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mTOR signaling in aging and neurodegeneration: At the crossroad between metabolism dysfunction and impairment of autophagy

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ABSTRACT

Compelling evidence indicates that the mammalian target of rapamycin (mTOR) signaling pathway is involved in cellular senescence, organismal aging and age-dependent diseases. mTOR is a conserved serine/threonine kinase that is known to be part of two different protein complexes: mTORC1 and mTORC2, which differ in some components and in upstream and downstream signalling. In multicellular organisms, mTOR regulates cell growth and metabolism in response to nutrients, growth factors and cellular energy conditions. Growing studies highlight that disturbance in mTOR signalling in the brain affects multiple pathways including glucose metabolism, energy production, mitochondrial function, cell growth and autophagy. All these events are key players in age-related cognitive decline such as development of Alzheimer disease (AD). The current review discusses the main regulatory roles of mTOR signalling in the brain, in particular focusing on autophagy, glucose metabolism and mitochondrial functions. Targeting mTOR in the CNS can offer new prospective for drug discovery; however further studies are needed for a comprehensive understanding of mTOR, which lies at the crossroads of multiple signals involved in AD etiology and pathogenesis.

1. Introduction

Considerable evidence indicates that Alzheimer disease (AD) may be classified as an age-related metabolic neurodegenerative disease. Indeed, the brain is an organ with highly active energy metabolism in human body. Although the adult brain accounts for only 2% of total body weight, it utilizes approximately 20% of total body oxygen consumption and 25% of total body glucose in the resting awake state (Sokoloff, 1999). Glucose is virtually the sole fuel for the adult brain under physiological conditions (Bouzier-Sore et al., 2006). However, in addition to glucose other alternative substrates, including ketone bodies, glycogen and amino acids may be used as energy fuel under certain circumstances. Intriguingly, among the mechanisms proposed to be central to neuronal loss, the impairment in energy metabolism is a pathophysiological feature of AD and its occurrence precedes cognitive dysfunction and pathological alterations even for decades (Cunnane et al., 2011; Jack et al., 2008; Reiman et al., 1996). Thus, much effort is given to elucidate the etiological factors and consequences associated with altered energy metabolism that may likely provide valuable clues for treatment strategies and diagnostic approaches in AD (Chen and Zhong, 2013).

The mammalian target of rapamycin (mTOR) signaling pathway is an attractive candidate to study with respect to both aging and energy balance because it has the potential to affect a large number of processes that could be crucial in age-related degenerative phenomena. For example, mTORC1 is activated by growth factors, amino acids, and cellular energy status to regulate protein synthesis, autophagy, mitochondrial function, lipogenesis, ketogenesis, and glucose homeostasis.

Neurons are differentiated cells with polarized cell bodies. Their viability and function is closely connected to the availability of trophic factors as well as active metabolism. In addition, neurons, because of their extreme polarization, size and post-mitotic nature, may be particularly sensitive to the accumulation of aggregated/damaged cytosolic compounds, or membranes, and depend on autophagy for survival (Tooze and Schiavo, 2008).

Insight into mTOR signaling may provide a comprehensive means to counteract both aging and age-related diseases. Here, we review the major regulatory roles of mTOR on autophagy, glucose metabolism and mitochondrial function in the brain and how these intricate pathways may affect aging and neurodegeneration.

2. mTOR: complexes and signaling network

2.1. mTORC 1/2 complexes

mTOR is a serine/threonine protein kinase of 2549 amino acids, that belongs to the phosphatidylinositol 3-kinase-related kinase protein

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(PIKK) family (Jung et al., 2010). mTOR is an ubiquitously expressed protein, mainly localized into cytoplasm that modulates among other cell processes proliferation, mortality, survival and protein synthesis (Abelaira et al., 2014). Phosphorylation at Thr-2446, Ser-2448 and Ser-2481 within the kinase catalytic domain (KIN) domain of mTOR are correlated with overall higher levels of mTOR activity. Adjacent to the KIN domain is the FKBP12 rapamycin-binding domain (FRB), the site of inhibitory interaction between rapamycin and mTOR. The binding of rapamycin to FKBP12 disturbs mTOR–protein complex formation, thus impairing mTOR activity (Hoeffer and Klann, 2010). mTOR is known to be part of two different protein complexes: mTORC1 and mTORC2, which differ in some components, in upstream and downstream signalling, and in responsiveness to rapamycin treatment (Fig. 1). Both mTOR complexes share the catalytic mTOR subunit, the mammalian lethal with sec-13 protein 8 (mLST8), the DEP domain containing mTOR-interacting protein (Deptor), and the Tti1/Tel2 complex (Wullschleger et al., 2006). In contrast, regulatory-associated protein of mammalian target of rapamycin (Raptor) and proline-rich Akt substrate 40 kDa (PRAS40) are specific to mTORC1, while rapamycin-insensitive companion of mTOR (Rictor), mammalian stress-activated map kinase-interacting protein 1 (mSin1), and protein observed with rictor 1 and 2 (Protor) are specific of mTORC2 (Laplante and Sabatini, 2012; Takei and Nawa, 2014).

