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Time for the systems-level integration of aging: resilience enhancing strategies to prevent Alzheimer's disease

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ABSTRACT

Systems biology and systems neurophysiology generate comprehensive mechanistic models of the spatial-temporal evolution of body system networks and their interplay during the dimensional *spectrum* from physiological to pathophysiological conditions.

Alzheimer's disease (AD)-related pathophysiological alterations converge with overexpressed age-related functional decline, i.e. aging, which is induced by genetic- and stochastic time-dependent events.

Accumulation of cellular senescence has a casual role in aging-related disease and senolytics drugs have already shown encouraging results for counteracting senescence detrimental effect.

However, the non-linear complex nature of AD pathophysiology calls for a systems-level integration of aging dynamics, from molecules until large-scale networks.

We need a holistic systems-wide comprehensive model of aging which is constituted by a non-linear spatial-temporal weakening of adaptive responses resulting in the activation of compensatory mechanisms that ensure biological robustness, resilience, and finally homeodynamics.

After exceeding the threshold of compensated (resilient) aging, a cascade of decompensatory events occurs, ultimately triggering irreversible systems failure that at some advanced stage reflect widespread “pathophysiological hallmarks of AD”.

The gap for the comprehensive understanding of aging, resilience, and AD pathophysiological evolution is the accomplishment of a quantitative, flexible, and integrative modeling approach to detect multiple spatial-temporal patterns and for dissecting causal mechanisms and downstream cascades throughout the aging-AD *continuum*.

Novel technological and conceptual advances, will enable the systems-level integration of aging signatures as well as compensatory mechanisms that provide resilience to early functional decline. This will provide new systems-scaled outcomes and endpoints to map and therapeutically enhance resilience and accomplish a long-lasting compensated aging.

Keywords: aging; senescence; adaptation; resilience; stress response; Alzheimer's disease

INTRODUCTION

Complex chronic diseases including cancer, immune diseases, diabetes, and neurodegenerative diseases – such as Alzheimer's disease (AD) – are characterized by: 1) a multifactorial nature, due to the coexistence of (epi)genetic, biological, and environmental susceptibility 2) concurrent pathomechanistic alterations due to the perturbation of multiplex pathways and networks resulting in several disease modules and interactomes 3) the involvement of highly conserved cross-talks among different body systems (Arnsten, 2015; Bassett and Sporns, 2017; Shine et al., 2019).

The continuous failure of late stage clinical AD drug trials, raises fundamental theoretical and conceptual questions (Cummings et al., 2018; Dourlen et al., 2019; Hampel et al., 2018b; Panza et al., 2019)

A critical step for developing effective drugs with disease-modifying properties is to explore and predict the comprehensive effect of a compound on three basic levels (i.e. proof of pharmacology), including 1) hitting the intended target, 2) altering the relevant pathophysiological mechanism, and 3) impacting clinical outcomes with a long-lasting effect. It seems crucial to enrich drug Research and Development (R&D) pipelines with the perspective of an integrative, exploratory, and holistic multi-paradigm or systems level concept, at both experimental and computational level and with a strong biomarker-drug co-development strategy throughout all development stages (Bokde et al., 2009; Cummings et al., 2018; Hampel et al., 2019)

Systems biology and systems neurophysiology paradigms enable to accomplish unbiased experimental predictions of pathophysiological dynamics through knowledge-driven or entirely explorative designs (Cummings et al., 2018; Dourlen et al., 2019; Hampel et al., 2018b; Panza et al., 2019). In this sense, integrative analyses allow to track meaningful biological signatures reflecting key pathomechanistic alterations as well as their spatial and temporal evolution over the course of a certain disease.

In the context of neurodegenerative diseases, the contribution of systems biology and systems neurophysiology is to generate comprehensive models of multilevel networks interactions, from molecular pathway(s), to cellular machineries, synaptic circuits, until large-scale network patterns, in either physiological or pathophysiological conditions and according different temporal and spatial scales (Arnsten, 2015; Bartsch et al., 2015; Bassett and Sporns, 2017; Medland et al., 2014; Richiardi et al., 2015; Shine et al., 2019). The objective of this article is first to provide a comprehensive overview of the major networks ensuring systems hemodynamics, at the different biological levels, and propose a theoretical framework to explore their changes along a system-wide aging, including cellular senescence, with regards to the risk of developing AD. We will list and describe the most relevant stress response and long-term resilience mechanisms as well as their relationship with lifespan, healthspan (the time during which the organism maintains its functional capacity), and age-related diseases such as AD. In addition, we will depict the main alterations that slow down adaptation and resilience in aging and AD, throughout a *continuum* from adaptation to compensation until systems failure, and from the molecular level to the body systems level. We will eventually deliver argumentation about the fact that a potential workflow, for the *in-vivo* systems-level integration of all aging variables, from molecules (encompassing senescence signatures) to large-scale networks, including adaptive and compensatory patterns will be feasible within a short time. The accomplishment of a holistic systems-scaled comprehensive model of aging is a key step for finally testing the Geroscience Hypothesis”... *any intervention that retards the aging process will simultaneously delay the onset of multiple diseases..*” (Kennedy et al., 2014).

In line with this postulate, an innovative pathway (mechanism)-based pharmacological paradigm aimed at developing effective biomarker-guided targeted and tailored therapies for ~~preventive~~ “resilience enhancing” and aging-freezing strategies. We will further clarify and define useful terminology and taxonomy relating to the discussed fundamental scientific concepts.

The *continuum* from homeostasis to adaptation, compensation, decompensation and system failure

Homeostasis or more correctly the homeodynamic space is essential for protecting all core physiological processes necessary for optimal functioning and survival and its preservation is highly related to the interplay between intrinsic (e.g., genetic and mechanistic factors) and environmental factors (Berg, 2016; Rattan, 2014). Adaptive responses and compensatory mechanisms account for the overall biological robustness and resilience of an organism and are hierarchically organized across molecular /cellular pathways, cell to cell and tissue dynamics, until large-scale networks, and body systems (Arnsten, 2015; Bartsch et al., 2015; Bassett and Sporns, 2017; Medland et al., 2014; Richiardi et al., 2015; Shine et al., 2019).

The preservation of homeodynamics under stressful conditions implies a functional shift to face a higher energetic demand, adaptation is essential to reallocate bioenergetic resources through a reprogramming which confers the proper robustness to cope with stressor(s). Biological adaptation (adaptive response) consists in a wide range of functional switches regarding a set of network(s) through different spatiotemporal scales according levels and systems in which they occur (Arnsten, 2015; Bashan et al., 2012; Harrison et al., 2009; Kaushik and Cuervo, 2015; Marder and Goillard, 2006; Shine et al., 2019)

Indeed, the bioenergetic shift which enables to maintain a proper effective functional output and ultimately homeodynamics, consists of distinct biological networks (spatial scales) as well as their sequential or simultaneous recruitment (temporal scales) (Arnsten, 2015; Hermans et al., 2011; Menche et al., 2015; Shine et al., 2019)

The magnitude and duration of an adaptive response match the downstream effect(s) induced by the stressor (see Figure 1). Moreover, all organisms from nematode to humans have an intrinsic property to develop a gradual self-regulation of the stress responses consistent with typology and time exposure of the stressor (Berg, 2016; Lu et al., 2014) For instance, at the cellular level, evolutionarily conserved transduction signals enable to sense the stress levels, thus triggering anti-stress responses which promote either cell survival or apoptosis, or senescence itself as stress escalates and becomes unsustainable. In addition, low exposure to a given stressor may trigger bio-positive effects representing appropriate cues which strengthen homeodynamics (hormesis) (Brose et al., 2018; Rattan, 2008; Ruhlmann et al., 2016).

This dynamic modulation of adaptive responses is important even at the extra-individual level, where adaptation is a pivotal point for natural selection to promote population-scaled evolutionary

transitions (evolutionary adaptation) optimizing large-scale survival and reproduction across ecosystem (Behrens et al., 2014).

Multidisciplinary approaches and *in-silico* modeling studies have proven that, besides molecules and cells, adaptive responses are elicited at different brain levels and that an interaction between neurobiological and synaptic network changes exists within short- long-term homeostatic mechanisms (Arnsten, 2015; Bartsch et al., 2015; Bassett and Sporns, 2017; Medland et al., 2014; Richiardi et al., 2015; Shine et al., 2019).

When maladaptive dynamics occur, compensatory mechanisms are essential to ensure resilience and maintain homeodynamics. Compensation is a protective process in which a morpho-functional alteration is counteracted by another morpho-functional alteration without any significant change in the biological output, thus ensuring resilience. Compensation provides a viable balance between the biological robustness and the degree of disruptive effect exerted by the stressor over time (Apostolova et al., 2018; Arenaza-Urquijo and Vemuri, 2018; Bokde et al., 2009; Hampel et al., 2018b; Marder and Goillard, 2006; Scheller et al., 2018; Stern et al., 2000).

Like adaptation, the compensatory dynamics are mainly described at the molecular and cellular level. However, in the last ten years, it has been shown that brain compensation range from neuronal / glial metabolic reconfiguration, to large-scale network dynamics, until complex inter-systems cross-talks (for instance the brain-innate system interface) (Apostolova et al., 2018; Elman et al., 2014; Kaushik and Cuervo, 2015; Marder and Goillard, 2006; Perez-Nievas et al., 2013).

Animal and human studies have untangled that compensation may be associated with a re-activation of developmental program, such as synaptogenesis, occurring within self-organized multilevel systems (Arendt, 2005). On the flipside, overcompensation may trigger a vicious cycle of feedback loops culminating in loss of function, i.e. decompensation (breakdown of resilient dynamics) (Arendt, 2005).

We argue that pervious failing drug R&D programs may have underestimated that an individual genetic-biological vulnerability to stressors exists as well as an individual genetic / epigenetic / sex driven threshold of failure of adaptive responses and compensatory capacities

Probing the *continuum* from aging to AD

Aging has been a vibrant topic of discourse among many generations of Philosophers and William Shakespeare defined the perceived polarity of young age and old age.

“Crabbed age and youth cannot live together; Youth is full of pleasure, age is full of care; Youth like summer morn, age like winter weather; Youth like summer brave, age like winter bare. Youth is full sport, age's breath is short; Youth is nimble, age is lame; Youth is hot and bold, age is weak and cold; Youth is wild, age is tame. Age, I do abhor thee; youth, I do adore thee.”

For the neuroscientist the question arises about the biological nature of aging and how does aging biologically relate or contribute to age-related brain diseases, such as AD.

It has previously been postulated that aging is characterized by genetic- and time-dependent decline in adaptive and compensatory properties (Lopez-Otin et al., 2013; McHugh and Gil, 2018).

A remarkable research effort has been generated in the last ten years to identify the hallmarks of aging which are currently related to the (sub)cellular level only. We propose a systems biology-based conceptual framework of brain aging i.e., non-linear functional-structural alterations due to a decline of biological adaptation, compensation, and ultimately robustness and resilience which ensure homeodynamics at the molecular, cellular, synaptic, and large-scale brain network level until the complex brain-body systems cross-talks (see Figure 2). Relying on several translational study results, we hypothesize that systems-scaled aging processes including but not restricted to cellular senescence, may be expressed either in AD cognitive decline or normal cognitive condition depending on the individual threshold of genetically and biologically determined resilience (Arenaza-Urquijo and Vemuri, 2018; Arnold et al., 2013; Hampel et al., 2018b; Perez-Nievas et al., 2013). Thus, aging and AD may belong to a *continuum* of network breakdowns, from decompensation to systems failure, inside brain and body systems.

We assume that the decompensatory cascade that draws aging towards AD has a nonlinear fashion since multiple pathomechanistic alterations evolve differently according to distinct spatial and temporal components, driven by complex genetic-epigenetic interplays.

Conversely, it is conceivable that as long as adaptation and compensation ensure homeodynamics systems networks survive to disrupting signals and AD pathophysiology does not evolve (i.e., “normal” or compensated aging). Hence, after exceeding the limit of compensated aging, a cascade of breakdown events occurs, and, at the final step, detrimental pathways trigger irreversible systems failure reflecting widespread pathophysiological hallmarks of AD: i.e. brain proteinopathies, synaptic failure, loss of brain plasticity, neuroinflammation, axonal damage, and neurodegeneration (Arnold et al., 2013; Bero et al., 2011; Bokde et al., 2009; Cirrito et al., 2005; Dourlen et al., 2019).

The devastating rate of failure of AD clinical trials within the last 25 years suggests that when decompensation commences across core biological networks and then systems failure takes place, every therapeutic intervention is likely to be less effective with no possibility to recover physiological functions and the homeodynamic space.

We hypothesize that the key points to prevent AD will be represented first by identifying those clusters of genetic, biological, and environmental factors that increase the vulnerability to stressors leading to an escalation of accumulation of senescence and other aging alterations, maladaptive responses, ineffective compensatory mechanisms that are likely to enter a vicious cycle of decompensation and generate irreversible systems failure. Second mapping *in-vivo* the adaptive capacity and the degree of resilience under initial pathophysiological dynamics (intermediate endophenotypes). We argue that the presence of active compensation ensuring resilience has the potential to reshape or even replace the current pathophysiological hallmarks of AD.

Achieving these two objectives would represent a crucial step for time-sensitive and effective **rejuvenation** therapies capable of strengthening resilience, i.e. “compensated aging”, counteracting its transition to systems failure and at the same time prolonging health span.

AGING, CELLULAR SENESCENCE, AND AD: a comprehensive overview

Even though aging is primarily a time-dependent stochastic process recent genetic manipulation studies have demonstrated that the “switch off” of specific genes can promote longevity (longevity genes) and prolong lifespan (Ayyadevara et al., 2008; Kennedy et al., 2014; Lopez-Otin et al., 2013; Xu et al., 2018) Such finding has raised the concept of a genetically driven (programmed) aging.

The discovery of an intrinsic (genetic) regulation of aging-related processes has raised the not yet unresolved intellectual conflict between evolutionary theorist which debate on whether aging may represent either an evolutionary adaptive response (adaptive aging ultimately called aging by design) or not (Goldsmith, 2008; Kyryakov et al., 2016). An adaptive aging counteracts the traditional evolutionary approach which rules out the possibility of an intrinsically determined self-defeating biological program (in terms of survival and reproduction) that does not bring about any short- or long-term benefit (non-programmed aging theory). According to the non-programmed theory only time-related stochastic insults account for the aging process. Answering such open question may represent a breakthrough in the development of aging targeted therapeutic avenues (Kowald and Kirkwood, 2016). However, we think that a deeper argumentation on this intriguing research topic goes beyond the aim of the present article.

