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Review

The p66^{Shc} gene paves the way for healthspan: Evolutionary and mechanistic perspectives

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ABSTRACT

Life expectancy in the last century has greatly increased although, in most industrialized countries, this has been paralleled by an increased incidence of neurodegenerative disorders, in addition to cardiovascular and metabolic pathologies. The p66^{Shc} gene has emerged as a novel gerontogene affecting health throughout life and during aging. In the last decade, studies on p66^{Shc} knock-out mice have indicated that this gene is a crucial regulator of reactive oxygen species (ROS) levels and is involved in age-related dysfunctions. p66$^{Shc-/-}$ mice show indeed a healthy phenotype characterized by greater brain and behavioral plasticity – associated to increased central levels of the neurotrophin Bran-Derived Neurotrophic Factor (BDNF) – in addition to reduced oxidative stress, fat accumulation and incidence of metabolic and cardiovascular pathologies. Studies performed in a semi-naturalistic setting, involving exposure to low temperatures and food shortage indicate that p66^{Shc} has been conserved through evolution because of its role as "thrifty gene" in energy metabolism. This feature, which allows survival in harsh natural conditions, can be deleterious when food is constantly available, as in westernized lifestyles, leading to fat accumulation and predisposing to metabolic, cardiovascular diseases and accelerating brain aging. Being at the crossroad of signaling pathways involved in both central and peripheral stress responses and in the regulation of energy homeostasis, p66^{Shc} is a good candidate molecule to address the mechanisms underlying healthy aging and to be targeted for the development of novel pharmacological tools for the prevention or cure of age-related pathologies.

Contents

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1. Introduction

Life expectancy in the 20th century has greatly increased in the Western Societies with the number of people aged 65 years and older being raised more than threefold since 1950, although at different rates in different parts of the world (Waite, 2004). Europe has currently the highest proportion of elderly people and will probably maintain this leading position for the next 50 years. However, this increase in longevity has not been accompanied by an increase in disease-free life expectancy. Cardiovascular disease (CVD), type 2 diabetes (T2D) and neurodegenerative disorders are highly prevalent in the elderly; these pathologies, frequently coexisting in the same aged individual, are often mutually reinforcing. The EU Commission, in the 2012 Aging Report on the economic and budgetary projections for the 27 EU Member States (2010–2060), has reported that "*ageing per se has a non-negligible effect on expenditure growth, however this is rather moderate. In effect, much depends on whether gains in life expectancy are spent in good or bad health. Optimistically, if all additional life years are healthy life years, the additional cost burden from ageing can be lowered*". Therefore, understanding the mechanisms underlying longevity, and the individual susceptibility to age-related diseases is of paramount importance since a better quality of life might help alleviating frailty of the old age, also contributing to decrease the cost burden for the National Health Systems related to the hospitalization of chronic patients.

Aging can be defined as a multifactorial degenerative process resulting from the organism's progressive loss of the ability to maintain homeostasis and less efficient adaptation to changes. The impact of this age-related breakdown of adaptation is far more disruptive when integrative homeostatic communication systems are also affected. In mammals, and other vertebrates, aging in healthy individuals is associated with a progressive decrease in the functionality of multiple systems and organs, compromising adaptation to environmental stressors and thus increasing vulnerability to stress-related neurodegenerative diseases. In this review the main focus will be on genetic determinants of aging – particularly the role of the $p66^{Shc}$ gene – and the integrated function of central and peripheral molecules involved in the response to environmental stressors and metabolic signals affecting health and longevity.

1.1. Genetic and environmental determinants of health and longevity

Evidence is mounting that modulators (genes) of the rate of aging exist and are conserved over large evolutionary distances. Most animal models in aging research rely on simple organisms such as the budding yeast or invertebrates as the rounded worm *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster* that, with their short life cycle and well defined physical or biological markers of aging, have immensely contributed to collect evidence on genetic-based pathways involved in the aging process (Guarente and Kenyon, 2000; Johnson et al., 2002; Lithgow, 1996; Partridge and Gems, 2002). Mutations in genes affecting endocrine signaling, stress responses and metabolism can all increase the lifespan of model organisms. Interestingly, many mutations that extend lifespan perturb, directly or indirectly, endocrine/metabolic signaling. Among these, the most extensively studied is that related to insulin/insulin-like growth factor (IGF-1) – and its orthologs – affecting lifespan in worms, flies, and mammals (Ding et al., 2013; Holzenberger et al., 2003; Kenyon et al., 1993; Kimura et al., 1997; Longo and Finch, 2003; Tatar et al., 2001). IGF-1 receptor heterozygous knock-out mice live approximately 30% longer than wild-types (Holzenberger et al., 2003), mutations in upstream genes that regulate insulin and IGF-1, such as the growth hormone (GH) receptor mutants, increase longevity (Coschigano et al., 2003). Likewise Ames and Snell dwarf mice, characterized by pituitary defects (and hence by low levels of GH and IGF-1), are long-lived (Brown-Borg et al., 1996; Ding et al., 2013; Flurkey et al., 2002). Reports of increased lifespan in mice with deletion of insulin receptor substrate (IRS)1, reduced expression of IRS2, or selective deletion of IRS2 in the brain specifically implicate the activity of IRS-forkhead O (FOXO) family pathway (Bartke, 2008). The FOXO family of transcription factors participates in diverse physiologic processes including stress resistance and those mediated by AMP kinase that in mammals regulates energy metabolism and food intake via phosphorylation of an array of substrates, including metabolic enzymes and transcription factors (Kenyon, 2005). Recently, the role played on longevity by the *Drosophila* gene Indy (which encodes for a protein involved in the regulation of the Krebs cycle) has been strengthened and extended to its homolog in mammals (Frankel and Rogina, 2012). In particular, the effect of decreasing INDY activity, as in the long-lived Indy mutants, seems to be related to altered energy metabolism in a manner that favors lifespan extension (Knauf et al., 2006). Indeed, mice lacking Indy (mIndy−/−) show increased hepatic mitochondrial biogenesis, lipid oxidation, and decreased lipogenesis. In addition, if fed with high-fat diet, they are protected from adiposity and insulin resistance (Frankel and Rogina, 2012). In the context of metabolism-related genetic determinants of lifespan is indeed worth mentioning sirtuins. These enzymes are part of a family of histone deacetylases regulating important biological processes including (among many others), apoptosis and cell senescence, adipocyte differentiation, energy expenditure and gluconeogenesis (Dali-Youcef et al., 2007). Increasing sirtuin levels through genetic manipulation extends lifespan of yeast, nematodes and fruitflies (Michan and Sinclair, 2007) and one of the mechanisms accounting for such an increase appears to be related to the insulin/IGF-1 signaling pathway (Tissenbaum and Guarente, 2001). Overexpression of Sir2 extends the lifespan of *Saccharomyces cerevisiae* (Kaeberlein et al., 1999) and growing yeast in a medium containing a reduced glucose content leads to the activation of this sirtuin and to the consequent increase in lifespan, suggesting a clear link between metabolism/energy homeostasis and longevity (Lin et al., 2002). Likewise, both in *Caenorabditis elegans* and *D. melanogaster*, extra copies of the Sir2 orthologs lead to increased longevity (Rogina and Helfand, 2004; Tissenbaum and Guarente, 2001). Worth noticing, in *C. elegans*, this effect has been shown to be strictly dependent on the forkhead transcriptional factor DAF-16, the downstream target of the insulin/IGF-1 signaling pathway (Tissenbaum and Guarente, 2001). Despite the large body of evidence showing a pro-longevity effect of sirtuins the mechanisms through which these enzymes – including the mammalian Sirt1–Sirt7, the seven homologues of Sir2 (Baur et al., 2010) – retard aging remain incompletely understood. In addition, the role of these longevity genes has been challenged by authors arguing that the observed effects can be biased by the genetic background of the organism (Burnett et al., 2011). Yet, a very recent publication provided novel evidence to support the role of sirtuins in modulating lifespan. In fact, Kanfi and co-workers showed that overexpressing Sirt6 in mice leads to extended longevity (though only in male subjects) and this was associated to lower IGF-1 serum levels, higher levels of IGF-binding protein 1 and altered phosphorylation levels of major components of IGF-1 signaling, a key pathway in the regulation of lifespan (Kanfi et al., 2012).