The main characteristic component of mTORC1 is raptor, a scaffold protein that regulates complex assembly and substrate recognition. Raptor is a 150 kDa protein that binds to TOR signalling (TOS) motif-containing proteins, carrying them to the mTOR catalytic domain in order to phosphorylate mTOR downstream proteins such as the p70S6Ks, 4EBPs, and STAT3 (Hoeffer and Klann, 2010). Moreover, raptor is essential as an amino acid sensor, thus regulating the subcellular localization of mTORC1 (Sancak et al., 2008). Raptor competes with rapamycin for binding to the FRB domain, assembles the mTORC1 complex and activates its catalytic activity (Hoeffer and Klann, 2010). PRAS40 and Deptor are negative regulators of mTORC1. PRAS40 regulates the interaction between mTOR and Raptor and negatively regulates mTOR signalling by blocking mTORC1 access to its substrates. When mTORC1 is activated, it is able to directly phosphorylate and reduce PRAS40 and DEPTOR function (Dunlop and Tee, 2009).

mTORC2 contains the rapamycin-insensitive companion of mTOR (Rictor) and as the name indicates is resistant to acute rapamycin treatment, although, prolonged rapamycin exposure impairs the mTORC2 complex. In addition, newly synthesized Rictor is susceptible to rapamycin, suggesting that only preformed mTORC2 is resistant to rapamycin, perhaps through steric occlusion, blocking access to the FRB. The function of Sin1, another unique protein component of the mTORC2 complex, is not clear but retains an essential function because deletion of Sin1 is embryonically lethal. TORC2 plays a role in organization of the actin cytoskeleton, but also phosphorylates Akt, which both activates TORC1 and inhibits FOXO nuclear recruitment (Crino, 2011; Hoeffer and Klann, 2010; Laplante and Sabatini, 2012).

The heterodimer consisting of tuberous sclerosis 1 and 2 (TSC1 and TSC2) is a key upstream regulator of mTOR. TSC1/2 functions to constitutively inhibit mTORC1/2 via Rheb (Ras-homolog expressed in brain), a Ras family guanosine triphosphatase GTPase by stimulating the conversion of active Rheb-GTP to inactive Rheb-GDP (Wullschleger et al., 2006). Several kinases control, by phosphorylation, the activity of TSC1/2, regulating the heterodimer formation. Depending on the phospho-acceptor amino acid residues, these phosphorylation events culminate in either mTOR inhibition or activation (Takei and Nawa, 2014). GSK3β (glycogen synthase kinase 3β) also phosphorylates TSC2, activating TSC1/2 and thus inhibiting mTORC1 (Takei and Nawa, 2014).

2.2. PI3K/AKT axis

The PI3-K/Akt/mTOR is involved in cell response to growth factors such as insulin, insulin-like growth factors (IGFs) and epidermal-derived growth factor receptors (EGFRs) through the activation of phosphoinositide-3 kinase (PI3-K). The growth factor signalling that regulates mTORC1 mainly involves the insulin/insulin-like growth factor (IGF-1), which binds to IGF-1R/IR, 2α–2β subunit tyrosine kinase receptors and triggers tyrosine phosphorylation and activation of the insulin receptor substrate (IRS) family to activate PI3-K. PI3-K bound to IRS converts phosphatidylinositol-4,5-phosphate (PIP_2) in the cell membrane to phosphatidylinositol-3,4,5-phosphate (PIP_3) (O'Neill, 2013). PIP_3 accumulation is antagonized by the lipid phosphatase PTEN (Wullschleger et al., 2006). Increased PIP_3 levels recruit Akt to the membrane, where it is activated by phosphorylation of Thr-308 and Ser-473 by phosphoinositide-dependent kinase-1 (PDK-1), mTORC2 and DNA-PK. In turn, Akt phosphorylates and inactivates TSC2, which as noted above is a negative regulator of mTORC1 (Fig. 2) (Franke, 2008a,b).

A primary negative-feedback inhibitory pathway exists whereby sustained activation of mTOR/p70S6K suppresses Akt activity (O'Neill, 2013). This mechanism acts through mTOR-mediated serine phosphorylation of IRS-1, to induce IRS-1 inactivation and degradation, thus eliminating coupling of PI3-K/Akt to the insulin and IGF-1 receptors and other activating receptors. This process is a major cause of insulin resistance (Shah et al., 2004; Tanti and Jager, 2009). In addition, mTORC2 phosphorylates Akt at Ser-473 to activate the system, but also promotes Akt degradation. Similarly JNK and inflammatory pathways (e.g. TNFα) antagonize and can turn off PI3-K/Akt, modulating IRS-1 activity (Tanti and Jager, 2009). Growth factors also activate mTORC1 through the Ras signaling pathway effectors ERK1/2 and p90 ribosomal S6 kinase 1 (Alayev and Holz, 2013).

mTORC2 controls the members of the AGC subfamily of kinases including Akt, serum- and glucocorticoid-induced protein kinase 1

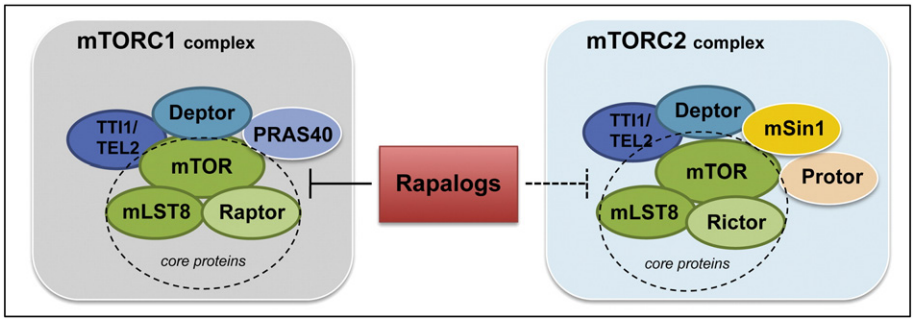

Fig. 1. mTOR complexes. TOR Complex 1 (mTORC1) is composed of mTOR, regulatory-associated protein of mTOR (Raptor), mammalian lethal with sec-13 protein 8 (mLST8) (core proteins) and the non-core components proline-rich Akt substrate 40 kDa (PRAS40), the DEP domain containing mTOR-interacting protein (Deptor) and the TTI1/TEL2 complex. This complex functions as a nutrient/energy/redox sensor and controlling protein synthesis. mTOR Complex 2 (mTORC2) is composed of mTOR, rapamycin-insensitive companion of mTOR (Rictor), mLST8, and the non-core proteins mammalian stress-activated protein kinase interacting protein 1 (mSin1), Deptor, TTI1/TEL2 and protein observed with rictor 1 and 2 (Protor). The mTORC2 signaling pathway is less defined than the mTORC1 signaling pathway.