It is noteworthy to highlight that a comprehensive understanding of the overall biological background of longevity and aging, especially with regard to the brain, has not been achieved yet.

Molecular and cellular hallmarks of aging: the antagonistic role of cellular senescence and the causal role of its accumulation

Cellular senescence is one the most important hallmark of aging alongside stem cell exhaustion, genomic instability, epigenetic aberrant dynamics, telomere shortening, proteostatic dysfunction, deregulation of intracellular signaling and nutrient-sensing pathways, and mitochondrial dysfunction (Lopez-Otin et al., 2013).

According the role each hallmark may play, a hypothetical categorization has been proposed: causal mechanisms (genomic instability, epigenetic aberrant dynamics, telomere shortening, loss of proteostasis), antagonistic mechanisms (nutrient-sensing pathways, mitochondrial dysfunction, cellular senescence), downstream detrimental mechanisms (deregulation of intracellular signaling and stem cell exhaustion).

Cellular senescence is a cell fate characterized by an irreversible cell cycle arrest coupled with improper activation of mitogenic and growth-promoting pathways (Munoz-Espin and Serrano, 2014).

Senescence represents an anti-tumor defense mechanism halting the propagation of damaged cells and setting off the immune system surveillance (i.e. antagonistic hallmark of aging) (Lopez-Otin et al., 2013; Munoz-Espin and Serrano, 2014). Therefore, senescence constitutes a resilient tissue-specific mechanistic alteration counteracting the functional biological aging-related decline. Furthermore, senescence plays a crucial role in embryogenic development, adult vascular remodeling, wound healing and lymphocytes reprogramming (Lopez-Otin et al., 2013; Munoz-Espin and Serrano, 2014).

Several experimental data suggested that an accurate stimulation of the senescence-inducing tumor suppressor pathways may extend longevity (Lopez-Otin et al., 2013; Munoz-Espin and Serrano, 2014).

Despite senescence's physiological aging-antagonistic roles, the accumulation of senescent cells has causal role for aging aging-related functional decline and is associated with chronic inflammatory state, increased risk of age-related disease, tumorigenesis, and shorter lifespan (Baker et al., 2016; Xu et al., 2018).

Experimentally induced accumulation of senescent cell through transplantation or stress-mediated induction can directly draw aging-related physical dysfunction and diseases while *post-mortem* human studies have confirmed such a strong association (Baker et al., 2016; Bussian et al., 2018).

By contrast, removal of senescence cells or the shutdown of pro-senescence pathways delay age-related pathologies and extend lifespan (Baker et al., 2016; Bussian et al., 2018) (see also “Cellular senescence and senotherapeutics: a pharmacological opportunity for extending heathspan”).

Cellular senescent phenotype can be tracked through well-accepted biomarkers (see Table 1).

There are three alternative mechanisms that may trigger senescent transformation and may have distinct phenotypic features (Lopez-Otin et al., 2013; Munoz-Espin and Serrano, 2014).

The replicative senescence is the first type of senescence described and is primarily induced by the age-associated and progressive telomere shortening and attrition taking place over multiple cell cycles. The stress-induced (premature) senescence is caused by either environmental stressors such as ionizing radiation, UV, chemotherapeutic drugs or endogenous factors mainly: oxidative stress (OS), oncogenes, epigenetic dysregulation, mitochondrial dysfunction.

Besides replicative and stress-induced mechanisms of senescence, a third mechanism was demonstrated with a genetically programmed senescence serving as key regulation factor for development pathways such as embryonic morphogenesis, vascular remodeling, and wound healing (Munoz-Espin et al., 2013).

The key process of senescence is represented by the cell cycle arrest which is **mainly** due to a tissue- and organ-specific hyperexpression of p16^{Ink4a}, p15^{Ink4b}, and p21^{Waf1} which are cyclin-dependent kinase inhibitors (CDKI) and key tumor suppressor factors.

Many of the pro-senescent stimuli lead to DDR which in turns induces the upregulation and hyperphosphorylation of p53 which promotes several downstream pathways including transactivation of p21^{Waf1} which has a CDK inhibitory effect that culminates in the hyperphosphorylation of the retinoblastoma tumor suppressor (Lopez-Otin et al., 2013; Munoz-Espin and Serrano, 2014).

The epigenetic deregulation of the Ink4a locus during replicative senescence is associated to hyperexpression of both p16^{Ink4a} and AFR. The latter reinforces the p53-mediated pathways through slowdown of p53 degradation machineries (Munoz-Espin and Serrano, 2014).

Upregulation of p16^{Ink4a}, p15^{Ink4b}, and p21^{Waf1} leads to permanent cell cycle arrest at G1/S transition (Lopez-Otin et al., 2013; Munoz-Espin and Serrano, 2014). The transcriptional activation of p16^{Ink4a} is currently one of the core biomarkers for the *in-vivo* assessment of **replicative** cellular senescence (see Table 1).

Another core feature of senescence is the apoptosis resistance, a phenomenon that has physiological implications for tissue homedynamics but detrimental consequences, in case of overaccumulation of senescent cells. Indeed, apoptosis resistance and pathway underlying its presence have been associated to chemotherapy resistance in cancer (Baar et al., 2017).

Several molecular factors can induce either senescence with resistance to apoptosis or apoptosis with a “dose”-dependent fashion and according to the cell line they originate from (Childs et al., 2015; McHugh and Gil, 2018). A reversible shift from the apoptotic resistant state to the non-resistant one has recently been demonstrated, thus disclosing a promising therapeutic target for **diseases with accumulation of senescent cells** (Baar et al., 2017).

Most senescent cells produce and release the senescence-associated secretory phenotype (SASP), in autocrine and paracrine manner, that is essential for tumour suppression, tissue regeneration and modelling. The SASP induces the local amplification of the senescence program and boosts the intercellular communication through the secretion of chemokines, cytokines, growth factors, metalloproteinase, and other proinflammatory response mediators (Acosta et al., 2013).

Overexpression of the SASP leads to a downstream pro-inflammatory cascade which associates with detrimental chronic inflammation and over accumulation of senescent cells in a self-reinforcing vicious cycle.

Cellular senescence and AD

In the context of neurodegenerative diseases, the attention towards cellular senescence and the related therapeutic potential, has been drawn by the fact that both dividing CNS resident immune cells and post-mitotic neurons develop senescence or “senescent-like” alterations respectively (Cohen and Torres, 2019; Jurk et al., 2012; Stojiljkovic et al., 2019).

Most of the prominent feature of cellular senescence reported for non-CNS cellular lines (see Table 1) match the senescent transformation of neurons and glia cells (Cohen and Torres, 2019; Jurk et al., 2012; Stojiljkovic et al., 2019). Indeed, in the last ten years, several studies characterized neurons, astrocytes, and microglia senescent phenotype the well-accepted markers, including telomere length, telomerase activity, expression of p16, p21, p53, senescence-associated β -galactosidase, and a SASP

However, given the poor anatomical accessibility of the brain, data on the link between cellular senescence and neurodegenerative disease are lacking compared with other medical field such as oncology. To date, whether cellular senescence plays a causal role in neurodegenerative disease is

still largely unknown, however, a rising evidence has shed light on the link between cellular senescence and early pathomechanistic alterations of AD.

First the stem cell exhaustion, an integrative hallmark of aging and a consequence of senescence, constitutes the biological background of the age-related decline in adult hippocampal neurogenesis which in turn is associated with impairment of synaptic plasticity essential for memory learning (see also "Senescence and synaptic aging: state of art") (Lazarov and Hollands, 2016).

Second, rodent model of AD **cellular** senescence and *post-mortem* human studies have shown that senescent microglia (also called dystrophic) is characterized by functional decline and associates (or colocalizes) with neuroinflammatory lesions and accumulation of tau-mediated threads and tangles which are likely to occur prior to neurodegeneration (Streit et al., 2009).

Several studies have demonstrated that p16^{INK4a}-positive astrocytes and microglia surround A β plaques and neurofibrillary tangles supporting the hypothesis of: i) a physiological contribution of astrocytes and glia in ensuring A β and tau proteostasis ii) a central role of brain **cellular** senescence for AD-related proteinopathies (Bussian et al., 2018).

Such findings have also been confirmed in preliminary and encouraging trials with senolytic drugs (see also "**Rejuvenation strategies: a focus on senotherapeutics as a pharmacological opportunity for extending heathspan**").

Senescence triggered by stressors such as OS, toxic cerebral byproducts (including A β and tau) associates with higher levels of SASP compared to replicative senescence. Moreover, animal and human investigations of AD showed that senescent immune cells undergo an upregulation of some cytokines, including IL-6, IL-1 β , TGF- β , and TNF- α that are core components of the SASP, inducing microglia overactivation and astroglyosis and eventually sustaining neuroinflammation, a core pathophysiological process of early preclinical stages of AD (Cohen and Torres, 2019).

Astrocytes and microglia contribute to synaptic homeodynamics therefore overaccumulation of senescent immune cells may account for the aging-related synaptic functional decline (see also "Senescence and synaptic aging: state of art").

Senescence can also occur in another line of non-dividing cells such as oligodendrocytes and their progenitors (OPC) supporting the evidence of an age-related increasing vulnerability to white matter damage as well as of a defective remyelination (Erten-Lyons et al., 2013). Moreover, a fine regulation of the myelination process is likely involved in hippocampal synaptic plasticity suggesting a distinct path whereby senescence may impair memory functions in aging and AD.

A significant spatial relationship between accumulation of senescent OPC and brain A β plaques has been reported in human AD patients as well as in a mouse model of AD, indicating that A β toxic

monomers induce senescent transformation in OPC whose role in AD still remains unclear (Zhang et al., 2019).

Third, endothelial cells of the blood-brain barrier (BBB) undergo senescence which in turn is associated to peculiar anatomical features of BBB breakdown such as, for instance, loss of tight junction with alterations in permeability. Also, the astrocytes senescence plays a role in the BBB dysfunction (Yamazaki et al., 2016). Indeed, astrocyte endfeet-terminated processes interact with endothelial cells of the brain capillaries and contribute to BBB integrity, perivascular homeostasis, crosstalk with the peripheral immune system, endothelial transport. Astroglia also mediates the neurovascular signaling to capillaries (to modulate the vasodilatation) and facilitate, through cell surface aquaporin-4, paravascular pathways for clearance of brain metabolites.

Brain immunosenescence and immunosurveillance: a brief overview on emerging concepts in AD

Regardless the presence of senescent phenotypic transformation, immunosenescence - age-related phenotypical and functional shift / decline of the innate and adaptive immune system - takes place during aging (Nikolich-Zugich, 2018). Immunosenescence itself is characterized by loss of brain homeostasis at multiple levels, including synapses, proteostasis machineries, BBB dynamics, and inflammatory responses (Malavolta et al., 2013; Rawji et al., 2016).

Experimental models of aging as well as in-human findings have recently suggested candidate hallmarks of immunosenescence: i) overall decrease of naïve T cells and circulating B cells (mainly CD19+), ii) accumulations of late-stage differentiated CD8 + memory cells and, decreased CD4+ T-cell, iii) macrophage and neutrophil defects in receptor expression or signaling linked to loss of homeodynamics and functions (including phagocytosis, chemotaxis, and ROS production), iv) a systemic low-grade inflammatory state (ultimately called inflammaging), v) phenotypical alterations of NK cells (including protein receptors expression), vi) lymphocytes and myeloid cells senescent transformation vii) lymphocytes impaired cytokine-responsiveness, viii) lymphocytes (T, NK, and NKT) lack of functional cytotoxicity (Baar et al., 2017; Nikolich-Zugich, 2018; Ovadya et al., 2018; Rawji et al., 2016; Stervbo et al., 2015a, 2015b).

An age-related deficit in immunosurveillance, - a complex mechanism whereby the immune system recognizes (pre-)malignant or senescent cell motifs and promote their suppression - has been reported (Ovadya et al., 2018).

Immunosenescence and loss of immunosurveillance are associated with decline of brain homeodynamics since, for instance, lymphocytes and myeloid cells derived interferon- γ , IL-4,

BDNF, among others are crucial effectors for social behavior and memory learning (Baruch et al., 2014; Meyer-Luehmann and Prinz, 2015).

Immunosenescence is a key feature of AD experimental models and recent explorative studies have also shown it occurring in AD human patients (Larbi et al., 2009; Meyer-Luehmann and Prinz, 2015; Pellicano et al., 2012).

The causal nexuses of brain immunosenescence are still poorly understood in humans. However, it has been shown that aging is associated with altered microglia functions and with an imbalance of brain-homing peripheral immune cells that populate meninges and choroid plexus (lymphocytes and dendritic cells) and form a direct interface with the brain parenchyma (Baruch et al., 2014; Li et al., 2018; Meyer-Luehmann and Prinz, 2015). Alterations of brain T cells subpopulation ratio and cytokines secretory patterns as well as priming (hyperactivation with sustained pro-inflammatory activity) of dysfunctional microglia have increasingly been recognized as key features of immunosenescence during early stages of neurodegenerative diseases (Baruch et al., 2014; Li et al., 2018; Meyer-Luehmann and Prinz, 2015).

Notably, recent Omic-based studies have indicated that microglia age differently from peripherally-derived macrophages in either healthy condition or disease (Malavolta et al., 2013; Rawji et al., 2016).

Moreover, since infiltrating peripheral immune cells contribute to brain homeostasis and immunosurveillance and that such cells go towards an age-related functional decline, a causative role of the peripheral immune for brain immunosenescence has also been hypothesized (Malavolta et al., 2013; Rawji et al., 2016).