Before the development of genetically modified organisms, reduced caloric intake without malnutrition (caloric restriction – CR (McCay et al., 1989)) was the only known model able to retard aging. CR causes a rearrangement of the metabolic set up of an organism and is the only known non-genetic intervention that robustly extends lifespan in every organism in which it has been tested (Klass, 1977; Lin et al., 2002; Loeb and Northrop, 1917; Mattison et al., 2007; McCay et al., 1989). In rodents, moderate CR

is able to induce alterations in the physiology of many organs and systems leading to: reduction of oxidative damage; reduced glucose and insulin levels (Lee and Yu, 1990; Masoro et al., 1983, 1992); slow the progression of a range of age-dependent pathologies (Anson et al., 2003; Klebanov, 2007; Maswood et al., 2004; Mattson and Wan, 2005; Wang et al., 2005) and reduce of the risk of coronary disease and stroke in humans (Mattson and Wan, 2005). Genetic and environmental/dietary factors may both affect the duration of life in different organisms. Interestingly, many mutations that extend lifespan involve the same signaling pathways mediated by CR. Even more interesting is that many cell signaling pathways of longevity are overall associated with altered metabolism and (oxidative) stress resistance and appear to converge on the FOXO family of transcription factors that regulate the expression of a battery of stress-responsive genes that affect antioxidant capacity, cell cycle arrest, DNA repair and apoptosis (Kenyon, 2005).

In humans, aging largely contributes to metabolic decline and to the etiology of related pathological conditions such as T2D, CVD, and stroke (Barzilai et al., 2012). Older individuals are often characterized by insulin resistance (IR) and abdominal obesity which are main components of the metabolic syndrome (MS) a pathological condition comprising multi-organ morbidity (Folsom et al., 1993; Morley, 2008). The age-associated accrual of visceral fat together with the increasing numbers of senescent cells might lead to increased secretion of proinflammatory cytokines that interferes with insulin action (Sepe et al., 2011). However, a growing body of evidence shows that the incidence of obesity, and the associated MS, is rising in the young population especially in western countries (Haffner and Taegtmeyer, 2003). These morbid conditions associate to a whole metabolic unbalance with high levels of oxidative stress (OS) and inflammation in addition to shorter telomeres (that correlate with increasing body mass index – BMI) overall resembling a form of precocious aging (Fadini et al., 2011). In addition, patients with MS are often characterized by a higher incidence of mood and cognitive dysfunctions than the general age-matched population that, in turn, emerge as significant risk factors for aggravation of MS and the related health outcomes, particularly CVD and T2D (Engum, 2007; Goldbacher et al., 2009; Muller et al., 2010; Raffaitin et al., 2011; van Reedt Dortland et al., 2010; Zeugmann et al., 2010). Thus, overall, alterations in metabolism and body fat distribution may certainly play a role in a vicious cycle that can precipitate the aging process and the onset of diseases (Barzilai et al., 2012). The mechanisms that account for these phenomena are incompletely understood, however longevity genes might be involved. Very recently, Fadini and co-workers propose SIRT1, $p66^{Shc}$, and the mammalian TOR (mTOR)/RSK/AMPK pathways to play a role in this clinical context because they integrate nutrient bioavailability, OS, and metabolism and because biological plausibility supports their reciprocal interconnections (Fadini et al., 2011).

1.2. Aging and oxidative stress

Oxidative stress is a common state characterizing biological systems in aerobic conditions derived from an imbalance between pro-oxidative and anti-oxidative molecules where the oxidants override defensive systems. Oxidative molecules, namely Reactive Oxygen Species (ROS), are produced primarily by the physiological metabolism of O_2 in cells (Kakkar and Singh, 2007) but also environmental stimuli (cytokines, ultraviolet radiation, chemotherapeutic agents, hyperthermia and even growth factors) might contribute to their generation shifting the normal cellular redox balance into a state of OS potentially leading to diabetes, ischemia/reperfusion, to mention only a few (Dalle-Donne et al., 2006; Dhalla et al., 2000; Jenner, 2003; Sayre et al., 2001; Finkel and Holbrook, 2000). In addition, it appears to play a role in the neuropathology of several age-related neurodegenerative disorders (Finch and Marchalonis, 1996; Finkel and Holbrook, 2000; Hensley et al., 1999; O'Banion and Finch, 1996; Zhu et al., 2001a,b).

The mammalian brain is characterized by poor antioxidant defenses, high metabolic rate, and reduced capacity for cellular regeneration resulting particularly susceptible to OS insults (Floyd, 1999; Floyd and Hensley, 2002). The major portion of the total ROS, mostly as H_2O_2 produced during aerobic metabolism, is a by-product of the electron transport chain operating in the mitochondria (Floyd and Hensley, 2002; Hensley et al., 1998; Papa and Skulachev, 1997; Perez-Campo et al., 1998). A recent paper from Giorgio and co-workers specifically indicates H_2O_2 as a common mediator of aging signals (Giorgio et al., 2007). Notably, among ROS, H_2O_2 is the only species that is generated by several specific enzymes in the cell suggesting that its intracellular concentration is tightly regulated and may serve specific cellular functions.

$p66^{Shc}$ is a peculiar protein acting specifically in the mitochondrion as a redox enzyme that generates H_2O_2 to trigger mitochondrial swelling and apoptosis (Giorgio et al., 2005). The H_2O_2 generated by $p66^{Shc}$ accounts for ~30% of the total pool of intracellular H_2O_2 and is biologically relevant, as shown by the finding that cells and tissues that are derived from $p66^{Shc}$-null mice accumulate significantly less OS, and because $p66^{Shc}$ can induce a mitochondrial apoptotic response (Giorgio et al., 2005; Trinei et al., 2002). Giorgio and co-workers further propose that genes that control H_2O_2 production are specific determinants of lifespan involving that aging should be considered as the expression of a specific genetic program that generates H_2O_2 as a signaling molecule. In fact, deletion of $p66^{Shc}$ gene in mice results in the decreased formation of mitochondrial H_2O_2 (Giorgio et al., 2005), which correlates with delayed aging (Francia et al., 2004; Menini et al., 2006), reduced incidence of aging-associated degenerative diseases (Francia et al., 2004; Menini et al., 2006; Napoli et al., 2003; Rota et al., 2006) and increased lifespan (Migliaccio et al., 1999). Thus, genetic mammalian models of increased scavenging or decreased production of mitochondrial H_2O_2 directly implicate mitochondrial H_2O_2 in aging and lifespan determination.