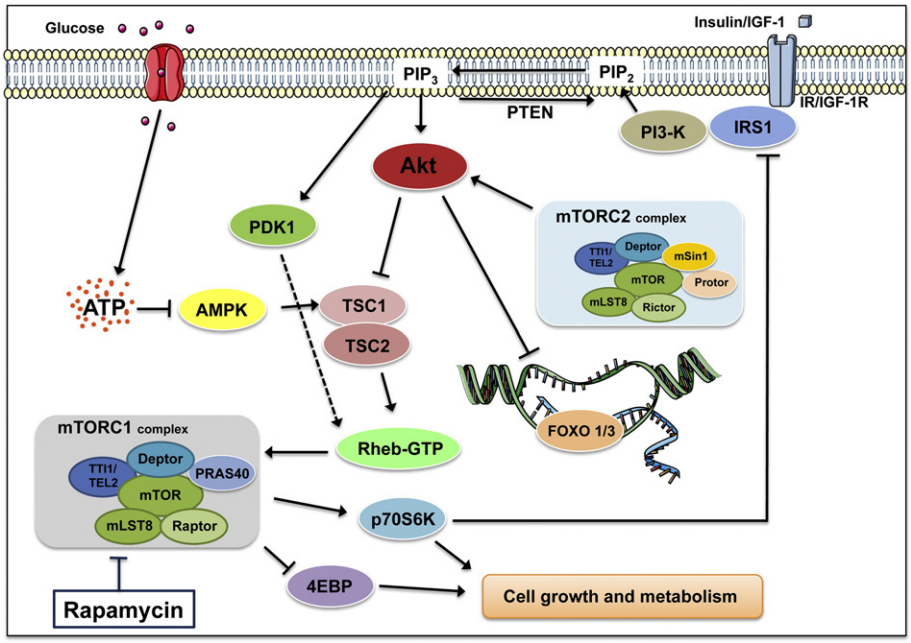

Fig. 2. mTOR and insulin signaling network. Growth factors, such as insulin, and nutrients, such as glucose, bind to membrane receptors to activate class I PI3K. This process generates PIP_3, which recruits protein kinase B (PKB/Akt) and its activator PDK1 (phosphoinositide-dependent kinase 1) to the plasma membrane, resulting in activation of PKB/Akt. Active PKB/Akt indirectly activates mTOR through inhibition of negative regulators [tuberous sclerosis complex (TSC1/2)] of mTOR and activating the mTOR activator Rheb (Ras homolog enriched in brain). mTOR phosphorylates and activates S6 kinase 1 (p70S6K1), that in turn promote protein translation. In addition, mTOR phosphorylates and inactivates eukaryotic initiation factor 4E-binding protein (4E-BP). A primary negative-feedback inhibitory pathway exists whereby sustained activation of mTOR/p70S6K suppresses Akt activity. This mechanism acts through mTOR-mediated serine phosphorylation of IRS-1, to induce IRS-1 inactivation and degradation, thus eliminating coupling of PI3-K/Akt to the insulin and IGF-1 receptors and other activating receptors. PI3K, phosphatidylinositide-3 kinase; Akt, protein kinase B; AMPK, AMP-activated protein kinase; 4EBP, elongation factor 4E binding protein; eIF2, eukaryotic initiation factor 2; eIF4E, eukaryotic translation initiation factor 4E; mTOR, mammalian target of rapamycin; S6, ribosomal S6; S6K, ribosomal S6 kinase.

(SGK1), and protein kinase C-a (PKC-a). mTORC2 directly activates Akt by phosphorylating its hydrophobic motif (Ser-473), a site required for its maximal activation (Sarbassov et al., 2005). Akt controls on mTORC1 support a potential signalling crosstalk between both the mTORCs complexes.

2.3. mTOR and glucose metabolism

An improved understanding of physiological and pathological regulation of glucose homeostasis is now considered a fundamental step for the management of chronic metabolic disorders of the brain such as AD (Chen and Zhong, 2013). Noting above that the brain energy production relies heavily on efficient glucose metabolism, any defects in glucose homeostasis would invariably threaten neuronal cells. In fact, both defects in hyperglycemia and hypoglycemia regulation significantly affect human brain health, especially cognitive function.

Cerebral glucose metabolism includes two main processes: glucose transport and intracellular oxidative catabolism (Chen and Zhong, 2013). Astrocytes participating in the composition of blood brain barrier control normal physiological glucose transport (Molofsky et al., 2012) in addition to various glucose transporters distributed in the brain (Duelli and Kuschinsky, 2001). The impairment of cerebral glucose metabolism can result either from altered transportation or oxidative catabolism dysfunction, which likely contribute to the metabolic abnormalities evidenced in AD brain. It has been hypothesized that glucose transportation abnormalities may be related to insulin resistance and intracellular metabolic alterations to mitochondrial dysfunction, both events well demonstrated to occur in AD patients (Chen and Zhong, 2013).