AGING, CELLULAR SENESCENCE, AND AD: THE ANTI-STRESS RESPONSE

Molecular and cellular mechanisms of adaptation and their decline during aging

To date, molecular, cellular and brain tissue homeostatic mechanisms have been explored more than higher complexity levels. Sub cellular / cellular adaptation refer to properly coordinated molecular machineries needed to face and prevent potential stress-induced damage, thus providing functional and bioenergetic stability for the whole organism and finally ensuring homeostasis. Cellular homeostasis is ensured by reprogramming bioenergetic / metabolic performance through: 1) proteostasis networks (mechanisms quality control, from protein synthesis to protein degradation) (Kaushik and Cuervo, 2015; Labbadia and Morimoto, 2015), and 2) highly conserved cellular transduction pathways, i.e. multiple intracellular regulatory signals acting at the (epi)genetic, pre and post translational level and ensuring preservation of core process including proteostasis, oxidative balance, cellular trafficking (Kaushik and Cuervo, 2015; Labbadia and Morimoto, 2015).

Proteostasis networks and age-related decline: general concerns

There is a complex interplay consisting of highly conserved feedback loops and cross-talks between an array of quality control mechanisms and protein clearance pathways across cells, tissues, and body systems. It has been demonstrated that proteostasis machineries and the related hierarchical organization across tissues and body systems decline with an age-related and tissue-specific fashion. These mechanisms can alter the entire network through a self-reinforcing detrimental downstream cascade which in turn accelerates aging (Kaushik and Cuervo, 2015; Labbadia and Morimoto, 2015).

Studies conducted in model organisms including nematodes (*Caenorhabditis elegans*), *Drosophila melanogaster*, and mice indicate that the capability to adapt through the maintenance of proteostasis is tightly associated with both lifespan and healthspan (Kaushik and Cuervo, 2015; Labbadia and Morimoto, 2015).

Experimental evidence suggest that the protection and preservation of the core properties of each proteostasis network as well as the reciprocal modulation among several machineries can significantly extend longevity (Kaushik and Cuervo, 2015; Labbadia and Morimoto, 2015). The understanding of how gradual mechanistic perturbation of proteostasis occur during aging is of widespread interest and now feasible through comparative gene expression-proteomic network

analysis and emerging high-throughput approaches. In Table 2 and Figure 3, we illustrate the prominent alterations that are likely to occur in aging

We expect that systems biology approach will help confirm and quantify such observations in neurons and neuronal circuits in reliable experimental models and even human individuals.

The anti-stress transduction signals along the dimensions of aging and neurodegeneration

Cellular stress-responsiveness consists of multiple evolutionarily conserved and highly-organized biological pathways (cellular stress response network). The whole organ systems rely on the efficiency of these regulatory signals which elegantly modulate gene expression patterns, epigenetic processes, DRR activity level, proteostasis, transmembrane transport and vesicles trafficking dynamics, microtubule stability and cytoskeletal remodeling, lipid membrane organization, mitochondrial biogenesis and turn over, bioenergetic / metabolic cycles and resources storage (Cole, 2012; Harrison et al., 2009; Mair et al., 2011). There is an elegant and complex interplay among transduction pathways, from ligand-receptor binding to downstream effector(s) activation, in which several pleiotropic master orchestrators modulate the biological output through up-down regulation of cross-talks and feedback / feedforwards loops.

The dysregulation (either hypoactivation or hyperactivation) of several distinct pathways has been associated with brain aging and to the increased risk of developing neurodegenerative diseases. In the case of AD, two pathways seem to be tightly linked to the disease etiology and pathophysiology: the mammalian target of rapamycin (mTOR) and the glycogen synthase kinase-3 β (GSK3 β).

mTOR is a nutrient-sensing cellular pathway activated by several cues as nutrients, hormones, growth factors and external stressors. mTOR is a central regulatory node upstream of the transcriptional regulation of autophagy shifting cellular metabolism to anabolic processes, including mitochondrial biogenesis (Kaushik and Cuervo, 2015; Labbadia and Morimoto, 2015) see Figure 4. mTOR complex 1 constitutively slows down autophagic processes and is activated by environmental nutrients (for example, amino acids) (Herranz et al., 2015). By contrast, mTOR is inhibited by sensors of cellular caloric restrictions such as by AMP-activated protein kinase (AMPK), a key orchestrator of the metabolic changes of cellular senescence. Overall, mTOR is upregulated in cellular senescence and plays an essential role for the production of the SASP (Herranz et al., 2015). GSK3 β is abundantly expressed in the CNS particularly in the hippocampus, where several physiologic processes such as neurogenesis (the process of generating functional neurons from precursors), neurotransmission and synaptic transmission and plasticity are tightly

linked to its physiological activity. GSK3 β has been linked to core pathophysiological processes occurring in AD such as tau hyperphosphorylation, A β overproduction, neuroinflammation and inhibition of cytoprotective signals leading to neuronal and synaptic loss (Beurel et al., 2015; Cole, 2012).

It has become clear that all the pathomechanistic alterations occurring in AD are generated by the aberrant regulation of physiological multiple transduction pathways leading to a loss of homeostasis. For instance, hyperphosphorylation of truncated proteins such as tau and A β is likely to result from an imbalance between phosphatases and kinases (such as GSK3 β) (Beurel et al., 2015; Cole, 2012). However, whether hyperphosphorylation occurs after or before misfolding and accumulation and which is the exact temporal sequence of this and other post-translational modifications has to be fully mapped yet.

Answering to this question would have a critical role in developing (multi-)targeted, upstream pathway-based therapies for preventive strategies.

The role of Calcium homeodynamics in cellular senescence, aging, and AD

Calcium and its downstream mediators - i.e. all Calcium-dependent enzymes including calpain, protein kinase C, and Calcium/calmodulin-dependent protein kinase - are essential, in neurons, microglia and astrocytes, for neurotransmission, the fine regulation of neural and synaptic plasticity (synapses/dendrites/terminals growth *versus* regression), synaptic adaptation, and overall synaptic homeostasis (Demuro et al., 2010; LaFerla, 2002; Yu et al., 2009).

The upstream role of neuroexcitability and Calcium dysregulation, in aging and AD, have been underestimated for long time even though a Calcium hypothesis of AD was proposed in 1994.

The upstream triggers and drivers of Calcium dysregulation have not completely untangled yet, especially in humans.

Also the role of Calcium in cellular senescence is not fully described, however, a recent *in-vitro* trials showed that treatment of senescent cells with Calcium chelators is associated with upregulation of autophagic pathways (whose dysfunction is associated to cellular senescence) and inhibition of senescent transformation under OS (Borodkina et al., 2016). Such study has indicated that Calcium intracellular levels indirectly contribute to the cellular transition towards a senescent state under exposure to OS. Previous studies showed that intracellular Calcium fluctuations modulate second messengers' activity in pathways associated with either replicative or other types of stress-induced senescent transformation.

At a more biologically complex level, Calcium dysregulation along with astroglia senescence are associated with BBB breakdown dysfunctions since Calcium transients in astrocyte endfeet are essential for the fine regulation of the vessels tone, and consequently the cerebrovascular blood flow and BBB permeability, which is essential for homeodynamics of circulating byproducts (Mulligan and MacVicar, 2004).

Moreover, accumulating evidence, have demonstrated that an initial perturbation of cellular Calcium homeodynamics can be both caused and downstream affect intracellular Calcium-harboring organelles such as the ER, mitochondria, lysosomes, and autophagosomes, which are involved in proteostatic pathways, membrane transport dynamics (including mitochondria), vesicles trafficking. As reported in previous sections, the above mentioned subcellular compartments have a critical role in anti-stress responses and cellular adaptation (Demuro et al., 2010; LaFerla, 2002; Yu et al., 2009).

Hence, Calcium dyshomeostasis is likely to play a crucial and upstream role in early AD-related pathomechanistic alterations.

Animal models of AD have indicated that Calcium dysregulation is characterized by elevated intracellular Calcium levels which might be due to: i) increased Calcium influx through L-type voltage-gated Calcium channels (VGCCs), ii) enhanced Calcium release from intracellular stores through Inositol 1,4,5-Trisphosphate receptor (InsP3R) and the ryanodine receptor (RyR), iii) reduced Glutamatergic N-methyl-D-aspartate (NMDA) receptor-mediated effect, iv) impaired ability of mitochondria to buffer or cycle Calcium, v) reduced cytosolic Calcium buffering capacity and activation of calcineurin and calpains (Demuro et al., 2010; LaFerla, 2002; Yu et al., 2009). Recent microarray-based trials, Genome-Wide Association Studies (GWAS), and gene expression profiling investigations indicated Calcium-linked susceptibility genes such as CLUSTERIN, ITPKB, RGS4, RAB3A, STMN1, SYNGR3, PICALM, RYR3, MRVI1, RGN, APOC2, AMY2B, and APOE (among others) may account for Calcium dysregulation as the causal nexus of AD and other neurodegenerative diseases (Saetre et al., 2011).

A long-lasting perturbation in intracellular Calcium-regulating systems is a candidate signature of aging. Biomarkers to track *in vivo* Calcium dynamics are currently under development in rodent model of aging and AD. In humans, brain-derived exosomes and metabolomics-based signatures seem promising *in-vivo* approaches (Mustapic et al., 2017; Varma et al., 2018).

In line with our hypothetical model of systems-wide aging processes, we argue that a Calcium imbalance hampers intracellular signals which in turn may affect the overall neural and synaptic plasticity until large-scale networks activity, even potentially before any cellular bioenergetic

failure, loss of homeodynamics, and neuronal damage occurs (Demuro et al., 2010; Mustaly-Kalimi et al., 2018; Yu et al., 2009).

Calcium dysregulation has been demonstrated to potentially occur upstream to brain proteinopathies with A β monomers production and tau hyperphosphorylation that can be triggered by sustained increase of cytosolic Calcium concentrations over basal levels (Demuro et al., 2010).

In particular, abnormal intracellular levels of Calcium may induce cytosolic pH fluctuations with downstream modification of protein thermodynamic core properties such as free surface energy, which are key determinants of proper folding process (Demuro et al., 2010). Moreover, Calcium plays a crucial role in A β -mediated synaptic dysfunction and neuronal loss since A β oligomers exert neurotoxicity through over activation of intracellular Calcium-release channels, massive Calcium influx which elicits OS, and mitochondrial dysfunction in a self-perpetuating cycle which culminates in pro-apoptotic signals (Demuro et al., 2010).

Calcium-dependent signaling pathways are crucial for hippocampal synaptic function and plasticity. Age-related Calcium-systems dependent alterations are likely to be responsible for aberrant neuronal and glial excitability causing long-term potentiation (LTP) and long-term depression (LTD) threshold shifts (see Figure 3 for more details). These alterations reflect those neural desynchronization and abnormal spatial and temporal brain networks oscillatory activity found in experimental aging models and which may account for the age-related risk of epilepsy, neuropsychiatric disorders and neurodegenerative disease including AD (Demuro et al., 2010; Yu et al., 2009).

Given the crucial role of Calcium in AD etiology and pathophysiological evolution, a sustained research into the therapeutic perspectives of restoring Calcium homeostasis is demanded.

From a pharmacological standpoint three upstream mechanisms accounting for cellular loss of Calcium homeostasis, have gained increased interest in recent years: the ER stress-response (see Table 1 for more details), ryanodine receptor (Lacampagne et al., 2017) and the Sigma 1 receptors (S1R) (Hayashi and Su, 2007; Mori et al., 2013)

Alongside mitochondria, ER is the most important Calcium buffering organelle playing a constitutive role in maintaining resting Calcium levels into cytosolic space. The ER membrane Calcium channel receptors, InsP3R and the RYR are activated by low Calcium contractions and promote its release into the cytoplasm. It has been demonstrated that aberrant post-translational remodeling of the RYR channels is associated with abnormal leak of Calcium and downstream to AD-related pathophysiological processes (Lacampagne et al., 2017).

The S1R is a ligand-regulated transmembrane protein localized to mitochondria-associated ER membranes/interface and is finely regulated by ER-mediated calcium dynamics (Hayashi and Su,

2007; Maurice and Su, 2009; Mori et al., 2013). Therefore, S1R are crucial molecules in the ER stress response, in the subcellular cross-talks between ER and mitochondria and play a key role in the calcium mobilization and homeostasis (Hayashi and Su, 2007; Maurice and Su, 2009; Mori et al., 2013).

The full understanding of the physiological mechanism of S1R is not achieved yet, however, it has been shown in animal models of AD, that under sustained ER stress activation, S1R 1) promote an increase of the mitochondrial calcium influx and 2) after translocation, may serve as enhancer of calcium-dependent intracellular transduction pathways, including phospholipase C and protein kinase C which in turn finally stimulate calcium homeostasis (Hayashi and Su, 2007; Maurice and Su, 2009; Mori et al., 2013). Stimulation of S1R is associated to significant inhibition of the NMDA receptor activity finally ameliorating dendritic spines and axonal sprouting, i.e. neural plasticity (Hayashi and Su, 2007; Maurice and Su, 2009; Mori et al., 2013).

For its large *spectrum* neuroprotective effect due to an extensive capacity to modulate intracellular calcium shuttling, S1R have been increasingly recognized as novel main candidate targets to prevent or delay AD-related pathophysiological processes.

SYNAPTIC AGING: the primary pathophysiological node of AD is the failure of synaptic networks.

Brain aging may associate with a decline in synaptic functions and overall robustness (Morrison and Baxter, 2012; Zhou et al., 2004) (for which we propose the term of synaptic aging). An age-dependent slowdown of the synaptic homeodynamics, including Calcium dysregulation, OS, and cellular senescence-related decline of adaptation is more likely to occur in some brain regions such as the neocortex which display increased synaptic vulnerability over time.

Indeed, it is well established that key brain networks are composed by neuronal subpopulations with a constitutive bioenergetic and electrophysiologic properties serving at specific physiological functions. For instance, the DMN neurons show a high rate of basal metabolic performance is coupled with low energy reserves and higher intrinsic stress levels which confers to them an intrinsic high degree of vulnerability to stressors compared with resistant neurons (Arendt, 2005; Bero et al., 2011; Cirrito et al., 2005; Machulda et al., 2011). Hence, a long-lasting synaptic overactivity is associated with a bioenergetic demand that can increase the risk of OS, Calcium dysregulation, and genotoxicity.

The DMN (among other networks) displays highly active connectivity dynamics that ensure flexible pattern re-organization and plastic remodeling (synaptic network compensation). On the flipside, over boosted compensation may drive the exhaustion of cellular resources which ultimately

escalates to failure of molecular, cellular and system itself (Hatanpaa et al., 1999; Masliah et al., 1993; Morrison and Baxter, 2012; Palop and Mucke, 2016).