2. The $p66^{Shc}$ as a novel gerontogene

$p66^{Shc}$ has emerged as a novel vertebrates' gerontogene (present only in mammals, *Xenopus* and *Botia Dario*) able to affect lifespan by controlling OS and metabolism (Trinei et al., 2009). In 1999 Pelicci and collaborators described the serendipitous discovery of unexpectedly long-lived knock-out mouse lacking the $p66^{Shc}$ gene (Migliaccio et al., 1999). This study was the first to link a deletion in a single mammalian gene to longevity without reporting any apparent phenotypic abnormality (Purdom and Chen, 2003b). Mice lacking this gene ($p66^{Shc-/-}$ knock-out mice – KO) live 30% more than their wild-type (WT, $p66^{Shc+/+}$) counterpart and are characterized by high resistance to OS, reduced trygliceride accumulation in the adipocytes, increased metabolic rate, decreased fat mass and resistance to diet-induced obesity (Berniakovich et al., 2008; Tomilov et al., 2011). Three *Shc* genes have been found in mammals: *ShcA*, *ShcB* (*Sli*) and *ShcC* (*Rai*) (Luzi et al., 2000). The *ShcA* gene encodes two mRNA species and three proteins: $p66^{Shc}$ and $p46^{Shc}$/$p52^{Shc}$. The $p66^{Shc}$ mRNA has an alternative transcription initiation site from that of the $p46^{Shc}$ and $p52^{Shc}$ isoforms (i.e., an alternative promoter) (Ventura et al., 2002). Each ShcA protein harbors three identical functional domains: an N-terminal phosphotyrosine-binding domain (PTB), which is slightly truncated in the $p46^{Shc}$ isoform, a central proline-rich domain (CH1), and a carboxy-terminal Src homology 2 (SH2) domain (Luzi et al., 2000). $p66^{Shc}$ differs from $p46^{Shc}$ or $p52^{Shc}$ by an additional N-terminal proline-rich domain (CH2) (Luzi et al., 2000). All three ShcA proteins (p46, p52 and p66) participate in mitogenic signaling and

oncogenesis by regulating receptor tyrosine kinase signaling. The participation of $p46^{Shc}$ or $p52^{Shc}$ is thought to enhance certain weak signals from growth factor receptors or G-protein coupled receptors while no evidence indicates that $p66^{Shc}$ activates the Ras signaling pathway (Bonfini et al., 1996; Foschi et al., 2001; Migliaccio et al., 1997).

$p66^{Shc}$ (mRNA and protein) is expressed at different levels in specific tissues, such as lung, spleen, liver, heart, kidney (Trinei et al., 2002) and skin (Lebiedzinska et al., 2009); transcriptional levels of $p66^{Shc}$ have been also identified in lymphocytes, mouse tymocytes and splenic T cells that acquire the capacity to express it in response to apoptogenic stimuli (Pacini et al., 2004). Most notably, it is highly expressed within the adipose tissue where it appears to control adipogenesis in addition to intracellular signaling events related to fat accumulation (Berniakovich et al., 2008; Tomilov et al., 2011). In addition, the $p66^{Shc}$-redox activity might control the response of myocytes and endothelial cells to glycaemia and ischemia (Bianchi et al., 2006; Camici et al., 2007; Rota et al., 2006). Concerning the expression of $p66^{Shc}$ in the mammalian central nervous system, scarce data are available. ShcA adaptor proteins are known to function as initiators of Ras mitogenic signaling cascade in various non neuronal systems where they are considered to be expressed ubiquitously. Conti and co-workers in 1997 first investigated the role of the ShcA gene during neurogenesis in neuronal cells namely, whether and how it is involved in the proliferative and differentiative phases of the developing brain (Conti et al., 1997). Analyses of ShcA mRNA and protein in the rodent developing brain (both mice and rats; embryonic day 14–18 to early post-natal day 2) revealed a progressive down-regulation of their expression during differentiation from neuroblasts to neurons suggesting that these proteins are lost from postmitotic neuronal cells. However, western blot analyses showed that $p66^{Shc}$ was the only isoform present at a low, although detectable level, in different adult brain regions (cortex, striatum, basal forebrain and hippocampus) (Conti et al., 1997). Data on the immunoreactivity of $p66^{Shc}$ in the mouse adult brain have been recently confirmed by our group for cortex, hippocampus, striatum, hypothalamus and pituitary gland (Berry et al., personal communication). Worth noticing, a very recent paper from Sone and co-workers quantified the expression levels of transcripts of Shc-related isoforms in the brain of young adult, middle-aged and aged rats and found that $p66^{Shc}$ expression is specifically up-regulated in the aged brain suggesting that this gene might play a role in oxidative stress-related decline of brain functions (Sone et al., 2012).

The function of the $p66^{Shc}$ gene appears to be mechanistically related to that of insulin/IGF1 (Clancy et al., 2001) and OS (Harman, 1998), which play a critical role in lifespan determination. $p66^{Shc}$ is a redox enzyme which contains a serine phosphorylation site, Ser36 in its CH2 domain, unique to this isoform. It becomes serine-phosphorylated upon activation by insulin, H_2O_2 or by other stress signals, produces intracellular ROS (H_2O_2) within the mitochondrial intermembrane space, decreasing the expression of ROS scavenging enzymes (Berniakovich et al., 2008; Nemoto and Finkel, 2002; Trinei et al., 2009) eventually shifting the intracellular redox balance toward oxidation and apoptosis. In fact, an important downstream target of Ser36-phosphorylated $p66^{Shc}$ are the FOXO proteins that, at least in mammals, can protect cells against OS damages by controlling the expression of antioxidant enzymes (Kops et al., 2002; Nemoto and Finkel, 2002) as well as by controlling the expression of proteins involved in the DNA repair mechanisms (Furukawa-Hibi et al., 2002; Tran et al., 2002). Nemoto and Finkel showed that in $p66^{Shc-/-}$ cells the activity of the mammalian forkhead homolog FKHR-L1 is partially increased resulting in a consequent increase in both H_2O_2-scavenging and OS resistance (Nemoto and Finkel, 2002). Thus, an increased expression of scavenger genes may account, at least in part, for the lower levels of ROS seen in $p66^{Shc-/-}$ mutant mice and for the consequent increased longevity (Kops et al., 2002; Nemoto et al., 2006; Trinei et al., 2002).

Most intriguingly, Berniakovich and co-workers have recently shown that in the adipocytes the H_2O_2 generated by $p66^{Shc}$ upon insulin or H_2O_2 stimulation, in a positive feed-back fashion, is able to specifically reinforce the insulin signaling pathway, favoring triglyceride accumulation (Berniakovich et al., 2008). Consequently mice lacking $p66^{Shc}$ are resistant to high-fat diet induced obesity, show increased basal body temperature and increased metabolic rate, suggesting that an uncoupled respiration mechanism in the mitochondria of the brown adipose tissue, might lead to increased energy expenditure and, consequently, to an increased resistance to body weight gain. Interestingly, Tomilov and co-workers have shown that $p66^{Shc}$ knock-out mice (ShcP) while not presenting changes in the levels of $p52^{Shc}$, are characterized by a four-fold increase in those of $p46^{Shc}$ in white adipose tissue, suggesting that $p46^{Shc}$ overexpression might play a role in decreasing adiposity and reducing insulin sensitivity in this tissue (Tomilov et al., 2011). Indeed it has been hypothesized that $p66^{Shc}$-generated OS might accelerate aging by favoring fat deposition (Berniakovich et al., 2008; Nemoto et al., 2006). The extent of longevity appears to be dose-dependent as levels of $p66^{Shc}$ expression correlate with lifespan in mice, since heterozygous subjects $p66^{Shc+/-}$ display an intermediate lifespan compared with wild-type and knock-out (Giorgio et al., 2012; Migliaccio et al., 1999).

