 2010), they might contribute to stem cell maintenance in adult tissues via regulation of mTORC1/2, autophagy, ROS production, senescence, and cell death. Accordingly, SESN1 and SESN2 expression was decreased in FOXO3A-deficient HSC. These cells were not able to effectively maintain their stemness and had decreased levels of autophagy (Warr et al., 2013). Sestrin role in maintenance of HSC and other types of stem cells may be especially important during caloric restriction and starvation, as Sestrins are activated by nutrient deprivation and might support cell viability and stress resistance (Dalina et al., 2018; Ding et al., 2016). Sestrins also potentially provide the beneficial effects of calorie restriction on lifespan extension by protecting stem cell functions. However, Sestrins might also support cell death of stem cells in the conditions of genotoxic stress preventing accumulation of DNA-damage, carcinogenesis and other age-related pathologies (Rossi et al., 2008). SESN2 expression is reduced in HSC of old mice, and SESN2 downregulation might compromises HSC regenerative potential (Warr et al., 2013). These data contrast with the observations by Lanna et al. demonstrating that expression of all members of the Sestrin family is increased in the T-cells from old individuals (Lanna et al., 2017). Therefore, Sestrins may play different roles in the biology of differentiated blood cells and HSC.

Sestrins can also control differentiation of particular cell types in vertebrates. Cell differentiation involves chromatin remodelling and upregulation of cell cycle inhibitors, directing cell transition into a differentiated state. Muscle tissue is composed of different cell types: differentiated cells – myocytes, dividing progenitors – myoblasts, and muscle stem cells – myogenic satellite cells (Wang et al., 2014). Muscle differentiation is driven by the transcription factors that control myogenic differentiation, such as myoblast determination protein (MyoD) and myogenic factor 5 (Myf5). These factors are the major myogenic determinative factors, while myogenin, myogenic factor 4, and MyoD accomplish the terminal differentiation programme through activation of muscle-specific genes (Ruijtenberg and van den Heuvel, 2016). In chicken myocytes, SESN1 supports both proliferation and differentiation of myoblasts. As demonstrated in this model, SESN1 expression is suppressed by the miR-16-5p microRNA that is transiently activated during embryonic muscle development and fibre formation. miR-16-5p inhibits myoblast proliferation and differentiation, antagonising SESN1 effect on differentiation (Cai et al., 2018). SESN1 may also contribute to muscle differentiation by supporting mitochondrial biogenesis and respiration through AMPK and PGC1α activation in mammals (Kim et al., 2020). The potential impact of SESN1 on muscle differentiation is indicated by its low expression in mouse myoblasts but elevation of its expression in differentiated myocytes (Kim et al., 2020). SESN3 also stimulate muscle differentiation via downregulation of myostatin, a TGFβ family member, resulting in muscle growth suppression (Nascimento et al., 2013). Therefore, Sestrins may support muscle remodelling during exercise and in response to some environmental conditions that inflict muscle damage and trigger muscle degeneration (Segales et al., 2020).

In future studies it will be important to determine how Sestrins contribute to regeneration of various tissues when environmental stress inflicts tissue damage. For example, lung tissue remodelling in response to tobacco smoke or other damaging factors is driven by alveolar progenitor cells (Zacharias et al., 2018), and SESN2 might suppress lung tissue regeneration by preventing cell proliferation or differentiation. Tobacco smoke exposure decreased the number of alveolar type II (AII) cells in wild-type mice, however, the effect of tobacco smoke was much
less detrimental in the Sesn2Δ/Δ animals, whose lungs had a bigger number of ATII cells and elevated C (SP-C) protein expression (Heidler et al., 2013). Sesn2 inactivation also leads to upregulation of PDGFRβ and TGFβ signalling in mouse lung and lung fibroblasts. PDGFRβ is responsible for activation of tissue remodelling via upregulation of keratinocyte growth factor (KGF/FGF7), elastin, αSMA, and collagen type IV (Heidler et al., 2013; Tomascovic et al., 2015; Wempe et al., 2010). SESN2 negatively regulates PDGFRβ expression via its antioxidant activity mediated by NRF2 activation (Tomascovic et al., 2015).

These data indicate that Sestrins may play opposite roles in tissue remodelling in different organs. Taking into account that PDGFβ plays an important role in maintenance of many types of neuronal cells (Funa and Sasahara, 2014), it would be interesting to determine the impact of Sestrins on neuronal tissue remodelling. Accordingly, SESN2 is upregulated in peripheral nerves after spared nerve injury in a model of neuropathic pain and its inactivation exacerbates neuropathic pain (Kallenborn-Gerhardt et al., 2013).

Another important Sestrin function is Akt activation that stimulates proliferation and migration of hair follicle stem cells but does not induce stem cell exhaustion, supporting skin regeneration and wound healing (Segrelles et al., 2014). Akt mediates TGFβ effects on tissue remodelling and plays an important role in control of differentiation of skin fibroblasts to myofibroblasts, facilitating wound healing (Li et al., 2016). Therefore, SESN2 might contribute to maintenance and regeneration of skin tissue through Akt activation in epidermal stem cells and dermal fibroblasts.