Translational findings have shown that non-neurodegenerative age-related cognitive decline is tightly linked to subtle synaptic alterations which may evolve into synaptic failure despite the absence of protein-mediated neurotoxicity, and neurodegeneration (Arnold et al., 2013; Hatanpaa et al., 1999; Masliah et al., 1993; Morrison and Baxter, 2012; Thiagarajan et al., 2005). To date, there is little insight regarding causal mechanisms between dysfunction of established vulnerable brain regions, such as those involved in the default mode network (DMN), and proteinopathies or neurodegeneration (Bero et al., 2011; Koch et al., 2010).

In spite of the presence of toxic proteins accumulation with evidence of initial neural dysfunction, individuals may display normal cognitive function suggesting that synaptic dynamics from the subcellular compartments to the synaptic cleft until large-scale connectivity networks (i.e. the dynamic integration and coordination of different functional areas of the brain), may underlie resilience compensating preclinical pathophysiological alterations (Cirrito et al., 2005; Franzmeier et al., 2018; Hatanpaa et al., 1999; Husi et al., 2000; Palop and Mucke, 2016; Perez-Nievas et al., 2013; Westlye et al., 2011).

From a functional standpoint, it has been proposed that “synaptogenic” mechanisms of AD-related cognitive decline, i.e. network activation and deactivation deficits, abnormal oscillatory rhythmic activity and network hypersynchrony have all recognized as key synaptic functional alteration that may account for AD-related synaptic failure (Palop et al., 2007; Palop and Mucke, 2016; Westlye et al., 2011)

For next-generation human studies on aging, resting-state and task related functional magnetic resonance studies (fMRI) is the gold standard technique to untangle signatures of synaptic aging at the large-scale network level (for instance the age-related decline of functional connectivity inside the DMN) as well as synaptic compensation (or synaptic resilience). Moreover, emerging hybrid techniques such as electroencephalogram (EEG) - or magnetoencephalogram (MEG) - fMRI, may provide the proper spatial and temporal frames to explore *in vivo* stress responses and short- long-term biological-neural compensation (Niessing et al., 2005; Scheeringa et al., 2009).

In this regard, some relevant insights have already been accomplished. fMRI studies have demonstrated that hippocampal and prefrontal hyperactivity may represent compensation of genetic or pathomechanistic predispositions that increase the risk to develop AD (Franzmeier et al., 2018; Putcha et al., 2011). Moreover, compensatory mechanisms seem to manifest over a connectivity anterograde shift as well, which is characterized by a functional slowdown in the posterior midline

cortex along with increased activity in medial frontal cortex (Davis et al., 2008; Koch et al., 2010; Park and Reuter-Lorenz, 2009).

Very recently, for the first time, it has been shown that genetic risk factors with a pleiotropic biological effect such as the APOE ϵ 4 allele impact the trajectories of the DMN changes over time in a cohort of cognitively healthy individuals at risk for AD (Chiesa et al., 2019).

In summary, we believe that it is of primary importance to identify the multilevel organization of resilient mechanisms, including synaptic compensation since, it has been shown that tuning some critical factors accounting for brain network dynamics, either in homeostasis or early decompensation, can improve network functional output and physiological properties, i.e. cognition and or behavior.

Moreover, overexpressed synaptic aging features and the related functional failure have been largely reported in experimental models of AD even in absence of brain proteinopathies, pathomechanistic expression of neurodegenerative processes, and clinical manifestations, suggesting a highly upstream role of synaptic homeostasis in AD pathophysiology.

State of art on established and candidate molecular- cellular- tissue-based biomarkers including cellular senescence

The *in-vivo* exploration of biomarkers of synaptic aging and synaptic failure is challenging because of the anatomical inaccessibility of the brain and the lack of a systematized approach.

Shrinking of dendritic spines is an established typical feature of synaptic aging (Morrison and Baxter, 2012; Zhou et al., 2004).

However, the primary features of synaptic aging and even more AD-related synaptic failure may encompass: selective (mainly hippocampal and prefrontal) imbalance in axonal remodeling (sprouting *versus* pruning), depletion of axonal boutons / dendritic spines / synaptic terminals with overall loss of synaptic density. Shrinking of dendritic spines is established as a typical feature of synaptic aging (Morrison and Baxter, 2012; Zhou et al., 2004).

At the-ultrastructural level, some highly frequent features of synaptic aging have been reported: (i) impairment of both post-synaptic and astrocytic-mediated and re-uptake; (ii) deficits in transmembrane transporter expression; (iii) abnormal binding to ligands; (iv) compromised cellular vesicle trafficking of neurotransmitters; (iv) decrease of synaptic proteins involved in neural adaptation and structural plasticity (remodeling) of axons and dendrites, (v) receptor profile shifts such as decrease of hippocampal NMDA (Hatanpaa et al., 1999; Husi et al., 2000; Morrison and Baxter, 2012; Piacentini et al., 2017).

All these alterations are associated with hippocampal neuronal hypoexcitability with stimulation thresholds for LTP induction shifted to higher frequencies.

The molecular drivers of such synaptic alterations are not completely elucidated yet, especially in humans. However, recent GWAS-based findings have indicated that the loss of synaptic plasticity is the core of AD pathophysiology and that a (epi)genetically driven intracellular signaling, may draw the synaptic failure (Dourlen et al., 2019).

Regarding the relationship between cellular senescence and synaptic decline in aging and AD, very recently, studies employing innovative cellular profiling approaches have delivered crucial insights. Hippocampal neurogenesis and neural plasticity are essential for memory learning and resilience to stress-induced neuroendocrine and behavioral impairment (Lazarov and Hollands, 2016). It has been demonstrated in humans that a decrease of adult hippocampal neurogenesis distinguishes the ventral dentate gyrus of AD patients compared to age matched cognitively normal individuals. Robust evidence indicated that the stem cells exhaustion, an integrative hallmark of aging and consequence of cellular senescence, is mechanistically linked to the impairment of adult hippocampal neurogenesis in brain aging and even more in AD (Lazarov and Hollands, 2016; Moreno-Jimenez et al., 2019).

Moreover, senescence may impact the quad-partite model of synapsis consisting of an axon terminal, a dendritic spine which is simultaneously contacted by an astrocytic and microglial process. Astrocytes and microglia are essential for CNS development and synaptic homeodynamics during adulthood. The former promotes synaptogenesis (axonal and dendritic spines sprouting), regulates the synaptic strength, take part in the spatial-temporal integration of multiple synaptic processes and modulate the neurotransmission. Microglia plays a crucial role for immune surveillance of the presynaptic microenvironment as well as for the synaptic remodeling towards dendritic and axon terminals pruning through remodeling of proteolytic and phagocytic mechanisms. The microglia can either recruit astroglia or being recruited by the latter (Cohen and Torres, 2019; Jurk et al., 2012; Stojiljkovic et al., 2019).

Senescent glial cells may draw the regional synaptic vulnerability *versus* regional synaptic resistance (for which we would suggest using the term “synaptic robustness”). Indeed, a recent study carried out *via* comparative proteomic profiling coupled to *in-silico* analyses have shown that p16^{INK4a}-positive astrocytes microglia are highly expressed in prefrontal cortex of AD subjects compared with non-AD individuals. Moreover, peculiar patterns of SASP composition, i.e. TGF- β in the case of senescent microglia, seems to guide a regionally selective age-related synaptic vulnerability (Bhat et al., 2012; Graham et al., 2019). Further studies are urgently need to fill the

gap in evidence regarding the link between **CNS-resident cells senescence** and synaptic dysfunction throughout aging and AD.

Further investigations are needed to unravel the dynamics between the senescence of CNS immune system resident cells and the synaptic shutdown occurring in aging and AD.

Synaptic aging: neurochemical compensation

Several neuropathological studies have provided crucial information on the synaptic compensation that may take place over the course of AD along its *continuum*. A few neurochemical remodeling occur during the progressive depletion of basal forebrain (BF) cholinergic neurons associated with a subsequent anterograde cortical cholinergic deafferentation particularly involving hippocampal and prefrontal regions (DeKosky et al., 2002; Ikonomic et al., 2007; Poirel et al., 2018). However, the progressive rate of functional impairment affecting large parts of the cholinergic system shows a nonlinear temporal and spatial evolution. Indeed, both *post-mortem* and *in-vivo* human studies have demonstrated that either cognitively normal or mild cognitive impairment individuals displaying AD-related pathophysiology, may have developed compensation for synaptic resilience. In these subpopulations, a regional hyperregulated choline acetyltransferase (ChAT) activity has been reported (particularly in the frontal cortex and in hippocampal) as well as a remodeling of BS cholinergic cortical projections (cholinergic axonal sprouting in hippocampi) (DeKosky et al., 2002; Ikonomic et al., 2007; Poirel et al., 2018). The degree of these pathomechanistic changes matches the rate of BF cholinergic neurons depletion. These mechanisms may compensate for impaired cholinergic firing, contributing in the maintenance of physiological synchronization of neural oscillations which is a key event for cortical and subcortical networks, particularly those regarding memory and learning (DeKosky et al., 2002; Ikonomic et al., 2007; Poirel et al., 2018).

Given the primary role of the cholinergic system in AD, it is essential to highlight that ACh projections may trigger several evolutionary highly conserved functional synergistic effects among different neurochemical systems involved in cognition and behavior (Higley et al., 2009; Poirel et al., 2018; Salamone et al., 2014). Such complex interactions seem to be fundamental for ensuring overall synaptic robustness and resilience. For instance, surface N receptors of astrocytes activate Ca²⁺ - mediated intracellular signaling essential for the spatial-temporal synchronization of astrocytes with the hippocampal LTP. Moreover, astrocytic and microglial N receptors activate autophagic processes, glutamate and serotonin synthesis and re-uptake as well as modulating neuroinflammatory response (Guerra-Gomes et al., 2017; Higley et al., 2009; Takata et al., 2011). It

has been shown that a remodeling of N astrocytic and microglial cell surface may occur during age-related gliopathy which can compensate for all the important physiological processes that involve glial cells (Guerra-Gomes et al., 2017; Higley et al., 2009; Takata et al., 2011).

Even the noradrenergic system undergoes compensatory remodeling during aging and given the widespread noradrenergic cortical projections the catecholaminergic system plays a prominent role in AD. It is well known that an age-related and pathological pre-cortical tau-deposition in the LC occurs (pre-tangle stage of AD) (Andres-Benito et al., 2017; Chalermpanupap et al., 2013). A progressive noradrenergic denervation is associated with higher expression levels of hippocampal and amygdala $\alpha 2A$ post-synaptic receptors which may represent a compensatory mechanism since stimulation of $\alpha 2A$ promotes dendrite sprouting (Andres-Benito et al., 2017; Chalermpanupap et al., 2013). Moreover, a progressive slowdown of cortical levels of noradrenaline is likely to be initially counterbalanced by upregulation of both tyrosine hydroxylases and NA transporter binding sites gene expression (Andres-Benito et al., 2017; Chalermpanupap et al., 2013). A cholinergic modulation of dopamine circuits has also been reported during aging and even more pronounced in AD where it may associate with typical pattern of dopaminergic-related synaptic plasticity (Higley et al., 2009).

OPEN ISSUES: overcoming a priori hypotheses and exploring the whole aging narrative

The road towards the possibility to dynamically track *in-vivo* aging within a systems-level integration paradigm poses considerable methodological challenges.

However, some conceptual crucial issues need to be addressed before setting up a blueprint for exploring aging applying a system-wide integrative approach.

1) The perspective of a ‘remote control’ of brain proteostasis **and immunosurveillance** through extra-CNS compensatory mechanisms (peripheral compensation) calls for **a paradigm shift: from a CNS-centric to a body-centric disease framework.**

Network analysis studies have shown that highly conserved cross-talks underlie the interactions between tissue-specific and ubiquitous housekeeping proteins. Experimental models of aging demonstrated that the modulation of this output can be mastered from either side the tissue specific interactome or the whole-body proteome components. These observations corroborate the existence of a complex systems-scaled proteostasis network which may synchronize and coordinate the proteome with a tissue specific manner (Labbadia and Morimoto, 2015; Wang et al., 2017).

In the context of neurodegenerative brain proteinopathies, recent systems biology studies have demonstrated that peripheral proteostasis machineries can compensate for incipient cerebral A β misfolding and toxic accumulation (Labbadia and Morimoto, 2015; Wang et al., 2017). On the flipside, the decline of peripheral protein clearance pathways is associated with higher risk of developing brain amyloidosis (de Leon and Blennow, 2018; Labbadia and Morimoto, 2015).

Discovering a reliable open-access to the brain is an essential step to overcome the high degree of inaccessibility of the brain to pharmacological therapies (Wang et al., 2017; Sala et al., 2017).

Approaching proteostasis from a systems biology perspective would allow to targeting the whole body systems-wide effect that a single aberrant proteostasis machinery may originally induce in a certain tissue (Bossi and Lehner, 2009; Miller-Jensen et al., 2007).

In Oncology, a peripheral approach to proteostasis is currently under experimental development (Bossi and Lehner, 2009; Miller-Jensen et al., 2007; Labbadia and Morimoto, 2015)

Moreover, recent human studies reported that medical procedures such as peritoneal dialysis may promote peripheral clearance of neurotoxic protein facilitating brain protein homeodynamics (de Leon and Blennow, 2018; Labbadia and Morimoto, 2015).

As further step, the system-scaled approach to aging can definitively corroborate (or even disprove) results from animal models of AD indicating A β -mediated key physiological signaling which is essential for modulating cellular trafficking, apoptosis, cholesterol homeostasis, neurogenesis, and angiogenesis (Abramov et al., 2009; Morris et al., 2018; Zimbone et al., 2018; Dourlen et al., 2019; Panza et al., 2019).

A systems-scaled approach is also required to untangle the multiple molecular cross-talks at the innate immune system-brain interface which may be therefore used for a peripheral rejuvenation of brain microglia eventually enhancing and sustaining immunosurveillance. In this context, studies conducted on mice models of AD have shown that the migration of bone marrow-derived cells into the CNS is associated with partial recover of brain damage in presence of brain proteinopathies and early neurodegeneration (Malavolta et al., 2013; Rawji et al., 2016). In humans, the proof-of-mechanism for rejuvenation strategies to enhance brain immunosurveillance has not been established yet (Malavolta et al., 2013; Rawji et al., 2016).