It is known that reduced signaling through the insulin/IGF-1 signaling pathway also extends lifespan (Bartke, 2008). Tissue-specific modulation of the insulin (IS) pathway is sufficient to delay aging. Insulin receptor knockout mice exhibit increased lifespan, reduced adiposity, and are protected against age-related obesity (Bluher et al., 2003). Similarly, tissue-specific modulation of mTORC1 or mTORC2 signaling demonstrates that each complex exerts different functions in different organs with regard to whole body glucose and lipid homeostasis.

Interestingly, inhibition of the IS pathway activates the transcription factor FoxO, and many of the lifespan extending effects of IS inhibition are indeed mediated by FoxO (Partridge and Bruning, 2008). Recently, it has been demonstrated that in mammalian cells and *C. elegans* FoxO/DAF-16 induces expression of glutamine synthetase (GS), and increased GS expression, in turn, inhibits TORC1 activity (van der Vos et al., 2012). Furthermore, in flies and mammals FoxO is able to inhibit TORC1 signaling by inducing expression of sestrins, which lead to activation of AMPK, a negative regulator of TORC1 signaling (Chen et al., 2010). Collectively, these findings suggest that FoxO may exert some of its positive effects on lifespan and tumor suppression via inhibition of TORC1 signaling.

To modulate insulin signaling in response to food-intake, mTOR directly phosphorylates the insulin receptor leading to its internalization (Romanelli et al., 2007); this, in turn, results in a decrease of mTOR signaling (Wullschleger et al., 2006). However, through the same mechanisms, chronic mTOR hyperactivity leads to insulin resistance, a key feature of type2 diabetes mellitus (Ueno et al., 2005). mTOR hyperactivity is also found in AD brains and in AD mouse models (Bove et al., 2011; Caccamo et al., 2010, 2011; Oddo, 2012).

One the major effects caused by glucose starvation (hypoglycemia) is the reduced ratio of ATP to AMP. Intracellular decrease in the ATP/AMP ratio activates the AMP-activated protein kinase (AMPK), a master sensor of intracellular fuel status that, similar to SIRT1, is activated by states of negative cellular energy (Gowans and Hardie, 2014). Once activated, AMPK down-regulates energetically demanding processes like protein synthesis, while stimulating ATP-generating reactions such as fatty acid oxidation. Thus, AMPK senses energy stress when cellular ATP levels are reduced. AMPK is activated by phosphorylation of Thr-172 in its activation loop by LKB1 (Mihaylova and Shaw, 2011). AMPK then phosphorylates TSC2 on Ser-1345 and Thr-1227, which promotes TSC2's ability to inhibit mTORC1. Regulation of the cellular energy

supply may also be a mechanism to activate mTORC1. Akt activates mTORC1 by maintaining a high ATP level that causes a decrease in the AMP/ATP ratio that in turn inhibits AMPK-mediated phosphorylation and activation of TSC2.

On the other hand, an increase in the ATP/AMP ratio directly activates the mTOR kinase while inhibiting AMPK. AMPK has been recently shown to mediate CNS actions of hormones and nutrients on food intake (reviewed in (Hardie, 2011)).

2.4. mTOR regulation of autophagy

Several substrates lead to diverse cellular responses downstream of mTOR. p70S6K and 4EBP that regulate translation, and Ulk1 and Atg13 that suppress autophagy are the best-characterized substrates for mTORC1.

Autophagy developed as a self-consuming mechanism with a key role in cell survival and in preserving cell metabolic balance (Di Domenico et al., 2014). This catabolic process involves the enclosure of cytoplasm by a double-membrane structure (autophagosome) and its subsequent delivery to the vacuole. Autophagy acts as a starvation response to maintain cellular nutrient levels and helps to regulate intracellular organelle homeostasis. Consequently, autophagy plays a crucial role in the removal of toxic/aggregated proteins and impaired organelles that could damage cells during stress and alteration of autophagy is reported in various human pathologies including neurodegenerative and lysosomal storage disorders (Dasuri et al., 2013; Mizushima et al., 2008). Autophagy involves several autophagy-related (Atg) proteins that coordinate vesicle formation in three different steps, initiation, elongation, and maturation. The autophagosome matures by fusing with an endosome and/or lysosome, thus forming an autophagolysosome (Ghavami et al., 2014). This final step permits the interaction of the autophagosome cargo with lysosomal hydrolases to allow its degradation. The initiation step involves the formation of a membrane structure termed the phagophore in the cytoplasm at the phagophore-assembly site(s) (PAS) (Yang and Klionsky, 2010). In physiological conditions (no cell starvation) mTORC1 suppresses the phagophore formation by direct interaction with the Ulk1 complex (Ulk1-At- g13-FIP200-Atg101). The mTORC1 complex phosphorylates and inhibits Ulk1 and its interacting partner Atg13 as well as AMBRA1, the key link between Ulk1 and Beclin-1 complexes (Wojcik, 2013). Under starvation conditions or rapamycin treatment, mTORC1-mediated phosphorylation of Atg13 and Ulk1 is inhibited, leading to dephosphorylation-dependent activation of Ulk1 and Ulk1- mediated phosphorylations of Atg13, FIP200, and Ulk1 itself that triggers autophagy initiation (Yang and Klionsky, 2010). The Ulk1/Atg13/FIP200/Atg101 protein complex is involved in autophagosome formation and plays an important role in Atg proteins recruitment and autophagosome syn- thesis. In turn, this causes the activation of another complex that comprises (among other proteins) the class III PI3 kinase Vps34 and the protein Beclin-1 (Ravikumar et al., 2010). The activity of Vps34, a class III phosphatidylinositol-3-kinase (PI3K), is necessary for the formation of new autophagosomes and is enhanced by its binding to Beclin-1 (Fig. 3).