Thus, this newly identified insulin–$p66^{Shc}$–H_2O_2 signaling pathway has emerged as a critical modulator of longevity in mammals linking together OS and metabolic signals accelerating the aging process by favoring fat deposition and metabolic-related disorders (Berniakovich et al., 2008; Tomilov et al., 2011).

3. Improving healthspan: lessons from the $p66^{Shc}$ knock-out mouse

3.1. The $p66^{Shc}$ knock-out mice are short lived under natural conditions

Lack of the $p66^{Shc}$ gene, at least in mice, leads to undeniable benefits ranging from increased healthspan during aging (Berry et al., 2007) to greater behavioral plasticity at adulthood (Berry et al., 2008), resistance to obesity (Berniakovich et al., 2008; Ranieri et al., 2010), atherosclerosis (Graiani et al., 2005; Napoli et al., 2003), ischemic injury (Carpi et al., 2009; Zaccagnini et al., 2004), and diabetes (Fadini et al., 2011; Menini et al., 2006; Ranieri et al., 2010). The trade-off theory of aging predicts that for each immediate benefit there is a price to pay later in life, for example a negative correlation between longevity and reproductive success has been reported. Thus, mutations producing beneficial effects early in life, but later increasing the rate of aging, will be incorporated into a population because natural selection will act more strongly on the early beneficial effects than on the later deleterious ones (Charlesworth, 1994; Partridge and Barton, 1993; Williams, 1957). In this context, the question arises as to why this gene has been selected and conserved during evolution and what is its physiological function. The issue of testing subtle variations in the biological fitness under controlled laboratory conditions is a tricky task, particularly when the significance of the gene for survival under natural conditions is unknown. To address these questions, we have investigated the effects of $p66^{Shc}$ deletion on survival and reproduction in a population of mice kept in outdoor conditions (a large enclosure) in a harsh environment for one year and exposed to natural selection (food competition and winter temperatures).

Following one year, when mice were recaptured and genotyped, we found that, in this wild-mimicking conditions, mice holding the p66^{Shc} deletion (p66$^{Shc-/-}$ and p66$^{Shc+/-}$) are strongly counter-selected demonstrating that the conditions of the 'Chisty Les' pen were deleterious in the absence of the p66^{Shc} gene (Giorgio et al., 2012). Successively, under controlled laboratory conditions, it was confirmed that, the simultaneous exposure to starvation and low temperatures (+12 °C) for 72 h led to 50% mortality of the p66$^{Shc-/-}$ mice, as compared to 0% of WT subjects. In this context, we also found alterations in fat-tissue functions to be responsible for the deficit in survival of the p66$^{Shc-/-}$ mice since they were characterized by reduction of all body fat depots investigated as well as decreased production and reduced circulating levels of adipokines, including leptin, adiponectin, TNF-alpha, and Pai-1 (Giorgio et al., 2012). These data clearly suggest that defects in fat accumulation and thermoregulation might have acted to counter-select mice holding the p66^{Shc} deletion in the semi-naturalistic setting. Reproduction – which involves, at least in mammals, gonadal function, pregnancy, and lactation – is a highly demanding process from an energetic point of view and relies substantially on fat functions (Hausman and Barb, 2010), suggesting that absence of the p66^{Shc} allele in the 'wild' might also account for reduced fertility. Interestingly, when tested in the laboratory p66$^{Shc-/-}$ mice are characterized by reduced fertility under stressful conditions and by abnormal or reduced maternal care (Giorgio et al., 2012).

Taken together these data strongly suggest that the p66^{Shc} gene might have been evolutionarily selected because of its specific role in energy metabolism.

3.2. A peculiar behavioral and neuroendocrine phenotype

3.2.1. Delayed behavioral aging in the p66$^{Shc-/-}$ mutants, focus on cognitive and emotional aspects

Longevity is not necessarily synonymous of health. In fact, the increased lifespan characterizing western societies has been paralleled by an increase in the incidence of age-related diseases, including neurodegeneration and dementia that heavily interfere with the quality of life (Barberger-Gateau and Fabrigoule, 1997; Prince et al., 2003).

As previously mentioned, CR is able to increase longevity in mammals (including humans) and to either slow down or prevent the progression of several age-related pathologies. Interestingly, most research in this field has primarily focused on peripheral targets, almost neglecting the brain, and this is possibly due to its direct and main involvement in specific metabolic-related pathologies (Anson et al., 2003; De Lorenzo et al., 2011; Klebanov, 2007; Mattson and Wan, 2005; Morris et al., 2011). However, emerging evidence suggests that a similar scenario might apply to neuronal damage and neurodegenerative disorders (Bishop and Guarente, 2007; Gillette-Guyonnet and Vellas, 2008). The effects of CR might be causally related to decreased OS in the brain (Ribeiro et al., 2012). The mammalian brain is characterized by poor antioxidant defenses, high metabolic rate, and reduced capacity for cellular regeneration resulting particularly susceptible to OS insults (Floyd and Hensley, 2002). ROS are generated as a by-product of normal metabolism (Chavko et al., 2003). Both pathophysiological conditions and emotional stress may increase their amount, leading to a condition of OS which represents an important mechanism contributing to aging in a wide range of organisms (Liu and Mori, 1999; Madrigal et al., 2006). In addition, ROS and OS have been found to be involved in the neuropathology of several neurodegenerative disorders associated with cognitive impairment (Finkel and Holbrook, 2000). A number of observations indicate increased levels of anxiety with age in mice both in non-social and in social contexts (Francia et al., 2006). In addition, Hovatta and co-workers specifically found a link between genes involved in the regulation of OS and emotionality (Hovatta et al., 2005).

The p66^{Shc} gene appears to be one of the converging points linking OS, metabolism and the genetics of aging. So far a number of animal models, developed to study the aging process, suggests a relationship between changes in general metabolism or in the cellular redox milieu and longevity (Bayram et al., 2012; Holzenberger et al., 2003; Kenyon et al., 1993; Kimura et al., 1997; Longo and Finch, 2003; Tatar et al., 2001; Treiber et al., 2011). p66$^{Shc-/-}$ mice are characterized by reduced OS and elevated resistance to high-fat induced obesity and, more importantly, are long-lived, representing a unique opportunity to study the aging process in vivo, and thus the relationship between longevity and health.

In this context, it is possible to hypothesize that reduced exposure to OS from early development and throughout life might protect the p66$^{Shc-/-}$ mutants from the negative effects of free radicals, resulting in a more efficient homeostatic control and in better abilities to cope with changes in the internal milieu, attenuating the effects of aging on the nervous system and resulting overall in a improved health status.

When we investigated whether mutant subjects might show reduced signs of behavioral aging we found p66$^{Shc-/-}$ mice to be characterized by reduced response to arousing or painful stimuli, increased exploration and reduced emotionality. The observed differences between KO and WTs become more pronounced with age, suggesting that deletion of the p66^{Shc} gene is able to slow the aging process (Berry et al., 2007, 2008). As for cognitive abilities, p66$^{Shc-/-}$ adult mice show better spatial memory and better behavioral plasticity in a hippocampal-dependent spatial memory task (Morris water maze) also showing an improved physical health at old age (Berry et al., 2008).