Moreover, studies based on bone marrow and parabiosis chimeras, as well as novel transgenic mice, have supported emerging rejuvenation strategies employing systemic (blood) factors, metabolic manipulations (such us manipulation of the host microbiota), and cellular reprogramming. All of these novel approaches can represent a pursuable path for preventing neurodegenerative diseases (Erny et al., 2015; Katsimpardi et al., 2014; Villeda et al., 2011).

2) The aging process as well as the individual thresholds of resilience / venerability are

(epi)genetically driven.

Genome-wide association studies (GWAS) are contributing to map the main genetic factors associated with increased risk of AD-related pathomechanistic alterations, thus decompensated aging.

APOE ϵ 4 allele is still the prominent genetic risk factor of late onset AD accounting for several pathophysiological processes not fully understood yet. It is well known that APOE plays a key role in cellular lipid turn-over, influencing membrane lipid raft composition. Lipid homeodynamics is fundamental for neurotransmission and synaptic plasticity (Bott et al., 2016; Seeley, 2011).

APOE ϵ 4 allele is associated with defective compensatory mechanisms, including the cholinergic hippocampal compensatory sprouting while cortical cholinergic deafferentation progresses (Bott et al., 2016). Moreover, distinctive large-scale brain network patterns are related to the APOE ϵ 4 allele (Bott et al., 2016; Seeley, 2011) in cognitively healthy individuals. Particularly, APOE ϵ 4 carrier individuals show specific and consistent features in the DMN connectivity, including decreased connectivity in the middle and posterior regions and increased connectivity in the frontal and lateral structures (Chen et al., 2015; Machulda et al., 2011; Westlye et al., 2011). Whether these features reflect compensatory patterns recruited to counterbalance the loss / gain of function of ApoE ϵ 4 has to be elucidated yet.

Several single nucleotide polymorphisms (SNPs) in different genes, related to distinct molecular pathways and intracellular signals, have been identified as upstream drivers of vulnerability to AD: PCALM, BIN1, CR1, CLU, SORL1, RAGE, AQP4, among others (Medland et al., 2014; Nho et al., 2013; Parikh et al., 2014; Wang et al., 2016).

Gene co-expression network analysis bridged to high-throughput RNA sequencing have provided evidence that different polymorphic variants of the same genes may be associated with either gain or loss of function of downstream interactomes with consequent detrimental or protective effect (Bashan et al., 2012; Padmanabhan et al., 2017; Starnawska et al., 2017). This observation has raised the intriguing and unresolved question if specific gene-gene interactions, gene encoding editing, transcriptional and splicing dysregulation may account for downstream molecular cross-talks which affect the whole network function (Johnson et al., 2018; Starnawska et al., 2017; Hidalgo et al., 2009; Mills et al., 2013; Rolland et al., 2014).

Also, cellular senescence itself is a complex, non-linear pre- post- transcriptional dysregulation of key genes.

We expect that multiplex omics-based single cell approach bridged with network analysis methodologies may soon provide a reliable and accessible blueprint for tracking the genetic, epigenetic, and downstream molecular underpinnings of aging and disease vulnerability versus

resistance, at the individual (Blokland et al., 2011; Glahn et al., 2010; Miller et al., 2008).

3) Adaptation and resilience are influenced by sex-biased biological factors. Therefore, it is conceivable that a systems-wide sexual dimorphism exists throughout aging (Ferretti et al., 2018; Hampel et al., 2018c). In the context of AD, a higher vulnerability of women to AD is well-known (Ferretti et al., 2018; Nyarko et al., 2018).

It is well established that highly conserved sex-biased genes and gene expression patterns underlie the sexual dimorphism through lifespan, including aging (both under physiological and pathophysiological condition) (Parsch and Ellegren, 2013; Penaloza et al., 2009; Reinius et al., 2008). The sexual dimorphism is associated with: a) different genetic architecture, b) sex-biased gene expression, c) sex-related regulatory mechanisms of gene-gene and gene–environment interactions (Parsch and Ellegren, 2013; Penaloza et al., 2009; Reinius et al., 2008; Ferretti et al., 2018). It is not understood yet whether genetic factors closely associated with sex are modifiers or primary determinants of the higher intrinsic vulnerability to AD that women express (Parsch and Ellegren, 2013; Penaloza et al., 2009).

Recently, it has been shown that sexual dimorphism impacts upstream regulator of disease hallmarks, including brain A β accumulation. For instance, an estrogen effect on the expression and activity levels of β -secretase 1 (BACE1). A sexual dimorphism exists also at the synaptic and network connectivity level (Ferretti et al., 2018; Hampel et al., 2018c).

Consistent with our theoretical model of systems-wide aging, it is expected to discover that the sex-biased genetic and biological background is coupled with sex-related differences across multi-level systems until large scale network organization, i.e. sex-biased endophenotypes of aging and AD (Parsch and Ellegren, 2013; Penaloza et al., 2009; Ferretti et al., 2018).

We assume that underestimation of the impact of sexual dimorphism on AD pathophysiology may partially explain the failure of previous clinical trials which did not assign sex-biased dosage of active treatment nor analyzed outcomes in a sex-wide manner.

Untangling distinctive weakness to stressors and identifying sex-related intrinsic deficiencies in adaptive and compensatory dynamics is expected to be a key step to achieve individualized therapies aimed at strengthening brain and body robustness over-time (Ferretti et al., 2018; Hampel et al., 2018c).

4) Time and space coordinates are differentially scaled within body systems as well as brain networks. The non-linear integration of multiple processes with peculiar spatial-temporal scales is necessary for any physiological output such a cognitive task (Buzsaki and Llinas, 2017). Aging and AD related dynamics, including compensatory mechanisms and network(s) failure, are highly dependent on the system levels (space) and the temporal context they are operating in.

The missing step for the comprehensive understanding of aging, resilience, and AD pathophysiological evolution is the accomplishment of a quantitative and flexible integrative multi-level modeling to detect multiple spatial-temporal patterns and for eventually dissecting causal mechanisms and downstream cascades (Hermans et al., 2011).

Implementing the computational analysis with brain / body system space and time coordinates will also help identify those primary disease-modules that significantly contribute to decompensatory cascades throughout the aging-AD *continuum* (Bartsch et al., 2015).

For instance, whether the neuronal bioenergetic failure associated with loss of cellular homeostatic dynamics is upstream or downstream to large-scale network desynchronization and the uncoupling of neural oscillator is likely to be predict by incoming next generation of *in-silico* modeling (Bartsch et al., 2015; Bashan et al., 2012; Bassett and Sporns, 2017; Miller et al., 2008; Shine et al., 2019).

Thus, predictive and disease-modifying strategies aimed at tuning and enhancing resilience-prone factors shall be able to match the different space scales and time windows that are disease-relevant.

PERSPECTIVES

Next steps along the roadmap for deciphering aging-related dynamics underlying the transition to AD, is the standardization and harmonization of a protocol for an accurate detection and profiling of CNS resident cells senescence (a suitable target for senotherapeutic) and then mapping the key drivers of brain adaptive responses and compensatory mechanisms, at the systems-level integration. A general consensus on the panel of markers of cellular senescence has not been achieved yet because of methodological limitation to discriminate between the several mechanisms and stimuli that may trigger the cellular program responsible for the phenotypic and functional transitional shift (Sharpless and Sherr, 2015).

Omics-based techniques such as proteomic profiling enables a cell-type approach are promising tools for detecting *in-vivo* cellular senescence and provide reliable outcomes for next generation of senolytic-based clinical trials. The evolution of functional neuroimaging-based hybrid techniques, including fMRI-EEG(-MEG) co-registration trials is significantly supporting the mapping of higher complexity levels of aging-related changes such as synaptic aging signatures.

In addition, unlikely adipose tissue, muscles, blood, or skin, the anatomical complexity of brain does not allow biopsy and tissue assessment for evaluating primary outcome measures such as changes in senescent cells accumulation rates.

Several lines of evidence suggest that peripheral lymphocytes and monocytes may reflect CNS

alterations due the intense crosstalk between the peripheral immune and the CNS resident immune cells. For instance, brain-derived toxic byproducts may enter the blood stream through a dysfunctional BBB, and trigger aberrant intracellular signaling in peripheral cells (Bartolome et al., 2007). It has been reported that immortalized lymphocytes from AD patients display apoptosis-resistance and upregulation of p21 CDKi (Bartolome et al., 2010).

Neural-Derived Exosome Protein Abnormalities represent an emerging possibility for prediction of preclinical AD, other neurodegenerative diseases and cerebrovascular disease is quantification of pathogenic proteins in plasma neural cell-derived exosomes (Goetzl et al., 2018; Mustapic et al., 2017).

Exosomes derived from any type of CNS cell may be harvested efficiently from human plasma by initial physical precipitation followed by immunoabsorption with antibodies that selectively recognize a surface antigen on that CNS cell-type. This two-step process permits separate enrichment of exosomes secreted by neurons, astrocytes, microglia and chondroitin sulfate proteoglycan type 4 (CSPG4) cells. We argue that reliable senescent signatures, previously assessed *in-vivo* in CSF, may be recovered from circulating plasma Neural-Derived Exosomes (Goetzl et al., 2018; Mustapic et al., 2017).

Next-generation sequencing and high-throughput immune profiling technologies offer the platform for characterizing innate immune responses during aging and AD (Larbi et al., 2009; Meyer-Luehmann and Prinz, 2015; Pellicano et al., 2012).

As a next challenge, the combination of aging-related variables (including cellular senescence biomarkers) into a systems-levels integration - integrative disease modelling (IDM) paradigm - will enable to identify aging modules and compensatory mechanisms (Bartsch et al., 2015; Bashan et al., 2012; Bassett and Sporns, 2017; Miller et al., 2008; Shine et al., 2019).

To date, IDM studies have suggested that while brain (sub)cellular adaptation occurs through a bioenergetic / metabolic molecular reprogramming, synaptic-scaled adaptation implies time-dependent neural oscillatory changes to synergistically modulate and recruit synaptic network for stress response dynamics and resilience (Bartsch et al., 2015; Bashan et al., 2012; Bassett and Sporns, 2017; Miller et al., 2008; Shine et al., 2019).

It is expected that cross-disciplinary and multi-omics approaches such as neuroimaging-omics will allow to depict which gene expression patterns and protein-protein interactions, in both cycling and senescent cells, are linked to distinct large-scale synaptic networks patterns (McIntosh et al., 2014; Medland et al., 2014; Richiardi et al., 2015) Neuroimaging-omics and computational approaches to identify and quantify networks of physiologic interactions, will also help mapping how (sub)cellular bioenergetic shifts can impact large scale neural oscillations and vice versa forecasting either

physiological or pathophysiological trajectories (McIntosh et al., 2014; Medland et al., 2014; Van Keuren-Jensen et al., 2014).

This step is essential to establish whether the trajectories of two / or more systems-scaled events are linked one another and also to cognitive outcomes. Network analysis of physiological and pathophysiological interactions across body systems and time will enable to accomplishing the “human map of brain resilience”

Rejuvenation strategies: a focus on senotherapeutics as a pharmacological opportunity for extending heathspan

Very recent proof-of-concept trials conducted in rodent models of aging and AD delivered evidence that senescent cells represent a druggable target **for rejuvenation strategies (treatments to delay and potentially even reverse the ageing process), and thus** for counteracting age-related disease in humans (Xu et al., 2018). **Two different promising senotherapeutic approaches (compounds able to target senescent cells) have been investigated so far: senolytic drugs (small compounds capable to selectively target senescent cells and promote their clearance) and senomorphic drugs (small compounds capable to slowdown senescent cells' phenotype).**

One of the most investigating senotherapeutic approach is the combination of dasatinib, a tyrosine kinase inhibitor approved for leukemia and quercetin which is an experimental compound with pleotropic effects including modulation of the NF- κ B, PI3K/Akt, and mTOR axis (Bussian et al., 2018; Xu et al., 2018).

A proof-of-concept study has recently demonstrated that treating mice transplanted with senescent cells with **a senolytic drug (the combination of dasatinib plus quercetin)** can recover from illness and prolong health- and lifespan (Xu et al., 2018).

The same senotherapeutic combination has recently shown a strong effect in inducing necrosis of senescent OPC surrounding cerebral A β plaque in brains of both human AD patients and mouse model of the disease. In the latter group, the senolytic effect was also associated with improvement of cognitive outcomes and neuroinflammation markers (Zhang et al., 2019).

The old compound AP20187, **an antibiotic ineffective in normal mice or humans**, has been proven with a **senolytic activity in transgenic mouse named INK-ATTAC where AP20187 promotes the dimerization of a pro-apoptotic factor expressed under p16^{INK4A} upregulation. AP20187 administration was associated with removal of p16^{INK4A}-positive senescent astrocytes and microglia coupled** with reduction of tau aggregation, neuroinflammation, and amelioration of the cognitive performance (Baker et al., 2011; Bussian et al., 2018).

This initial proof-of-concepts have indicated a therapeutic path to pursue. However, the translation of senolysis into a large-scale human clinical trial needs to first identify the most suitable target population(s) that may benefit of the treatment as well as a standardized biomarker panel to estimate target druggability, evaluate treatment response, and anticipate potential resistance mechanisms.

As above mentioned, another potential senotherapeutic approach may be represented by the pharmacological induction of a senomorphic effect, i.e. the **suppression of the cell senescence phenotypes, such as the compound capable of apparently “rejuvenate” senescent cells.**

An example of senomorphic effect has been demonstrated with trials investigating compounds capable to modulate senescent cells’ pro-inflammatory and self-reinforcing activity through the downregulation or even the suppression of the key factors of the SASP.

In this context, emerging SASP inhibitors are represented by drugs capable of slowing down the molecular factors upregulating the SASP production and secretion.

The proinflammatory transcription factor NF- κ B, the upregulation of the mTOR axis, the ROS induced upregulation of p38MAPK cascade, the upregulation of the JAK/STAT pathway, among others are the main trigger of the SASP (Acosta et al., 2013; Herranz et al., 2015).

Small compounds with a JAK/STAT inhibitory effect (ruxolitinib), or mTOR suppressing effect (rapamycin) or combined, mTOR plus NF- κ B, inhibitory activity (metformin) among others, have shown a good efficacy in reducing tissue damage due to SASP (Moiseeva et al., 2013; Xu et al., 2015).