The second ubiquitination-like reaction involves the conjugation of microtubule-associated protein 1 light chain (LC3) to the lipid, phosphatidylethanolamine (PtdEtn). LC3 is cleaved at its C-terminus by Atg4 to form the cytosolic LC3-I, which is conjugated with PtdEtn through the action of Atg7 (E1-like) and Atg3 (E2-like) to generate LC3-II (Garcia-Arencibia et al., 2010; Metcalf et al., 2012). LC3-II is bound to both sides of the membrane until fusion with lysosomes and after it the LC3-II on the cytosolic face is recycled (to LC3-I) by Atg4, while LC3-II on the inner face of the membrane is degraded (Garcia-Arencibia et al., 2010). Autophagosomes are then transferred along microtubules in a dynein-dependent manner to lysosomes, where the fusion forms the autolysosome. Once the autophagosomes fuse with the lysosomes, their cargo is degraded by lysosomal hydrolases. In addition to inhibiting autophagy, mTORC1 also negatively regulates the biogenesis of lysosomes, multifunctional organelles that have the capacity to degrade most cellular components.

Recently, it has been demonstrated that mTORC1 regulates lysosomes through the transcription factor EB (TFEB), a basic helix-loop-helix leucine zipper transcription factor that controls many genes with key roles in lysosomal function (Settembre et al., 2012). mTORC1 phosphorylates TFEB, which prevents its nuclear entry, so that starvation-induced mTORC1 inhibition promotes the nuclear accumulation of TFEB and thus its activity. TFEB also promotes autophagosome formation and their fusion with the lysosome thus playing a significant role in promoting autophagy when nutrient levels are low (Settembre and Ballabio, 2011; Settembre et al., 2011).

2.5. mTOR and mitochondria

Mitochondria are highly metabolic organelles that in response to changes in nutrient levels and growth signaling regulate health span by modulating energy production, Ca^{2+} homeostasis and also apoptosis (Godoy et al., 2014). Indeed, mitochondrial functions are sensitive to a number of signaling pathways, such as those activated in response to oxidative and inflammatory insult, that, in turn, act to modulate the transcription of a set of mitochondrial genes (Godoy et al., 2014).

Whereas the upstream signals regulating mTOR complex remain poorly understood, accumulating evidence suggests that mTOR senses mitochondrial activity and cellular energetic status (Schieke and Finkel, 2006). This feedback of the mitochondria to mTOR and its downstream targets p70S6K1 and 4EBP1 may support a molecular link between growth signals and cellular metabolism (Dennis et al., 2001; Inoki et al., 2003). The stability of the raptor-mTOR association and the kinase activity of the complex, measured by phosphorylation of the downstream targets p70S6K1 and 4EBP1 is sensitive to nutrients such as glucose and amino acids (Inoki et al., 2003). Similarly, numerous studies have suggested a connection of the raptor-mTOR complex with mitochondrial function.

However, the molecular basis linking mTOR with mitochondria is not known. Interestingly, mTOR was found to be associated with mitochondria, suggesting a direct physical interaction (Desai et al., 2002). mTOR activity and raptor mTOR interaction are regulated in a redox-sensitive manner (Sarbassov et al., 2005). This redox-based regulation of the mTOR pathway activity might be part of a potential mechanism by which mTOR senses nutrients and mitochondrial activity. Studies suggest that mTOR signaling might also be active in the reciprocal direction to regulate mitochondrial metabolism (Schieke and Finkel, 2006). It was observed that the stability of the Raptor-mTOR complex correlated with mitochondrial oxygen consumption and oxidative capacity. Disruption of the complex by rapamycin and RNAi-mediated knockdown of raptor resulted in decreased levels of mitochondrial oxygen consumption, whereas TSC2 knockdown cells showed increased mitochondrial activity. mTOR also regulates the balance between glycolysis and mitochondrial metabolism, with increased ATP production by oxidative phosphorylation after stimulation of mTOR activity. This is an example of how this regulatory loop between mTOR and mitochondria could explain at molecular level cellular energetics needs with growth stimuli. Based on these findings, which underlie that mTOR signaling stimulates mitochondrial respiration and regulates the balance between glycolytic and mitochondrial ATP generation the effects of TOR signaling on life span are evident.

Investigators have started exploring the role of the mTORC1 pathway in mitochondria energy production, demonstrating that mTORC1 activation increases both oxygen consumption and mitochondrial number (reviewed in (Finley and Haigis, 2009)). In particular, mTORC1 controls mitochondrial oxidative function by positively regulating the activity of PGC1-α (PPARγ coactivator 1), a nuclear cofactor that critically modulates mitochondrial biogenesis and oxidative metabolism (Fig. 4) (Cunningham et al., 2007). Thus, mTORC1 might have a significant role