A number of studies support the concept that ROS may be involved in memory impairment (Abidin et al., 2004; Pellmar et al., 1991; Williams and Bliss, 1989) and, within this context, this piece of data strengthens this evidence showing that affecting the redox balance in favor of an antioxidant milieu may improve cognitive abilities already at adult age. In addition, KO old mice also show a better physical performance than WTs suggesting that a reduced exposure to OS at old age might maintain muscle strength allowing them to climb onto the platform (Berry et al., 2008). In this context, the reduced pain sensitivity observed, particularly at senescence, might also have played a role contributing to alleviate the nociceptive burden that often associates to the physical decline observed at old age.

It is interesting to note that one of the main features emerging from the analysis of the behavioral phenotype of the p66^{Shc} mutants is indeed represented by their reduced emotionality, both in social and non-social contexts, associated to an increased explorative behavior at all ages tested. This piece of data is further confirmed by studies performed on adult females and on old male and female subjects (Berry et al., 2012; Giorgio et al., 2012). In fact, 24-month-old mice when tested in a social context – Intellicages – show a prompt exploratory activity of the novel environment with no specific gender difference (Berry et al., 2012). A similarly increased exploratory/locomotor activity can also be observed in adult lactating mutant females. This finding is quite intriguing since such a behavioral pattern conflicts with the maternal care needed by the offspring (Giorgio et al., 2012). However a fine analysis reveals that females are able to redirect all activities – other than maternal behavior – on foraging. From an evolutionary perspective metabolic needs (hunger) may increase locomotor activity resulting in an adaptive foraging behavior (Cabanac, 1985; Overton and Williams, 2004). Thus these data suggest that the increased exploratory behavior characterizing the mutants is an adaptive response to their increased metabolic rate.

3.2.2. Neuroendocrine and immune function

Aging is characterized by a low-grade chronic inflammatory state deriving from a higher propensity to mount inflammatory responses due to a decreased control of the neuroendocrine function, in addition to a reduced efficiency of anti-inflammatory networks (Franceschi et al., 2007). A prolonged exposure to OS and/or to the stress hormones glucocorticoids (GC), as a result of the hyperactivity of the neuroendocrine system (particularly of the hypothalamic–pituitary–adrenal – HPA – axis) throughout life, may all contribute to exacerbate the age-related decay of organism's functions.

In vitro evidence have shown that increased ROS generated upon inflammatory challenges may attenuate the GC negative feed-back suggesting that neuroendocrine function may be finely modulated upon OS/inflammatory signaling pathways (Asaba et al., 2004; Okamoto et al., 1999). As the key to successful aging and longevity could rely on an efficient cross-talk between the immune and the neuroendocrine systems and $p66^{Shc-/-}$ mutants are characterized by a lower susceptibility to OS, an appealing hypothesis is that this feature might contribute to the observed delay in the aging process (Franceschi et al., 2007; Sapolsky, 1999). Indeed, lack of this gene results in a mild hyperdrive of the HPA axis, in addition to an elevated central resilience to changes upon inflammatory stimuli (Berry et al., 2010). A rise in plasma GC levels upon inflammation and/or infection is necessary to prevent the overshooting of the immune system (Gaillard, 2001; Wiegers and Reul, 1998) while the hyporesponsiveness of the HPA axis has been associated to a higher susceptibility to autoimmune diseases (Bakker et al., 2000). Thus, the HPA escape from suppression specifically observed in KO mice could indicate a better prepared system to cope with the inflammatory challenges (Berry et al., 2010). This ability is further reflected in the resilience of central responses to the immunogenic stress specifically shown by KO mice when considering OS markers. In fact, mutant mice are characterized by overall reduced hippocampal levels of Isoprostanes (15-F2t-IsoP), are not affected by an immunogenic challenge and show decreased hippocampal levels of prostaglandins (PGE_2), possibly reflecting a reduced neuronal activation of this genotype in response to a stressful challenge (Berry et al., 2010).

Lastly, Minghetti and co-workers have shown that the measure of the peripheral antioxidant capacity (AOC) i.e., the total peripheral antioxidant defense may be considered as reliable index of the overall health status and prognosis in Alzheimer's patients, possibly reflecting the extent to which vulnerable neuronal populations are protected from oxidative processes (Minghetti et al., 2006). Thus, in a translational approach, peripheral AOC (which comprise scavenging enzymes as well as many small anti-oxidant molecules) measured in this mutant model showed high antioxidant capacities also following a challenge, possibly suggesting an overall lower exposure to OS (Berry et al., 2010).

3.3. The plastic brain of $p66^{Shc-/-}$ mice

3.3.1. Hippocampal neurogenesis during aging in the $p66^{Shc-/-}$ mutants

The mammalian brain is characterized by poor antioxidant defenses and high metabolic rate, resulting particularly susceptible to OS insults (Floyd and Hensley, 2002). This susceptibility is further increased during senescence, a vulnerable life stage characterized by a progressive decrease of homeostasis. At this age, proliferation and integration of adult-born neurons in the hippocampus – a brain area involved in cognitive functions and in the control of neuroendocrine responses to stress – is a rare event. Thus, even few young neurons might contribute to the maintenance of hippocampal homeostasis, representing a mechanism that can modify neuronal networks in response to external stimuli (Lee and Son, 2009; Snyder and Cameron, 2012). A number of studies have proposed that OS and inflammation may represent a critical early event in normal aging and neurodegeneration affecting the plastic potential of the hippocampus related to neurogenesis (Bastos et al., 2008; Finkel and Holbrook, 2000; Floyd, 1999; Russo et al., 2011).

$p66^{Shc-/-}$ mice are characterized by reduced OS levels, show an improved health status at senescence and better cognitive abilities already at adulthood (Berry et al., 2008). Thus, it is possible to hypothesize that during senescence these mutants might also show a slower decay of brain functions and increased neuronal plasticity. Our data indicate that neurogenesis-related variation in the hippocampus of senescent mice is mainly driven by sex, in fact all females (regardless of genotype) show an increased number of young neurons. However, and most intriguingly, $p66^{Shc-/-}$ female mice are characterized by increased proliferation, suggesting overall to be protected from precursor cell loss, an indirect evidence of brain plasticity.

As for sex differences, a large body of evidence indicate that gender differences exist in the rate of aging. In particular, estrogens appear to attenuate age-related cognitive decline in female subjects of species from rodents to primates (Gibbs and Gabor, 2003). This effect might be related to neuronal protection mediated by an anti-inflammatory action (Bruce-Keller et al., 2000; De Nicola et al., 2009) and it is possible that females might benefit more from lack of $p66^{Shc}$ gene than males by way of synergistic effects of reduced OS and estrogen (Berry et al., 2012).

$p66^{Shc-/-}$ mice are also characterized by decreased number of granule cells in the hippocampus and by overall smaller brains. The observed reduction in brain size suggests that the lack of $p66^{Shc}$ results in a neurodevelopmental defect: given the main expression of $p66^{Shc}$ during embryonic life (Conti et al., 1997) a reduced number of granule cells in the hippocampus of KO mice is likely to result from processes occurred early during development (Berry et al., 2012). Worth noticing, a very recent paper from Sone and co-workers quantified the expression levels of transcripts of Shc-related isoforms in the brain of young adult, middle-aged and aged rats and found that $p66^{Shc}$ expression is specifically up-regulated in the aged brain suggesting that the repression of this gene could become a potential target for an anti-aging strategy (Sone et al., 2012), especially in female subjects.