3.2. Sestrins and cancer propagating cells

Sestrins may also play an important role in carcinogenesis by controlling the biology of cancer propagating cells with stem-like properties (CPC) (also called cancer stem cells). Carcinogenesis is often driven by CPC that feature ceaseless proliferation potential, resistance to stress, and capability to differentiate to different sub-types of cancer cells. CPC also have a higher resistance to anticancer therapy (Batlle and Clevers, 2017). Sestrin role in maintenance and proliferation of CPC is not well known. However, some tumour-modulating effects of Sestrins may be attributed to prevention of conversion of stem cells to CPC via mutation acquisition which grant stem cells the ability to escape senescence and cell death. Sestrins may also suppress stemness in cancer cells minimizing their proliferation potential and resistance to anticancer therapies (Batlle and Clevers, 2017).

The only cancer type where Sestrin activity on their control of CPC function was tested is liver cancer. Liver tumours from Sesn2-deficient mice demonstrate higher Akt and Stat3 activity and upregulation of stem cell markers, such as Acta2, Cd44, and Cd133, comparatively to wild-type animals (Liu et al., 2019). Therefore, SESN3 suppresses stemness in liver CPC by retaining the Gli2 transcription factor, a mediator of the Sonic Hedgehog signalling pathway, in the cytoplasm via direct protein-protein interactions. However, the conclusion about the potential SESN3-Gli2 interaction was based on the overexpression of these proteins in 293 T cells and needs verification with endogenous proteins from the liver cells (Liu et al., 2019). Therefore, stemness repression in cancerous or pre-cancerous cells might be an important mechanism of suppression of liver carcinogenesis by SESN3.

Sestrins might also regulate stemness in other cancer types, although this assumption has to be tested in future studies. For example, SESN1 works as a tumour suppressor in follicular lymphomas. Lymphomagenesis is often driven by the gain-of-function Y641X mutant form of the EZH2 histone methyltransferase. EZH2Δ641X suppresses SESN1 expression resulting in mTORC1 activation that facilitates lymphomagenesis (Oricchio et al., 2017). Interestingly, EZH2 is highly expressed in CPC of different origin and contributes to cancer propagation and resistance to chemotherapy and radiotherapy (Wen et al., 2017). Therefore, inhibition of SESN1 expression by hyperactivated EZH2 forms may support maintenance and proliferation of CPC in lymphomas and other cancer types. SESN2 works as suppressor of lung and colon carcinogenesis (Ding et al., 2019; Ro et al., 2016). Similar to the role of SESN3 in liver carcinogenesis, SESN2 might suppress stemness phenotype in lung and colon cancers through downregulation of TGFβ, Hedgehog, and some other signalling pathways responsible for the stemness phenotype (Liu et al., 2019; Wempe et al., 2010; Zakaria et al., 2015). On the contrary, in skin, where SESN2 works as an oncogene, this protein may support the viability of epithelial and melanocyte stem cells, which bear UV-induced DNA-damage, through Akt activation (Zhao et al., 2014, 2017). These cells may give rise CPC leading to development of skin cancers and acquisition of drug resistance. Therefore, Sestrins may play opposite roles in carcinogenesis through control of maintenance and viability of precancerous stem cells and CPC in different tissues.

4. Conclusion

The evolutionarily conserved Sestrins are critically important for stress resistance suppressing accumulation of age-related abnormalities and supporting well-being and longevity in different Metazoa species. Until recently, Sestrin functions were attributed to the regulation of physiological processes in adult parenchymal cells. While it might be true for invertebrate organisms, such as C. elegans and D. Melanogaster, Sestrin functions in vertebrates are more complex. Proper maintenance of vertebrate tissues depends on stem and progenitor cells that drive tissue remodelling, replacing worn, damaged, and dysfunctional cells with functional ones. To maintain high regenerative potential, stem cells must be well-protected against stress, and Sestrins presumably support stem cell homeostasis preventing damage accumulation, modulating cell viability, maintaining the quiescence state, and facilitating differentiation. These Sestrin functions potentially contribute to proper and timely control of ageing. Sestrins might also modulate carcinogenesis through regulation of stemness in CPC. Understanding the role of Sestrins in stem cell biology will shed a light on the mechanisms of ageing and provide a ground for development of new therapies for prevention and treatment of age-related diseases.

Acknowledgements

This work was supported by the Wellcome Trust ISSF grant and the grant 17-14-01420 from the Russian Science Foundation to AB. We thank Nadusha Pryadilova for the help with the manuscript.

References


A. Haidurov and A.V. Budanov
Mechanisms of Ageing and Development 192 (2020) 111379


Mechanisms of Ageing and Development 192 (2020) 111379

A. Haidurov and A.V. Budanov