Trials with transient administration of rapamycin have shown good outcomes in mice transplanted with senescent cells (Bitto et al., 2016; Harrison et al., 2009).

Navitoclax (ABT-263) is an experimental compound with a BCL-2 inhibitory effect that has recently shown a remarkable **senolytic** effect (Chang et al., 2016).

The translation of senotherapeutics approaches into human clinical trials has recently started to explore safety and efficacy of dasatinib plus quercetin in subjects displaying organ illness and tissue-specific accumulation of senescent cellular population (for instance ClinicalTrials.gov identifier: NCT02848131).

The anatomical challenges posed by the CNS must be overcome before setting the first in human anti-senescence R&D pipeline.

Systems pharmacology for enhancing adaptation and compensation: towards a pharmacological modulation of aging

Identifying compensatory mechanisms which ensure normal biological and functional outputs during aging as well as understanding the key points to modulate compensation upstream to pathophysiological failures, will open up novel therapeutic avenues to target aging and draw it towards a prolonged compensated phase.

A blueprint for the systems-scaled in-vivo exploration of drug-induced effects and trades off is urgently needed.

Systems pharmacology, an integrative interdisciplinary modelling approach, is an emerging conceptual paradigm that aims at exploring and predicting the entire effect of a drug modeling several multidimensional variables encompassing body systems and their biological interactions across inter-individual variability.

Systems pharmacology provides a blueprint for generating comprehensive experimental designs, especially where the systems encompass complex cross-talks. Systems pharmacology enables to model snapshots of human drug-induced interactomes and intracellular signals, drug-related (epi)genetic regulation, transcription regulatory networks, drug-induced immune responses, drug-drug interactions and drugs-multi targets interaction (Harrold et al., 2013; Zhao and Iyengar, 2012; Geerts et al., 2013).

Thus, systems pharmacology explorative strategy generates a unique and innovative opportunity to extend traditional pharmacological test and analysis to construct a holistic drug effect modeling grounded on bioinformatic tools and computational algorithms capable to provide the overall output. This integrative approach is in line with the perspective of multi-targets treatment for complex diseases, as AD, and is essential to overcome the limitation inherent the traditional "one-drug-one-target" model (Harrold et al., 2013; Zhao and Iyengar, 2012; Geerts et al., 2013).

This paradigm can lead to the characterization of pathway-based molecular signatures that will allow a mechanistic stratification of individuals by clustering a given phenotype for underlying hidden pathomechanisms. Thus, disease affected individuals will be able to access stratified pathway-based therapies (Harrold et al., 2013; Zhao and Iyengar, 2012; Geerts et al., 2013).

Precision pharmacology is a novel conceptual paradigm that aims at accelerating drug R&D programs informing all decision-making processes, from the proof of pharmacology to the clinical trials development, and until the real-world approval. This, in turn, will dramatically lower the rate of attrition, generating a high quality and cost-effective support to researchers for accomplishing individualized treatments.

Indeed, applying precision pharmacology is assumed to serve at the following long-term goals: I) developing multi-target therapeutic approaches for multifactorial polygenic diseases, such as AD,

II) providing predictive models / quantitative frameworks of therapeutic efficacy and risk of adverse events for individuals, and III) developing “molecularly” biomarker-guided targeted therapies (in an unbiased fashion) – i.e., treatments specifically adapted (“tailored”) to the individual and the disease stage, in line with the precision medicine paradigm (Hampel et al., 2019, 2018a)

The broad goal of PM is to optimize the effectiveness of disease prevention and therapy starting from customized interventions tailored on the individual’s biological make-up – e.g. genetic, biochemical, phenotypic, lifestyle, and psychosocial characteristics – through the “P4 Medicine” (P4M) model that is predictive, preventive, personalized, and participatory (Hampel et al., 2018a, 2017). To ensure the development of the PM paradigm in AD, the international Alzheimer PM Initiative (APMI) and its connected Cohort Program (APMI-CP) were launched and conceptually associated with the large-scale U.S. Precision Medicine Initiative (PMI) (available at <https://obamawhitehouse.archives.gov/precision-medicine>) and the U.S. “All of Us Research Program” – originated from the previous U.S. PMI Cohort Program (available at <https://allofus.nih.gov/>).

Therefore, we think that it is now essential to bring aging at the systems-level integration untangling how molecular and cellular pathways dynamically interact with large-scale network to substantially transform the R&D pipelines and paving the way for developing “molecularly” biomarker-guided aging targeted therapies – i.e., treatments specifically adapted (“tailored”) to the individual aging phenotype.

Pathway-based therapies are reality in oncology where several phenotypes of cancer diverging for histology, cytology topography and clinical manifestations are already treated with the same drug after targeting the molecular pathway they share (Drilon et al., 2018).

We believe that systems pharmacology and *in-silico* drug development may support future senotherapeutic-oriented R&D pipelines.

For instance, *in-silico* modeling can deliver key insights to cut down the risk of adverse off-target effects on non-senescent cells. Furthermore, systems pharmacology may generate reliable model of most suitable target population determining optimal benefit/risk trade-off according genetic, biological, and functional variables.

Indeed, the precision pharmacology paradigm shift is expected also to facilitate the drug repositioning process – also known as drug re-profiling or drug repurposing process – indicating that a drug registered for a specific disease *spectrum* may prove potential off-target biological effects that may be beneficial for diseases for which it has not been registered (Harrold et al., 2013; Zhao and Iyengar, 2012). Drug repositioning programs may provide molecules capable of inducing metabolic / bioenergetic-rewiring as well as hermetic responses (for instance, caloric restrictions)

and or promoting compensatory mechanisms to alterations in the proteostasis machineries, calcium homeostasis, oxidative balance, among others.

In this sense, GSK3 β inhibitors as lithium chloride (a therapeutic approved for bipolar disorders), have been found to attenuate aberrant overactivation of GSK3 β thus increasing resilience and slowing down those downstream pathways leading to decompensated aging and AD (Hampel et al., 2009; Machado-Vieira, 2018; Phiel et al., 2003).

In conclusion, we assume that the system-level integration of aging processes will allow to attain the “human map of brain resilience “whereas systems pharmacology can probe, in a near future, multi-target drugs capable of targeting senescence and simultaneously other aging-related detrimental mechanisms (“aging targetome”), thus prolonging or even freezing aging at the compensated phase.

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CONFLICTS OF INTEREST

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HH is co-inventor in the following patents as a scientific expert and has received no royalties:

- In Vitro Multiparameter Determination Method for The Diagnosis and Early Diagnosis of Neurodegenerative Disorders Patent Number: 8916388
- In Vitro Procedure for Diagnosis and Early Diagnosis of Neurodegenerative Diseases Patent Number: 8298784
- Neurodegenerative Markers for Psychiatric Conditions Publication Number: 20120196300
- In Vitro Multiparameter Determination Method for The Diagnosis and Early Diagnosis of Neurodegenerative Disorders Publication Number: 20100062463
- In Vitro Method for The Diagnosis and Early Diagnosis of Neurodegenerative Disorders Publication Number: 20100035286
- In Vitro Procedure for Diagnosis and Early Diagnosis of Neurodegenerative Diseases Publication Number: 20090263822
- In Vitro Method for The Diagnosis of Neurodegenerative Diseases Patent Number: 7547553
- CSF Diagnostic in Vitro Method for Diagnosis of Dementias and Neuroinflammatory Diseases Publication Number: 20080206797
- In Vitro Method for The Diagnosis of Neurodegenerative Diseases Publication Number: 20080199966
- Neurodegenerative Markers for Psychiatric Conditions Publication Number: 20080131921

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AV, and **CN** declare no conflicts of interest.

Table. Established and emerging (*in italics*) biomarkers of cellular senescence

Mechanism	Biomarker	Key notes	References
Cell cycle arrest	<ul style="list-style-type: none"> ▲ CDKI p16 ▲ CDKI p21 	The upregulation of p16 ^{Ink4a} , a cyclin-dependent kinase inhibitor (CDKI), associates with epigenetic mechanism (through inhibition of a DNA methyltransferase) which causes the demethylation of the p16Ink4a promoter.	(Baker et al., 2016; Lopez-Otin et al., 2013; Munoz-Espin et al., 2013; Munoz-Espin and Serrano, 2014)
SASP	<ul style="list-style-type: none"> ▲ IL-6, IL-8 ▲ CCL2 ▲ MMP-1, MMP-3 	Cytokines, chemokines, and metalloproteinases are key components of the senescence-associated secretory phenotype (SASP).	(Acosta et al., 2013; Herranz et al., 2015)
DNA damage repair network	<ul style="list-style-type: none"> ▲ γ-H2AX foci ▲ phosphorylated p53 	<p>The presence of γH2AX (H2A histone family member X) foci is a marker of sustained DNA damage.</p> <p>The phosphorylation of p53 triggers a crucial pathway culminating in the activation of the DNA damage response (DDR) .</p> <p>*A set of unrecoverable DNA damage including double strand breaks may lead to either senescence or apoptosis in a “dose”-dependent fashion. In the senescent cell, the resulting genomic instability triggers a sustained activation of DNA damage response (DDR), also <i>via</i> p53, until it turns into a defective process.</p>	(Lopez-Otin et al., 2013; Sharpless and Sherr, 2015)
Lysosomal function	<ul style="list-style-type: none"> ▲ GLB1 ▼ Lamp2 ▼ lysosomal function ▲ Lipofuscin 	<p>LAMP-2A ((Lysosomal-associated membrane protein), a transmembrane component for protein translocation to the lysosome. It is a key regulator of the high selective chaperones-mediated autophagy</p> <p>GLB1 (Lysosomal-β-galactosidase) hydrolyzes β-galactose from glycoconjugates</p> <p>Intracellular lipofuscin aggregates are composed of oxidized proteins plus lipid degradation residues</p>	(Kaushik and Cuervo, 2015; Labbadia and Morimoto, 2015; Lopez-Otin et al., 2013)
Nuclear changes	<ul style="list-style-type: none"> ▼ LaminB1 ▲ CCF ▲ SAHF ▲ H3K9me3 ▲ HP1γ 	<p>Reduced levels of LaminB1, a structural protein of the nuclear lamina, are due to its genetic downregulation exerted by p53 and p16</p> <p>Hypo-condensed constitutive heterochromatin (chromatin enriched in repressive marks)</p> <p>Presence of cytoplasmic chromatin fragments (CCF)</p> <p>Senescence-associated heterochromatin foci (SAHF) composed by repressive histone marks such as H3K9me3 (epigenetic downregulation of the pro-apoptotic factor Bax) and HP1γ. SAHF are peculiar of oxidative stress induced senescence</p> <p>* The most relevant epigenetic alterations leading to cellular senescence are represented by the overactivity of DNA methylases, histones methylases and deacetylases, including H3K9me3, H3K27me3, and H4K20me3 (age-related changes in epigenetically silenced loci)</p>	(Baar et al., 2017; Munoz-Espin et al., 2013)
Cell morphology	<ul style="list-style-type: none"> ▲ cytoplasm nucleus ratio ▲ size ▲ flatness and 	<p>Morphological changes are prominently due to improper activation of mitogenic stimulation with a decrease in the rate of protein degradation, cytoskeletal remodeling, and electrolytes dyshomeostasis</p> <p>mTOR pathways and the endoplasmic reticulum unfolded protein response (ER-URP) ATF6α play a central role in driving senescent transformation</p>	(Munoz-Espin et al., 2013; Munoz-Espin and Serrano, 2014)

	wideness		
Mitochondria ultrastructure and morphology	<ul style="list-style-type: none"> ▼ volume ▼ fusion and fission abnormally elongated 	Age-related decline in mitochondrial trafficking due to decreased size, number, density mitochondrial dynamics: fusion, fission, movement, and mitophagy	(Munoz-Espin and Serrano, 2014; Wang et al., 2009)
Apoptosis resistance	<ul style="list-style-type: none"> ▲ BCL-2 ▼ Bax 	<p>Upregulation of the antiapoptotic protein BCL-2 due to chronic activation of the transcription factor cAMP response element-binding protein (CREB), a stress response key mediator</p> <p>Epigenetic downregulation of the proapoptotic gene Bax</p> <p>*Other factors: modulation of the PI3K-AKT-mTOR-FOXO axis, the Mek-Erk axis, the p53-p21 axis (p53 levels and p53 post-translational modifications), and the rate of oxidative stress.</p>	(Baar et al., 2017; Lopez-Otin et al., 2013)
ER-UPR	▲ <i>ATF6α</i>	Upon stress conditions, as accumulation of unfold proteins, the ER stress-response, restore ER homeostasis through: a) promote the translocation of chaperones from the ER lumen to the cytosol, b) to stimulate three ER transmembrane sensors triggering downstream complex signals i.e. ER-UPR: PERK, IRE1α and ATF6α. Upregulation of ATF6α is the strongest candidate biomarker for hyperactivation of the ER-UPR in senescent cells.	(Pluquet et al., 2015)

Notes: Most of the prominent feature of cellular senescence reported for non-CNS cellular lines (in the table) match the senescent transformation of neurons and glia cells. Indeed, in the last ten years, several studies characterized neurons, astrocytes, and microglia senescent phenotype the well-accepted markers, including telomere length, telomerase activity, expression of p16, p21, p53, senescence-associated β-galactosidase, and a SASP (Bussian et al., 2018; Jurk et al., 2012; Stojiljkovic et al., 2019)