3.3.2. BDNF paves the way for plasticity in the $p66^{Shc-/-}$ brain

The brain may change structure and function in response to a variety of stimuli and this ability is commonly referred as to "brain plasticity". In this context, neurotrophins are indeed ideal candidates for playing a role since they are expressed in the appropriate "loci" of plasticity, their expression is activity-dependent and they appear to affect synaptic function, membrane excitability as well as neuronal morphology and connectivity (Cirulli and Alleva, 2009). Expression of both nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) has been localized to the hippocampus and neocortex, two target areas for basal forebrain cholinergic neurons and sites where both developmental and adult synaptic plasticity occurs (Cirulli and Alleva, 2009). BDNF in particular is involved in specific aspects of cognitive abilities and behavioral plasticity, while playing also an important role in neuronal survival/differentiation, neuroprotection, emotionality, pain sensitivity and even in energy homeostasis and weight control (Cirulli et al., 2000, 2004; Thoenen, 1995, 2000).

BDNF is a prominent signaling molecule stimulating mitochondrial biogenesis and energy metabolism. It is able to trigger adaptive response of the nervous, cardiovascular, and energy regulating organ systems as a result of changes in metabolic needs by coordinating central and peripheral signals (Cheng et al., 2010). Accordingly, its expression and signaling is increased in response to environmental features such as physical exercise, cognitive

stimulation and CR that increase cellular energy demand (Mattson et al., 2004a). Thus BDNF provides all-system plasticity which is pivotal to survival and extends far beyond its main role in the brain. In animal models, central infusion of BDNF induces weight loss and decreases food intake (Rios et al., 2001). In mice carrying a mutation in the BDNF gene, high fat diet administration leads to hyperphagia, obesity and decreases early satiety (Fox and Byerly, 2004). By contrast, CR in BDNF heterozygous mice increases its expression in the brain (Duan et al., 2003) and reduces obesity, anxiety and aggressive behavior (Koizumi et al., 2006) in combination with serotonin (5HT) signaling.

In this context BDNF appears an ideal candidate molecule underlying behavioral and brain plasticity and most of the features so far described for $p66^{Shc-/-}$ mice which are characterized by a rearrangement of the metabolic set-point. Intriguingly, these features are shared by most of the (genetic and CR-related) mammalian models of extended longevity. In these models, the disruption of metabolic pathways leads to a general hormetic response involving the activation of stress-related signaling pathways including those of cell survival and resistance to OS etc., aimed at protecting the organism (Gems and Partridge, 2008) eventually resulting in a plastic phenotype promptly adaptable to changes. Thus, signaling pathways involved in both central and peripheral stress responses and regulation of energy homeostasis may share common brain circuitry and biochemical pathways, which may promote successful aging or contribute to the pathogenesis of age-related disease.

In humans, plasma or serum BDNF levels are decreased in obese compared to lean subjects (El-Gharbawy et al., 2006) and elevated in obese diabetic patients compared to controls (Suwa et al., 2006). This neurotrophin has also been associated with eating disorders related to severe psychiatric symptoms such as in patients affected by anorexia nervosa (Ribases et al., 2005); its involvement in MS remains controversial (Bullo et al., 2007; Suwa et al., 2006). Very recent evidence show that pre-pubertal obese children tend to have decreased plasma BDNF levels than lean controls although the exact role of BDNF in the pathophysiology of obesity in these subjects needs to be elucidated (Corripio et al., 2012). Furthermore a specific association between BDNF Val66Met polymorphism and obesity in children and adolescents has been found (Skledar et al., 2012).

Obesity in children and adolescents is a worldwide health problem, characterized by a plethora of somatic complications (hypertension, dyslipidemia, inflammation, atherosclerosis, elevated insulin levels, heart disease, increased tendency for blood clotting, kidney and liver dysfunction, neurological complications and T2D (Ebbeling et al., 2002)), but also psycho-social problems including high-risk behaviors, decreased self-esteem, loneliness (Swallen et al., 2005) and eating disorders (Babio et al., 2008; Sancho et al., 2007; Skledar et al., 2012). This pathologic condition has the potential to evolve in a much more catastrophic scenario leading to adult obesity (Bouchard, 1997) and MS associated to mood and cognitive dysfunctions, overall accelerating the aging process (Engum, 2007; Goldbacher et al., 2009; Muller et al., 2010; Raffaitin et al., 2011; van Reedt Dortland et al., 2010; Zeugmann et al., 2010). Thus, alterations in metabolism and body fat distribution may play a role in a precipitating the aging process and the onset of diseases. In this context targeting BDNF signaling may result in novel therapeutic interventions for a range of metabolic and neurological disorders.

$p66^{Shc-/-}$ mice are characterized by increased basal hippocampal levels of BDNF. This piece of data is paralleled by improved cognitive abilities, reduced emotionality and pain sensitivity (Berry et al., 2008) in addition to elevated neuronal protection against inflammatory insults (Berry et al., 2010). Indeed, when assessing spontaneous behavior in the KOs, they showed an uncommon motor pattern namely "backward walking" (involving stepping backwards rather than foreword). Worth noticing, this behavior has been specifically related to higher BDNF tone and to hyper-activity of both the serotoninergic and dopaminergic systems (Bert et al., 2006; Martin-Iverson et al., 1994). Thus an interesting hypothesis is that BDNF might not be the only player of plasticity, but that it rather acts in combination with 5HT to shape the emotional and metabolic phenotype of the $p66^{Shc}$ mutants. Indeed, BDNF and 5HT co-regulate one another and their signaling pathways are central to mood disorders since selective serotonin reuptake inhibitors, commonly used to treat anxiety in humans, have been found to increase the expression of BDNF and of its receptor, in the brain (Castren et al., 2007). Moreover they both act to modulate synaptic plasticity, neurogenesis and neuronal survival in addition to emotionality and metabolism in the adult brain (Mattson et al., 2004a).

4. A neuronal signaling quartet affecting quality of life during aging: $p66^{Shc}$-BDNF-5HT-IGF1

Signaling pathways involved in both central nervous system and peripheral stress responses and regulation of energy metabolism may play important roles in lifespan determination (Mattson et al., 2002). The brain has been proposed to play a crucial role in promoting longevity in metazoans (Bishop and Guarente, 2007). Intriguingly, Mattson and colleagues speculate that complex brain circuits involved in learning, memory, emotions and higher cognitive functions, could have evolved as energy adaptations (Mattson et al., 2004b). In particular the ability to locate food and to efficiently store and dispense energy are driving forces in the evolution of complex multicellular organisms that can decrease the probability of dying for environmental hazard (Mattson et al., 2004b).

In this regard IGF1, BDNF and 5HT might act as a "triumvirate" that, with their cooperative influence on energy metabolism, food intake, stress responses, emotionality and cognition plays a central role in healthspan during aging (Maswood et al., 2004). $p66^{Shc-/-}$ mice show increased brain BDNF levels and this might account for many of the features characterizing the $p66^{Shc-/-}$ phenotype so far described (Berry et al., 2010). In addition, we found higher levels of 5HT in the frontal and pre-frontal cortex (two brain regions known to play a role in mood disorders) in KO subjects (Berry et al., personal communication). In the $p66^{Shc}$ scenario, our data on the behavioral phenotype, and the associated changes in BDNF and 5HT, emerge as novel and intriguing, suggesting that the lack of a source of ROS affects specific signaling pathways in the brain related to energy homeostasis and emotional behavior, which appear as important determinants of health during aging (healthspan).