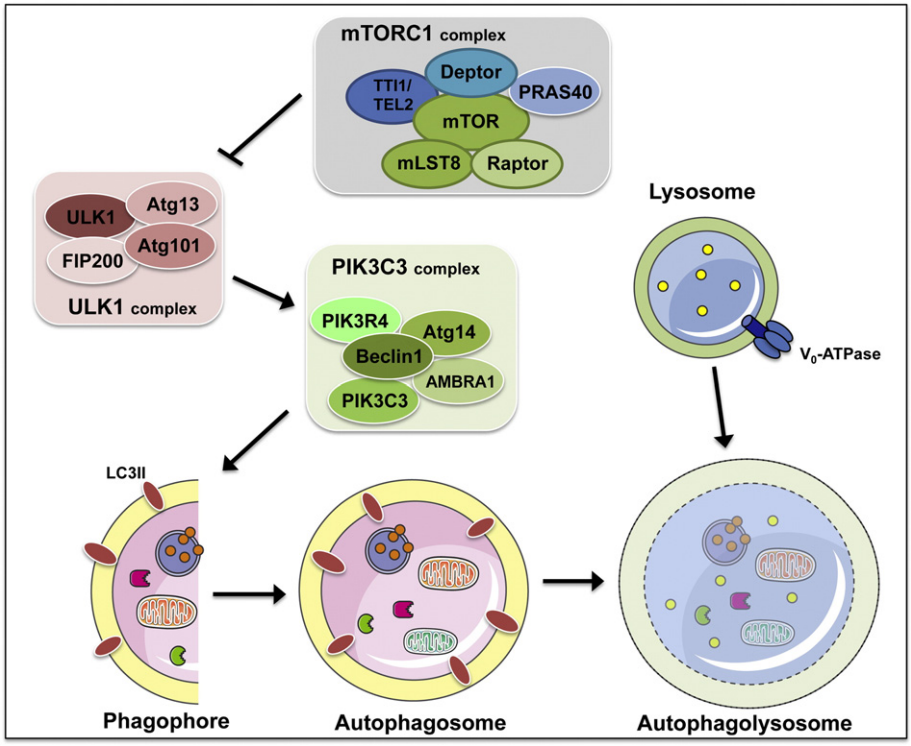

Fig. 3. mTOR and autophagy. mTOR is a key regulator of autophagy, that is inhibited in response to multiple stressful events, including deprivation of nutrients or growth factors. mTOR directly phosphorylates ULK1 and mAtg13 and inhibits ULK1 kinase activity, which is essential for autophagy induction. Autophagy involves several autophagy-related (Atg) proteins that coordinate vesicle formation in three different steps, initiation, elongation, and maturation.. The beclin-1 complex contributes to the nucleation of the phagophore. Beclin-1 complex is regulated positively by AMBRA1. Elongation of the phagophore membrane is dependent on the Atg12 and LC3 conjugation systems. LC3 is a hallmark of autophagy and require a multi-step conjugation process. Closure of the autophagosome is dependent on the activity of the LC3-conjugation system. The autophagosome matures by fusing with an endosome and/or lysosome, thus forming an autophagolysosome.

in the coordination of cell growth and mitochondrial metabolism. However, in apparent contrast to these data, NAD^+-dependent deacetylase SIRT1, which controls mitochondrial number and fuel oxidation by activating PGC1-α, is reported to inhibit mTORC1 activity in a TSC2-dependent manner (Ghosh et al., 2010). Although further work is required to clarify the role of the mTORC1 pathway in the regulation of mitochondrial function, these findings could be reconciled by taking into account the need for a context-specific regulation of mitochondrial substrate usage and activity (Catania et al., 2011). In fact, during cellular stress and energy depletion, anabolic and energy-consuming pathways like the mTORC1 cascade need to be turned off in favor of energy-producing processes.

In addition, mitochondria and their physical dynamics play a vital role at several stages of autophagy from initial assembly of the autophagosome to autophagy-mediated cell death (Rubinsztein et al., 2012). Recent studies show that the mitochondrial outer membrane recruits the autophagy proteins Atg5 and LC3. They are recruited not for the autophagic removal of mitochondria, mitophagy, but to provide the anchorage site and share the lipid moieties required for the elongation of the initial phagophore. In the same study, the authors illustrate that the cells that lack the mitochondrial protein Mfn2, which mediates mitochondria anchoring to the endoplasmic reticulum, do not show such recruitment of Atg5 or LC3 in the vicinity. This observation suggests a crucial role of mitochondria and endoplasmic reticulum in the initiation of autophagy (Hailey et al., 2010). Moreover, mitochondria form tubular structures by connecting to one another (mitochondrial fusion), during serum starvation, which also promptly induces autophagy.

3. mTOR in the brain

mTOR is expressed at high levels in the brain, mainly in neurons but also in glial cells. During embryonic development, mTOR signalling serves as a potent neuronal survival and division signal responding to growth factors and guidance cues, including IGF-1 and insulin. Although the precise mechanisms are still not fully understood, regulated and coordinated activities of mTORC1 and 2 are necessary for normal development of neurons and brain (Fig. 5) (Meijer et al., 2014; O'Neill, 2013). The mTOR pathway promotes extension of neuritis (dendrites and axons) in brain development (Heras-Sandoval et al., 2014). In terms of dendritic morphogenesis, increased activation of mTOR, results in increased dendrite branching, higher numbers of immature filopodia-like protrusions in the dendrites, and a decrease in the density of mature dendritic spines (Troca-Marin et al., 2014). mTORC1 activation induces protein and lipid synthesis so that it increases cellular mass with expansion of plasma membrane which favour the axon guidance during development. Local protein (and possibly lipid) synthesis mediated by mTORC1 may participate in the extension of an axon and dendrites of a neuron (Takei and Nawa, 2014). mTORC2 may be the a putative facilitator of growth cone motility, including neuritis path finding and elongation because it is known to affect actin dynamics.

The same system that controls division/migration programs during brain development evolves to control synaptic plasticity and processes underlying memory and learning in adult brain (Arendt, 2003). Thus, in post-mitotic neurons the mTOR pathway has significant functional impact on synaptic plasticity, neuronal polarity, neurotransmission, proteostasis, metabolic control and stress responses including DNA repair (O'Neill, 2013).