Table 2. The main alterations of proteostasis machineries in aging

Proteostasis networks	Aging-related features	Key points	References
Protein folding	<ul style="list-style-type: none"> ▲ protein insolubility (aggregation) ▲ folding in non-native states 	<p>Age-related decline in protein solubility (risk of aggregation), partially due to over-production of proteins with different thermodynamic features. Other contributing factors: electrolytes dyshomeostasis, pH fluctuations</p> <p>Misfolded proteins over-expose hydrophobic stretches that alter solubility coefficients</p> <p>Age-related intrinsic tendency to aggregate is related to defective DRR with abnormal aminoacidic sequences</p>	(Daggett and Fersht, 2003; Knowles et al., 2014)
HSPs (chaperones)	<ul style="list-style-type: none"> ▼ chaperones gene expression ▼ chaperones transcription factor ▲ degradation (cellular imbalance) 	Chaperones: 1) ensure correct folding to native states; 2) bind to molecular clients thus inducing allosteric (or orthosteric) changes, 3) are highly selective mediators of cytosolic small proteins degradation in lysosomes	(Kaushik and Cuervo, 2015; Labbadia and Morimoto, 2015)
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Autophagy	<ul style="list-style-type: none"> ▼ macroautophagy (even mitophagy) ▼ microautophagy ▼ chaperones-mediated autophagy (?) accumulation autophagosomes 	<p>Autophagy strongly contributes to life span while aging negatively impacts on autophagy machineries (vicious cycle).</p> <p>Autophagy ensure proper protein and mitochondrial (mitophagy) turn-over essential for coping with continuous energetic-metabolic demands and is highly controlled for recycling of non-critical proteins to a pool of amino acids.</p>	(Komatsu et al., 2006)
Proteasome	<ul style="list-style-type: none"> ▼ proteasomal subunits (turn-over or assembly) ▲ inactivation of the proteasome (?) Alterations ubiquitin enzymatic cascade. 	<p>Proteasome (large multisubunit complexes) activity can be either ubiquitin mediated or not.</p> <p>Proteasome is responsible for clearance of abnormal, denatured, short-lived or damaged proteins</p> <p>The ability to maintain a functional proteome declines during the aging process</p> <p>Inactivation of the proteasome occurs through direct interaction with age-induced protein-aggregates.</p>	(Kaushik and Cuervo, 2015; Komatsu et al., 2006)
Post-translational modifications	<ul style="list-style-type: none"> ▲ covalent modifications by-products ▲ cleavage end-products 	<p>PTMs of a protein alters its native structure with: 1) loss of function and a change in thermodynamic properties (i.e. insolubility, misfolding and aggregation) 2) prevents ubiquitin binding to protein and thus degradation</p> <p>Covalent additions to protein side chains: phosphorylation, acylation, alkylation, glycosylation, and oxidation</p>	(Labbadia and Morimoto, 2015; Sala et al., 2017)

HSPs: heat shock proteins; SAHFs: Senescence-associated heterochromatin foci; ER-UPR: endoplasmic reticulum unfolded protein response; PTMs: post-translational modifications

Figure 1. Trajectory of pathophysiological mechanisms across the *continuum* of systems multi-scale hierarchical self-organization, from systems homeostasis to systems failure: conceptual basis for molecular pathway-based therapies.

The homeodynamic space i.e. genome / epigenome and ecosystem factors. The homeodynamic space is ensured by adaptive responses and compensatory mechanisms scaled in time and space across multi-level system networks: from molecular pathways, to cellular stress responses, to brain cell-to-cell and synaptic dynamics, to large-scale brain network activity, to brain-periphery cross-talks. If a decompensatory cascade occurs, homeostasis progressively breaks down until final systems failure.

Unrevealing the spatial-temporal coordinates of multilevel adaptive and compensatory dynamics (from molecules to large-scale networks) is essential to enhance resilience and prevent diseases

The orange circle represents the entire *spectrum* of pathophysiological mechanisms across all systems levels, during the disease evolution.

Such nonlinear interactions support the hypothesis that the network failure might originate at any level initiating a detrimental cascade.

The **subcellular level** may show, for instance, compartmental impairment of proteostasis or mitochondrial functions dysfunction of membrane dynamics and cellular trafficking.

The **cellular level** dysfunction originates from the sum of distinct aberrant molecular pathways with the impairment of anti-stress responses and activation of signaling driving the cell fate.

The **tissue level** may display loss of structural and functional organization in terms of cell-to cell communication.

At **brain system level**, functional shifts in large scale network organization allow a spatial and temporal resources redistribution to cope with stress (i.e. systems-wide adaptation and comepeation).

The **organ systems level**. The existence of many cross-links-talks between CNS and the periphery might account for the hypothesis that brain diseases can originate or be substantially related to peripheral systems failure.

The colored pyramid represents the potential drug response at each level (from green to red and from the base to the peak there is a decreasing amplitude of effect) from adaptation to compensation towards decompensation and finally systems failure, a hypothetical “point of no return”.

Edited from Hampel H et al. Precision pharmacology for Alzheimer’s disease. Pharmacol Res. 2018. doi 10.1016/j.phrs.2018.02.014. Reproduced with permission.

Figure 2. Hypothetical trajectories of aging according individual threshold of resilience and vulnerability.

Each individual has a genetically, biologically, and environmentally determined thresholds of resiliency capacity. Also, an individual vulnerability to stressors and disease exists. We illustrate hypothetical trajectories of aging according individual threshold of resilience and vulnerability across spatial-temporal system-wide shifts and along 4 stages: from adaptation to irreversible failure.

STAGE 1 - ADAPTATION STAGE – STRESS RESPONSES:

Metabolic and energetic reconfiguration associated with functional switch in Molecular / Cellular / Tissue / Brain systems / body system network activity

STAGE 2 – COMPENSATION STAGE - RESILIENCY MECHANISMS:

Structural and functional counterbalancing of one or more initial pathomechanistic alterations.

STAGE 3 – BREAKDOWN OF RESILIENCY MECHANISMS:

Initial and progressive loss of compensatory effect (resilience) or over sustained compensation which may be neither protective nor homeostatic.

STAGE 4 (FAILURE STAGE) – FAILURE OF RESILIENCY MECHANISMS:

Hypothetical point of no-return. The colored curve line from green to red indicates a decreasing magnitude of drugs efficacy.

Orange arrows: drivers (genome / epigenome and interplay with environment)

Blu lines: didactic and schematic illustration of different hypothetical trajectories of brain aging dynamics. The continuous line shows hypothetical normal brain aging which will never reach the individual threshold of decompensation and failure. The dashed blue lines indicate two different degree of decompensated dynamics.

The colored column from green to red indicates a decreasing magnitude of drugs efficacy according to the timing of treatment.

Figure 3. Proteostasis networks ensuring cellular adaptation and their declining over aging.

Proteostasis is ensured by evolutionarily conserved networks which act at different levels. The translation control level is regulated by ribosome and ribosomal factors. The protein folding quality control (transition from unfolded to intermediates until native states) is orchestrated by the endoplasmic reticulum “unfolded protein response” (ER-UPR) and the chaperones. The latter also serve as regulator of protein trafficking across different cellular-subcellular compartments.

The final step is represented by protein degradation or autophagy. Two pathways allow protein degradation control: the endosomal/lysosomal degradation and proteasome degradation that can undergo either a ubiquitin dependent (UPS) or independent fashion.

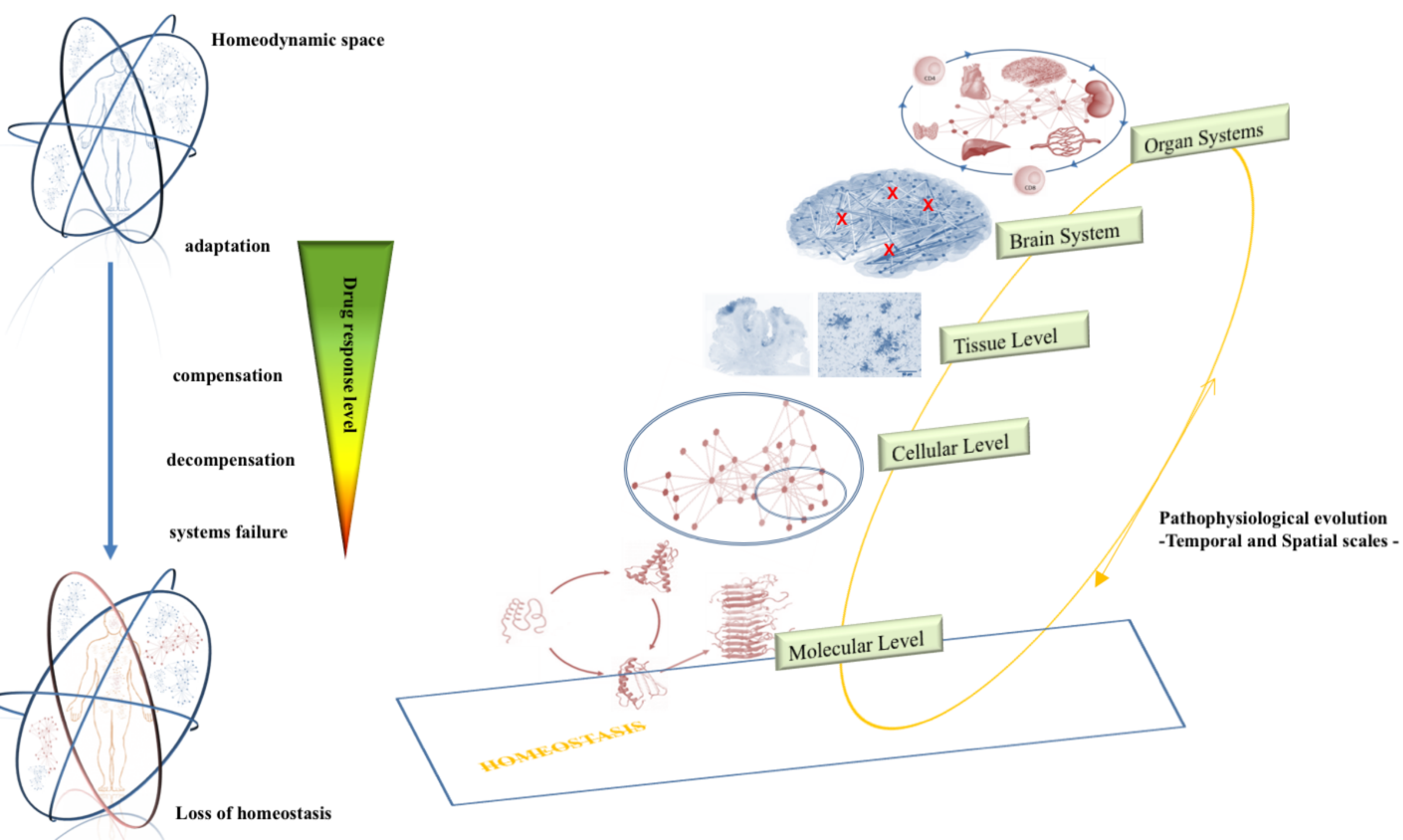
There is a complex, interplay consisting of highly conserved feedback and cross-talks between protein quality control machineries and clearance pathways and inside each component. The proper functioning of each proteostasis network accounts for longevity and potentially all the components ensuring the integrity of proteome are affected by aging.

See also Table 2 for detailed and integrative information [*Adapted from Kaushik S and Cuervo AM. Proteostasis and aging. Nat Med. 2015 Dec;21(12):1406-15*]

Figure 4. mTOR, its downstream factors, and physiological functions

mTOR is a nutrient-sensing cellular pathway activated by several clues as nutrient, hormones, growth factors and external stressors. mTOR is a central regulatory node upstream of the transcriptional regulation of autophagy shifting cellular metabolism to anabolic processes. mTOR constitutively slow down autophagic processes and is stimulated by cellular sensors of caloric restrictions. Increased cytoplasmatic concentrations of amino acids and grow factors, activate the so-called lysosomal nutrient-sensing complex with subsequent lysosomal membrane-tethering of transducer of regulated CREB (mTORC1) which, in turn activates the mTOR kinase complex (binding to mTORC2). Active mTORC1 exerts a tonic autophagy-inhibitory function downregulating, via phosphorylation, two crucial autophagy-inducing transcription factors, i.e. the helix-loop-helix transcription factor (TFEB) and the forkhead family of transcription factors (FOXO). In addition, mTOR enhances Nrf-1 activity associated with expression of proteasome subunit and promotes cellular shift to anabolic metabolism, including mitochondrial biogenesis. Aging is tightly associated with the risk of a broad range of activating gene mutations, either upstream or downstream mTOR pathways or specifically concerning the kinase DNA sequence, leading to a sustained over enhancement of mTOR pathways through the cross-talking and feedback interacting networks.

[*Adapted from Cho CH. Frontier of epilepsy research - mTOR signaling pathway. Exp Mol Med. 2011 May 31;43(5):231-74*]