But how does $p66^{Shc}$ mechanistically relate to these actors? Berniakovich and co-workers have reported that not only OS, but also insulin (through the phosphorylation of a specific serine residual – Ser36), may stimulate the redox enzyme-activity of $p66^{Shc}$. ROS (H_2O_2) produced upon this stimulation potentiate, in turn, insulin receptor signaling, suppressing the expression of uncoupling proteins and beta-oxidation enzymes, leading to triglyceride accumulation (Berniakovich et al., 2008; Nemoto et al., 2006). In this scenario $p66^{Shc}$ might well represent an additional actor leading to a quartet that modulates longevity and age-related diseases.

It is well established that environmental and genetic factors that increase longevity also increase cellular resistance to stress (Bakker et al., 2000; Duan et al., 2001; Johnson et al., 2000) while a growing body of evidence suggests that hormesis (i.e., beneficial effects resulting from a low dose exposure to a generic stress) might represent a common shared and conserved mechanism. Insulin/IGF1 (Clancy et al., 2001) and OS (Harman, 1998) play a critical role in lifespan determination and recently these lifespan pathways have been mechanistically related by the $p66^{Shc}$ gene

(Berniakovich et al., 2008). Thus knocking-out the p66^{Shc} gene may trigger the activation of stress-related signaling pathways including those of cell survival, resistance to OS etc., aimed at protecting the organism (Gems and Partridge, 2008). Again, from an evolutionary perspective, organisms with the ability to cope with stressors such as fasting, physical exercise related to foraging and "cheating" on conspecific and/or other species (cognitive activity), held a survival advantage suggesting that the nervous system was shaped through evolution so as to respond to mild stressors and to increase their resistance to more severe stressors (i.e., hormesis) (Mattson, 2008). In line with Mattson's evolutionary scenario we propose that the p66^{Shc}-dependent IGF1/metabolic-OS pathway might impinge upon signaling pathways related to mood and, more in general, to motivated behavior (such as BDNF and 5HT) to cooperate and determine survival strategies which ultimately can also affect the quality of life during aging (Mattson, 2008; Mattson et al., 2004b).

In humans, mood and cognitive dysfunctions are often associated with morbid conditions overall resembling a form of precocious aging such as MS (Fadini et al., 2011), creating a vicious circle involving, in turn, CVD and T2D (Engum, 2007; Goldbacher et al., 2009; Muller et al., 2010; Raffaitin et al., 2011; van Reedt Dortland et al., 2010; Zeugmann et al., 2010). Very recent data indicate that obesity per se can lead to plasticity-related changes in the reward circuitry that are associated with a depressive-like phenotype (Sharma and Fulton, 2013). As increases in BDNF and CREB activity in the striatum are well implicated in depressive behavior and reward, the authors suggest that these molecules may mediate the effects of high-fat diet and diet-induced obesity to promote negative emotional states and depressive-like symptomatology (Sharma and Fulton, 2013). This evidence further supports the hypothesis of a tight link between metabolic and emotional pathways in determining health and longevity (see Fig. 1). Future studies should address the role of p66^{Shc} in mood disorders and neurodegeneration associated with obesity and MS.

5. The role of p66^{Shc} in human health

Evidence collected so far strongly point to p66^{Shc} as a novel gerontogene able to modulate longevity, at least in animal models.

Mooijaart et al. (2004) tested the only known non-synonymous polymorphism of the Shc gene (Met410Val) in two independent cohorts of Dutch elderly of respectively 730 and 563 subjects aged 85 and over, for association with longevity using a prospective follow-up design. Overall an increase in Valine (Val) allele