In the adult CNS, mTOR is crucial for many forms of synaptic plasticity such as long-term potentiation (LTP) in the hippocampus and, thereby, plays an important role in the process of learning and memory via protein synthesis-dependent strengthening of synapses. Furthermore, the mTOR pathway is involved in synaptic plasticity by coordinating the timing and location for the synthesis of new proteins (Russo et al.,

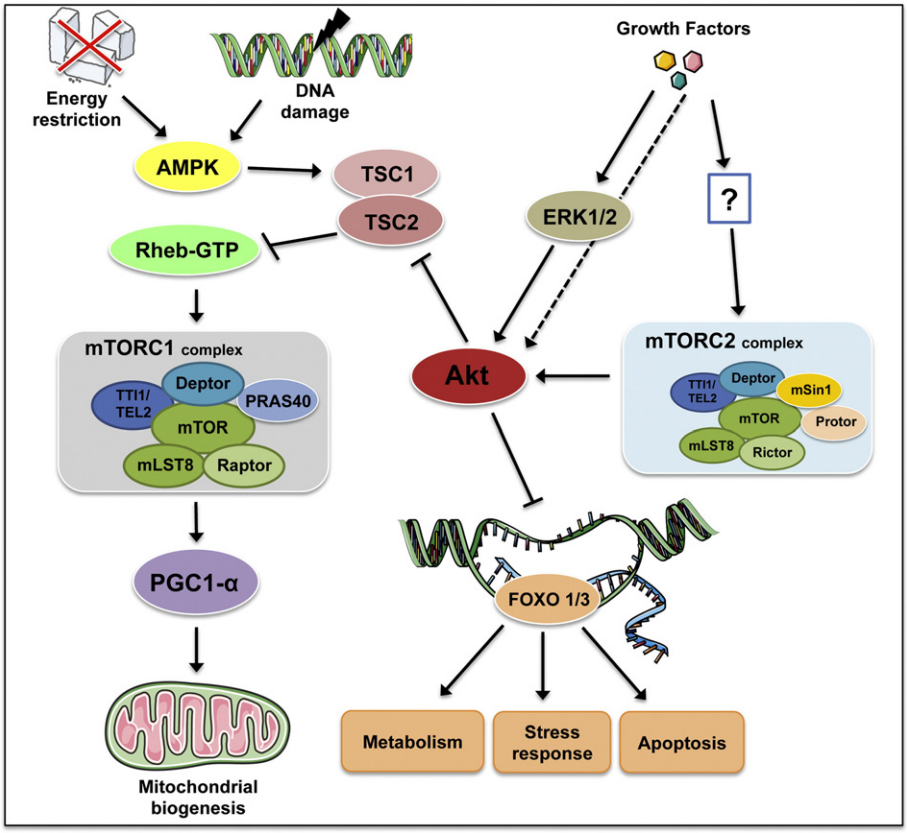

Fig. 4. mTOR and mitochondria. mTORC1 activation increases both oxygen consumption and mitochondrial number. mTORC1 controls mitochondrial oxidative function by positively regulating the activity of PGC1-α (PPARγ coactivator 1). Akt inhibits FOXO through direct phosphorylation, and indirectly activates mTORC1, which in turn elevates protein synthesis. When activated FOXO induces the expression of Sestrin 3, which activates AMPK to inhibit mTORC1. FOXO maintains cellular energy homeostasis by coordinating cellular supplies and demands. Under conditions of growth factor limitation or other cellular stresses, FOXO transcription factors are activated, and inhibit the anabolic energy consuming functions of mTORC1, while activating Akt to facilitate energy producing processes.

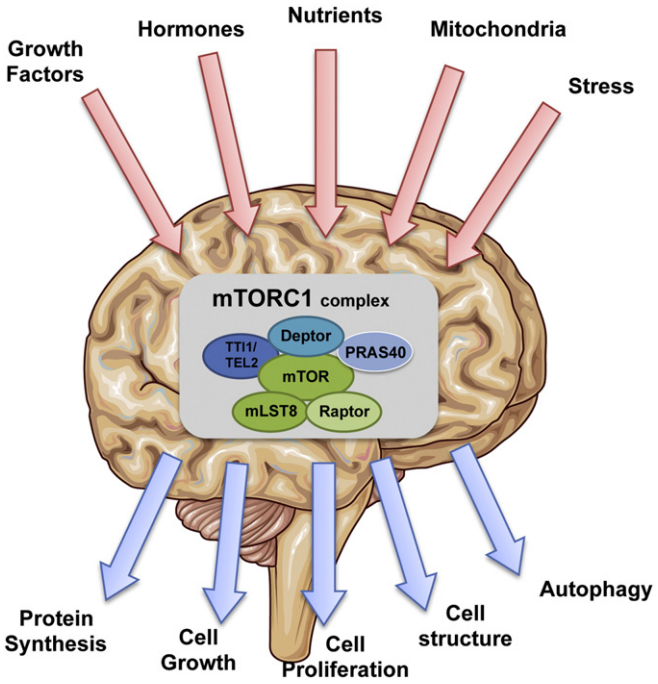

Fig. 5. mTOR in the brain. mTOR is expressed at high levels in the brain and serves as a potent neuronal survival and division signal responding to different signals including growth factors, nutrients, hormones and stress. All these nutrient- and energy-dependent events activate different downstream targets that mainly regulate protein homeostasis (protein synthesis and degradation) and cell proliferation. Deregulation of proteostasis plays a crucial role in the neurodegenerative process.

2012). Potential molecular mechanisms by which mTOR can regulate synaptic plasticity are very broad. Analysis of RNAs regulated by brain-derived neurotrophic factor (BDNF) through mTOR identified several proteins previously studied for their role in synaptic plasticity, learning, and memory (Russo et al., 2012; Takei and Nawa, 2014; Troca-Marin et al., 2014). Other proteins regulating synaptic plasticity, such as CamKIIα or PSD-95, also are dependent on mTOR activity (Abelaira et al., 2014; Yang and Klionsky, 2010).