→ Aging trajectory

--- fast aging decompensation

- · - · - medium aging decompensation

— slow aging decompensation

α : high vulnerability, low resilience

β : medium vulnerability, medium resilience

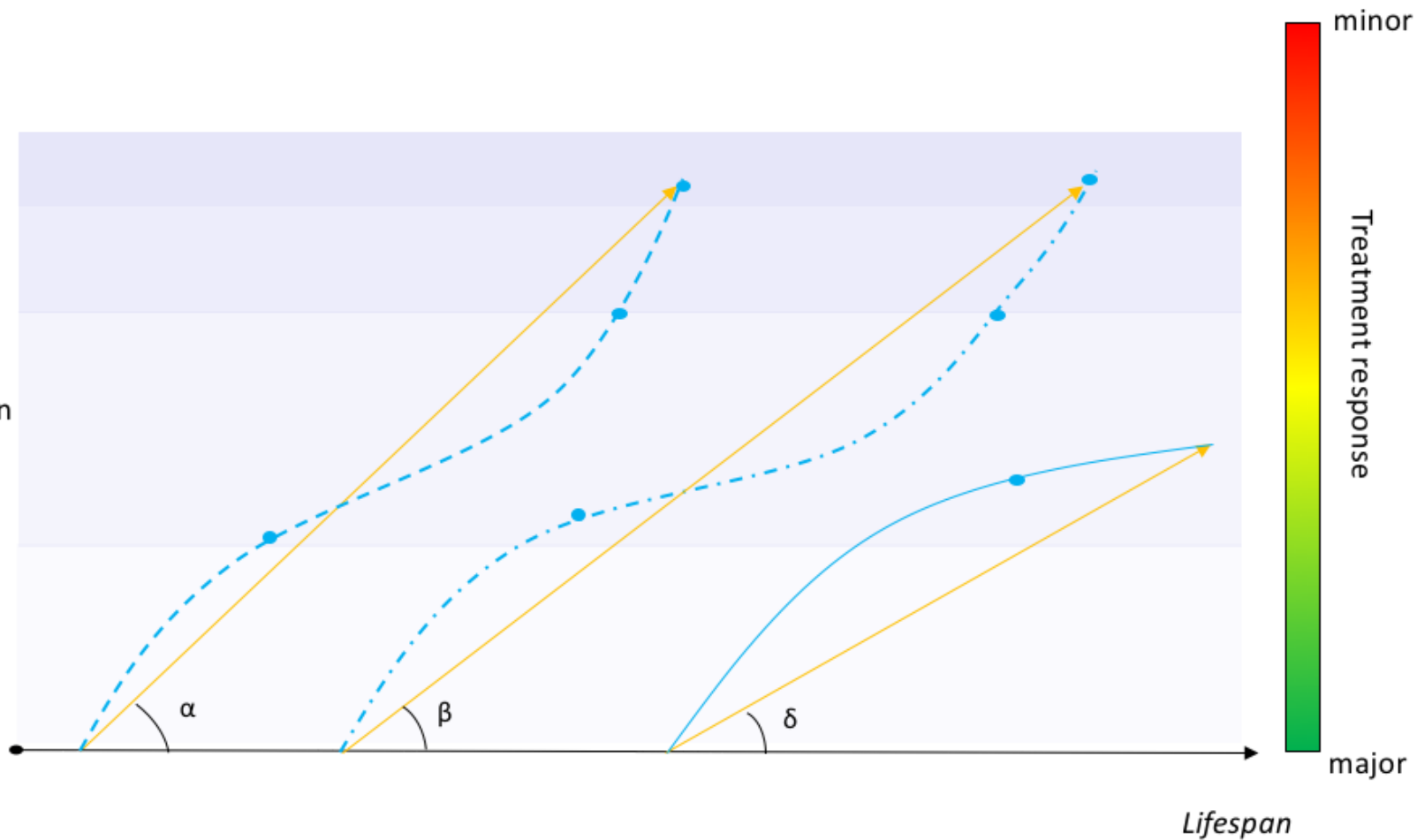
δ : low vulnerability, high resilience

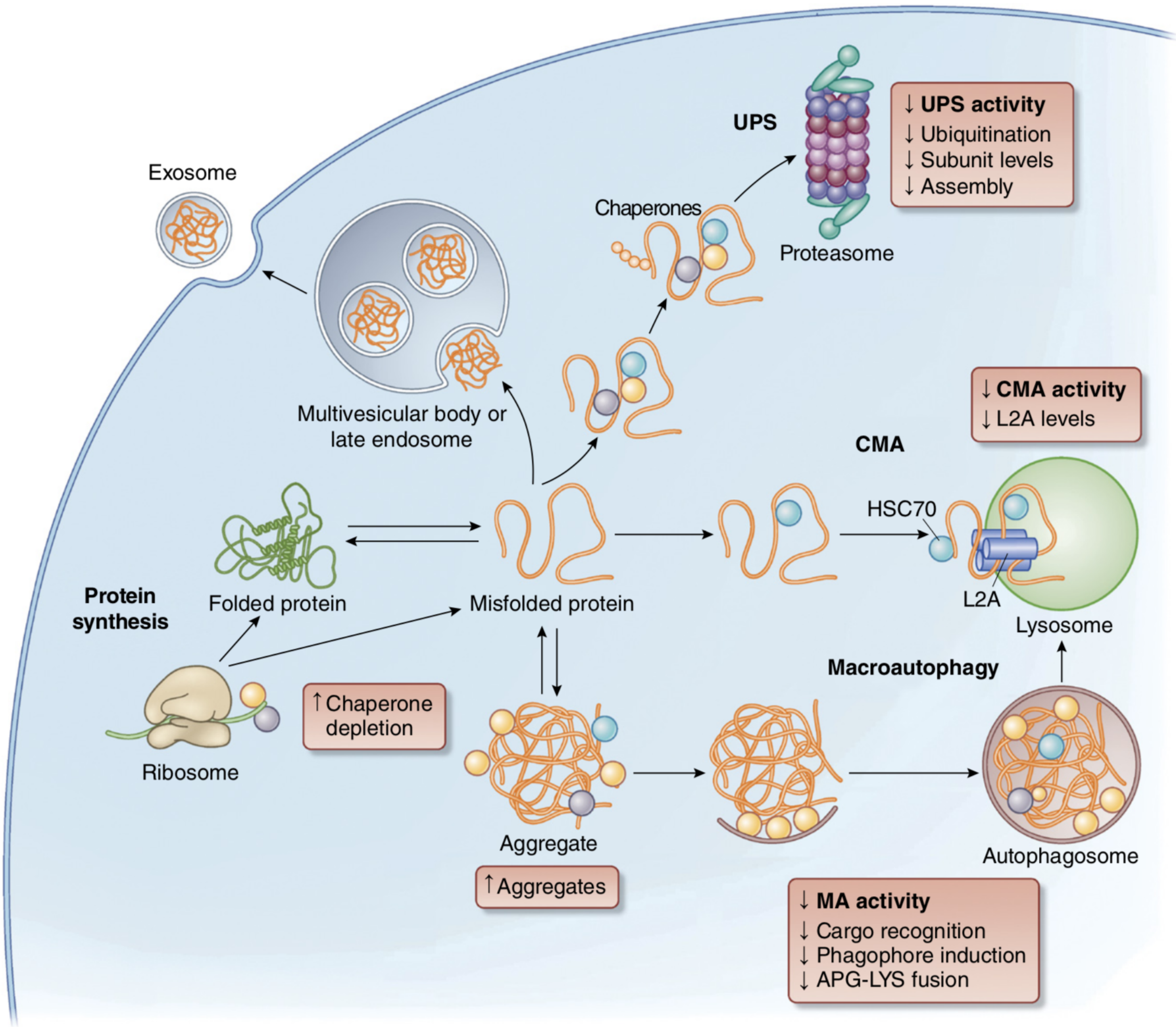
Threshold stage 4: systems failure

Threshold stage 3: decompensation

Threshold stage 2: compensation

Threshold stage 1: incipient decline of adaptation





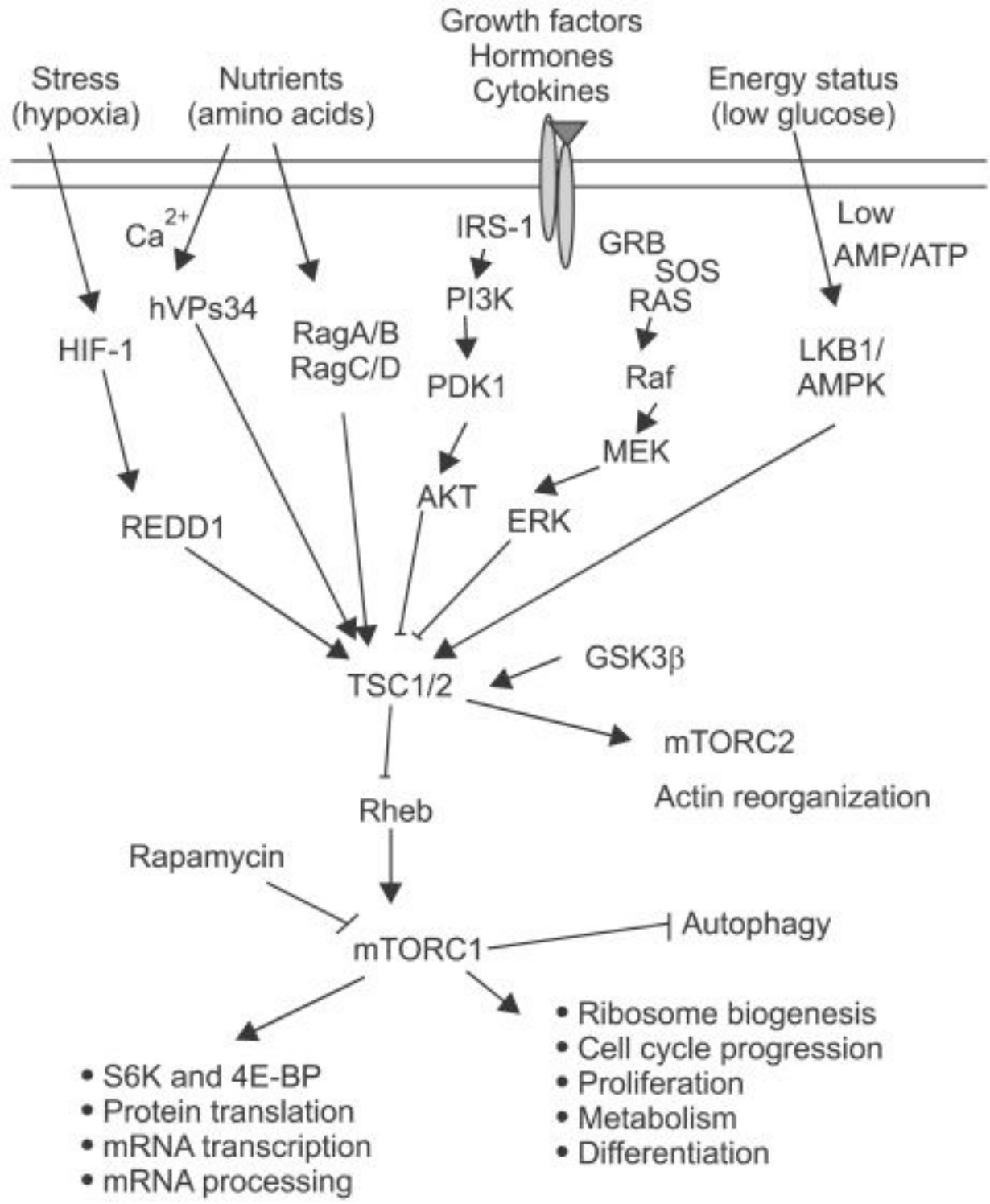


Table. Established and emerging (*in italics*) biomarkers of cellular senescence

Mechanism	Biomarker	Key notes	References
Cell cycle arrest	▲ CDKI p16 ▲ CDKI p21	The upregulation of p16 ^{Ink4a} , a cyclin-dependent kinase inhibitor (CDKI), associates with epigenetic mechanism (through inhibition of a DNA methyltransferase) which causes the demethylation of the p16 ^{Ink4a} promoter.	(Baker et al., 2016; Lopez-Otin et al., 2013; Munoz-Espin et al., 2013; Munoz-Espin and Serrano, 2014)
SASP	▲ IL-6, IL-8 ▲ CCL2 ▲ MMP-1, MMP-3	Cytokines, chemokines, and metalloproteinases are key components of the senescence-associated secretory phenotype (SASP).	(Acosta et al., 2013; Herranz et al., 2015)
DNA damage repair network	▲ γ -H2AX foci ▲ phosphorylated p53	The presence of γ H2AX (H2A histone family member X) foci is a marker of sustained DNA damage. The phosphorylation of p53 triggers a crucial pathway culminating in the activation of the DNA damage response (DDR) . *A set of unrecoverable DNA damage including double strand breaks may lead to either senescence or apoptosis in a “dose”-dependent fashion. In the senescent cell, the resulting genomic instability triggers a sustained activation of DNA damage response (DDR), also <i>via</i> p53, until it turns into a defective process.	(Lopez-Otin et al., 2013; Sharpless and Sherr, 2015)
Lysosomal function	▲ GLB1 ▼ Lamp2 ▼ lysosomal function ▲ Lipofuscin	LAMP-2A ((Lysosomal-associated membrane protein), a transmembrane component for protein translocation to the lysosome. It is a key regulator of the high selective chaperones-mediated autophagy GLB1 (Lysosomal- β -galactosidase) hydrolyzes β -galactose from glycoconjugates Intracellular lipofuscin aggregates are composed of oxidized proteins plus lipid degradation residues	(Kaushik and Cuervo, 2015; Labbadia and Morimoto, 2015; Lopez-Otin et al., 2013)
Nuclear changes	▼ LaminB1 ▲ CCF ▲ SAHF ▲ H3K9me3 ▲ HP1 γ	Reduced levels of LaminB1, a structural protein of the nuclear lamina, are due to its genetic downregulation exerted by p53 and p16 Hypo-condensed constitutive heterochromatin (chromatin enriched in repressive marks) Presence of cytoplasmic chromatin fragments (CCF) Senescence-associated heterochromatin foci (SAHF) composed by repressive histone marks such as H3K9me3 (epigenetic downregulation of the pro-apoptotic factor Bax) and HP1 γ . SAHF are peculiar of oxidative stress induced senescence * The most relevant epigenetic alterations leading to cellular senescence are represented by the overactivity of DNA methylases, histones methylases and deacetylases, including H3K9me3, H3K27me3, and H4K20me3 (age-related changes in epigenetically silenced loci)	(Baar et al., 2017; Munoz-Espin et al., 2013)
Cell morphology	▲ cytoplasm nucleus ratio ▲ size ▲ flatness and wideness	Morphological changes are prominently due to improper activation of mitogenic stimulation with a decrease in the rate of protein degradation, cytoskeletal remodeling, and electrolytes dyshomeostasis mTOR pathways and the endoplasmic reticulum unfolded protein response (ER-URP) ATF6 α play a central role in driving senescent transformation	(Munoz-Espin et al., 2013; Munoz-Espin and Serrano, 2014)

Mitochondria ultrastructure and morphology	▼ volume ▼ fusion and fission abnormally elongated	Age-related decline in mitochondrial trafficking due to decreased size, number, density mitochondrial dynamics: fusion, fission, movement, and mitophagy	(Munoz-Espin and Serrano, 2014; Wang et al., 2009)
Apoptosis resistance	▲ BCL-2 ▼ Bax	Upregulation of the antiapoptotic protein BCL-2 due to chronic activation of the transcription factor cAMP response element-binding protein (CREB), a stress response key mediator Epigenetic downregulation of the proapoptotic gene Bax *Other factors: modulation of the PI3K-AKT-mTOR-FOXO axis, the Mek-Erk axis, the p53-p21 axis (p53 levels and p53 post-translational modifications), and the rate of oxidative stress.	(Baar et al., 2017; Lopez-Otin et al., 2013)
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Notes: Most of the prominent feature of cellular senescence reported for non-CNS cellular lines (in the table) match the senescent transformation of neurons and glia cells. Indeed, in the last ten years, several studies characterized neurons, astrocytes, and microglia senescent phenotype the well-accepted markers, including telomere length, telomerase activity, expression of p16, p21, p53, senescence-associated β -galactosidase, and a SASP (Bussian et al., 2018; Jurk et al., 2012; Stojiljkovic et al., 2019)

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References Table 2

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