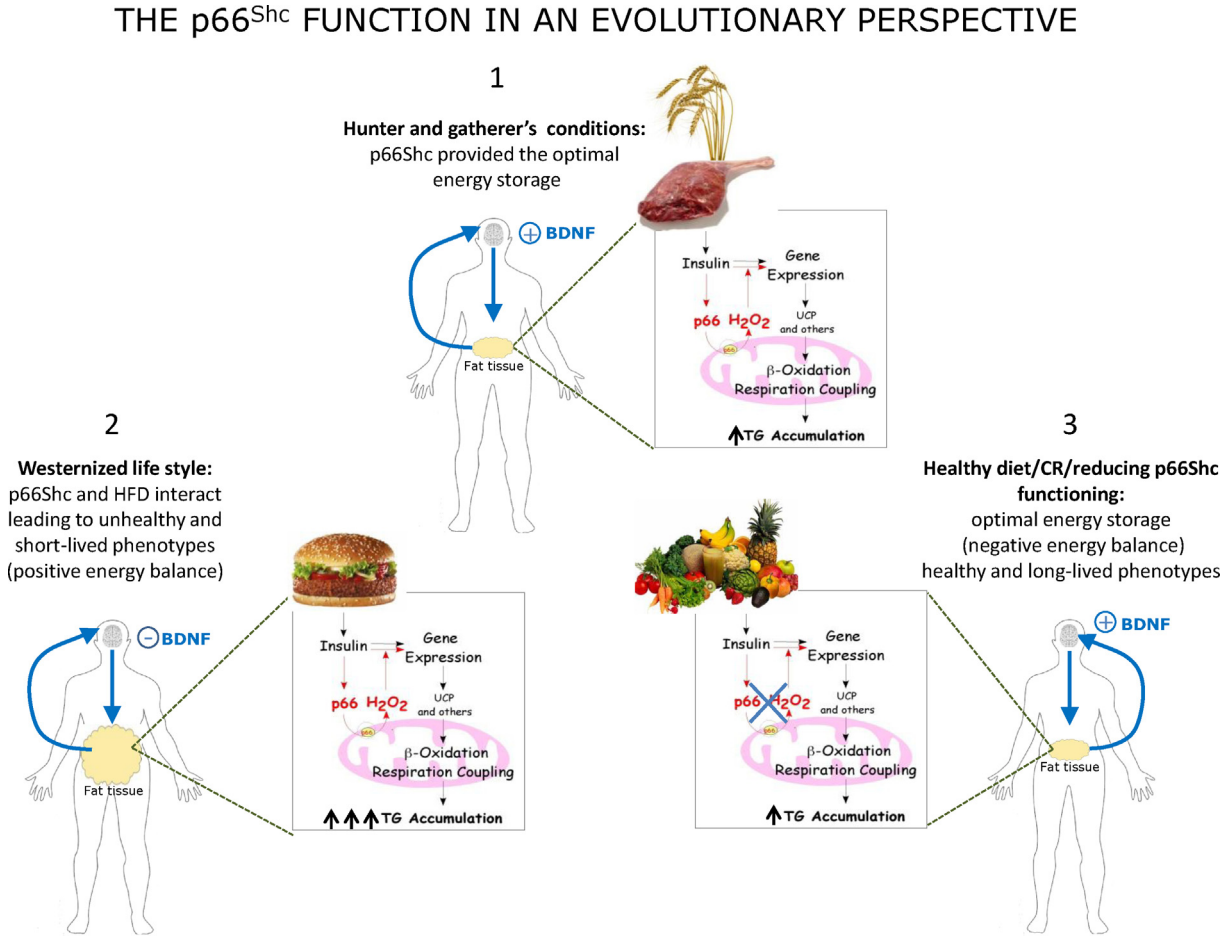

Fig. 1. The p66^{Shc} role in an evolutionary perspective. Longevity and healthspan might result from a finely tuned crosstalk between central and peripheral targets mediated by p66^{Shc} and BDNF. p66^{Shc} has probably evolved as a thrifty gene in order to store lipids and to accumulate fat playing a pro-survival role in the environment experienced by our ancestors (unpredictable changes in weather conditions, intermittent food availability, etc.). In this context mammals bearing the p66^{Shc} gene might have experienced conditions resulting in a favorable energy balance (triglyceride – TG – accumulation and black arrow close by in the inset) with increased BDNF levels upon endocrine signaling from fat tissue (blue arrow from fat tissue to brain). BDNF, given its influence on energy metabolism and food intake (blue arrow from brain to fat tissue) in addition to stress responses, emotionality and cognition, might thus play a positive role on healthspan and longevity (1). Westernized lifestyle is characterized by continuous availability of food, rich in fat (high-fat diet – HFD) and poor in nutrients leading to excessive fat accumulation also mediated by the thrifty action of the p66^{Shc} gene and by consequently reduced BDNF levels. This condition might result in an overall unhealthy and short-lived phenotype (2). A healthy hypocaloric diet (caloric restriction – CR) or pharmacological strategies aimed at reducing p66^{Shc} levels might provide an overall negative energy balance reinforced also by central signaling pathways involving BDNF (3) (partially modified by Trinei et al., 2009).

frequency with age and reduced mortality rate in Val allele carriers was observed, these data indicating that an association between the Met410Val polymorphism and longevity in humans may exist. It is interesting to note that such an association was not found in Japanese long-lived people (Kamei et al., 2003).

DNA methylation in CpG islands is known to be associated with gene silencing. Very recent evidence from Gentilini and co-workers investigated the role of epigenetics in the modulation of human longevity by evaluating the DNA methylation from peripheral leukocytes of female centenarians, their female offspring, the offspring of both non-long-lived parents and young women (Gentilini et al., 2012). Interestingly, these authors found that, among others, genes involved in the regulation of metabolism are differentially methylated between centenarians' offspring and offspring of both non-long-lived parents, suggesting a role for these genes in human longevity and for preserved DNA methylation as a potential mechanism accounting for better health status during aging (Gentilini et al., 2012).

Ventura and co-workers reported that the p66^{Shc} promoter contains a relatively high CG content and that cell lines with the least amounts of methylation had the highest levels of p66^{Shc} expression, and vice versa (Ventura et al., 2002). The finding that p66^{Shc} can be up or down-regulated in cell lines due to changes in promoter methylation status raises the possibility that differences in the methylation status of p66^{Shc} promoter could be linked to aging, especially if the same phenomenon can be reproduced in whole animals or human beings. Purdom and Chen hypothesize that differences in p66^{Shc} promoter methylation status could account for some of the epigenetic basis for variations in longevity among centenarians characterized by lower levels of p66^{Shc} protein in their fibroblasts (Purdom and Chen, 2003a). Indeed, a very recent evidence shows that the promoter hypomethylation of the p66^{Shc} gene, as well as enhanced H3 acetylation are the epigenetic markers that trigger its persistent overexpression despite glucose normalization in long-lasting diabetic patients suggesting that p66^{Shc} is a crucial mediator of sustained vascular hyperglycemic stress in diabetes and that this epigenetic mechanism might account for the phenomenon of "hyperglycemic memory" which leads to cardiovascular complications (Paneni et al., 2012).

Pandolfi and co-workers investigated the effects of oxidative or hypoxic stress on p66^{Shc} expression and protein levels in fibroblasts from young people, elderly and centenarians. Unexpectedly, centenarians showed the highest basal levels of p66^{Shc} mRNA (Pandolfi et al., 2005). In the same year, Pagnin and co-workers reported that p66^{Shc} expression is increased in the peripheral blood mononuclear cell (PBMC) of insulin resistant patients with T2D. In addition, these authors found a significant linear correlation between p66^{Shc} mRNA levels and plasma total isoprostanes (a marker of systemic OS) suggesting a role for p66^{Shc} in the morbidity associated with T2D, obesity and MS and the resulting lifespan shortening (Pagnin et al., 2005).

More recently, Pinton and Rizzuto proposed an intriguing reconciling hypothesis i.e., some defects in mitochondrial import of p66^{Shc} in centenarians might prevent its pro-aging properties, while leaving other biological functions (e.g., the production of low levels of ROS necessary in differentiation) unaffected (Pinton and Rizzuto, 2008).

Overall, existing evidence suggests that the role of p66^{Shc} in human longevity is more complex than previously thought. As an example, although in vitro evidence as well as evidence provided by animal models clearly point to p66^{Shc} as a main signaling pathway involved in fat accumulation, there is no definite evidence that this same signaling pathway regulates fat mass development and is involved in obesity in humans (Fadini et al., 2011). In addition, most of the above mentioned studies on human cohorts suffer from limitations, such as insufficient sample size, lack of power, sampling bias and population stratification. Some studies could not be replicated or gave different results when performed in a different population. Indeed further studies are needed. However, many of the associations observed support the hypothesis that p66^{Shc} may increase lifespan in humans and emerging evidence support a main involvement of p66^{Shc} in human health related to central and peripheral target molecules involved in regulation of metabolism and OS.

As already previously mentioned, MS often associates to higher incidence of mood and cognitive dysfunctions that in turn contribute to precipitate this pathology and the related health outcomes, particularly CVD and T2D (Engum, 2007; Goldbacher et al., 2009; Muller et al., 2010; Raffaitin et al., 2011; van Reedt Dortland et al., 2010; Zeugmann et al., 2010). These data overall strengthen the hypothesis that alterations of metabolism and body fat distribution, as those involving the p66^{Shc} gene, may play a role in a loop involving central pathways (most importantly BDNF) that can modulate the aging process and the onset of diseases (Barzilai et al., 2012).

Future studies should address the issue of the cause-effect involvement of p66^{Shc} in MS. In addition, the assessment of changes in the p66^{Shc} protein levels, or in its functional state (through peripheral measurements in the PBMC) might represent a sensitive tool to detect early changes in the metabolic/health status in humans. In this context also BDNF might represent an additional prognostic marker of psychiatric complications related to metabolic dysfunctions. Therefore, both p66^{Shc} and BDNF have the potential to be used in clinical practice as specific peripheral biomarkers of a pathological metabolic state indicating a potential risk for mental health and precocious aging.

6. Final remarks

The lack of the p66^{Shc} gene leads undeniably to a plethora of positive effects, accounting not only for retarded aging but also for a better health status (Berry et al., 2007, 2010, 2008). These effects rely on OS, metabolism, emotional and cognitive aspects related to specific signaling pathways in the brain involving a fine interplay of a "quartet" made up of BDNF, IGF1, 5HT and p66^{Shc}.

Overall the "thrifty function" of p66^{Shc} appears desirable when food is scarce and resources need to be stored. However, fat accumulation promoted by p66^{Shc} can be detrimental when food is constantly available – a condition characterizing westernized lifestyle – and may lead to increased vulnerability to develop degenerative diseases such as diabetes, CVD and cancer, accelerating the aging process (Neel, 1962; Neel et al., 1998).

Data discussed in this review set the conditions for future studies aimed at better understanding the role of p66^{Shc} and BDNF in the adaptive response to metabolic and environmental stressors in order to develop novel diagnostic and therapeutic tools aimed at promoting mental health and healthy aging.

Authors contribution

Alessandra Berry and Francesca Cirulli wrote this paper.

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