In aged brain mTOR is deeply involved in the regulation of the proteostasis network, through the on/off switch of translation and autophagy, in order to avoid the accumulation of toxic protein aggregates that might result in brain degeneration (O'Neill, 2013). In addition, mTOR is also involved in the control of food uptake. In the hypothalamus, mTOR acts as an energy sensor to control animal food intake and regulate body energy balance (Laplante and Sabatini, 2012). Moreover, mTOR controls the gonadotrophic axis and the onset of puberty, the response to external light and the regulation of circadian clock neurons in the suprachiasmatic nuclei (Russo et al., 2012).

3.1. mTOR signaling in aging

In the last decade, research has demonstrated the ability of mTOR to modulate lifespan, possibly through two of the main processes regulated by TOR, protein synthesis and autophagy. These events are both nutrient- and energy-dependent through at least two characterized downstream targets, 4EBP1 and p70S6K. Collectively, studies in diverse model organisms have shown that the impairment of TOR signaling leads to increased life span (Hands et al., 2009).

The effect of TOR on lifespan operates downstream of the insulin signaling pathway, as indicated by the observation that the increase in

lifespan in an insulin signaling mutant cannot be further extended by mutations in components of the TOR pathway (Donati et al., 2008). A link between insulin signaling, aging and autophagy was first described in rodent liver in which it was observed that the age-dependent increase of insulin caused an inhibition of autophagy while the ability of glucagon to upregulate autophagy was reduced with increasing age (Cavallini et al., 2001). Recently, much attention is given to the regulation of FoxO, that has been shown to directly control the transcription of autophagy genes, including members of the Atg8 family (LC3) and regulators of autophagy (Mammucari et al., 2007). Upregulation of FoxO is able to activate autophagy in *Drosophila* (Juhasz et al., 2007), *C. elegans* (Mammucari et al., 2007) and mouse muscle fibres (Zhao et al., 2007). In addition, the results obtained in *C. elegans* showed that the upregulation of autophagy in skeletal muscle via DAF-16 was independent of mTOR, as demonstrated by inhibition of mTOR by rapamycin or knockdown (Mammucari et al., 2007). Knockdown of a component of mTORC2 (Rictor) did, however, result in FoxO-mediated induction of autophagy.

As described above, S6K is a target of mTORC1 that phosphorylates IRS-1 at inhibitory sites, thus blocking the activation of Akt (Shah et al., 2004), and upregulating autophagy. Conversely, mTORC2 has been shown to phosphorylate Akt at Ser-473, hence activating Akt and downregulating autophagy (Sarbassov et al., 2005). The balance between mTORC1 and mTORC2 signaling therefore could be critical in the regulation of Akt, autophagy and aging. It seems that Akt signaling can be both positively and negatively regulated by mTOR, depending on the TOR complex.

Further evidences showing the direct link between insulin signaling and autophagy were obtained from a mouse with targeted deletion of PTEN in the liver. PTEN is a lipid and protein phosphatase, a widely known negative regulator of insulin/PI3-K activity, whose downregulation will result in activated Akt and thus mTORC1. Thus, autophagic flux in the liver of this KO-mouse was significantly reduced (Ueno et al., 2008). In *C. elegans*, downregulation of the autophagy gene Bec1 inhibited the longevity phenotype of the DAF-2 insulin receptor mutant (Melendez et al., 2003), indicating that the extension of lifespan due to alterations in insulin signaling may occur, at least in part, via autophagy.

With respect to aging, increasing evidence suggests that the mTOR/autophagy axis plays a dual role in the cellular response to oxidative stress. From one side, reduced autophagy, due to aging and age-related disorders, could lead to accumulation of oxidized proteins in aged cells under normal growth conditions (Rubinsztein, 2006). This hypothesis is supported by genetic studies that demonstrated in old *Drosophila* brains that enhanced Atg8 expression extends life span, induces resistance to oxidative stress and reduces the accumulation of oxidative damage (Simonsen et al., 2008). Furthermore, the age-dependent reduction in autophagy is responsible of the build-up of severely damaged mitochondria, thus exacerbating oxidative stress and tissue damage with age. It is conceivably that autophagy may provide protection against oxidative stress. This is in agreement with data showing that Atg4, an essential protease that controls the lipid modification of Atg8 and autophagosome formation, is a direct target for oxidation by hydrogen peroxide.

On the other hand, it has also been suggested that low levels of ROS provide a signal to regulate autophagic survival and death processes (reviewed in (Scherz-Shouval and Elazar, 2007)). Collectively, accumulated evidence suggests that free radicals are upstream and downstream of mTOR and numerous feedback and feed forward loops exist.

In addition to its role in promoting cell survival and increasing life span, autophagy also result in cell death (autophagic or type II cell death) (Shintani and Klionsky, 2004) that is observed under conditions of oxidative stress. Hydrogen peroxide is reported to induce autophagy via a novel signaling mechanism that links PARP-1 activation to the LKB-1-AMPK-mTOR pathway. Hence, PARP-1 activation appears to promote autophagy.

3.2. mTOR in Alzheimer-like dementia

As discussed above, the role of mTOR signalling in protein homeostasis (synthesis and degradation) appears to be particularly important in the brain. Several neurodegenerative disorders are characterized by the abnormal accumulation of aggregated and proteins and these disorders are collectively known as proteinopathies or protein misfolding diseases. Among these, AD, Parkinson disease, and Huntington disease, demonstrate the impairment of the mTOR-dependent autophagic pathway, one of the main pathologic mechanisms that drive the neurodegenerative process. In agreement, the decreased autophagic function with aging suggests its involvement into the chronic build-up of aggregates in neurons (Di Domenico et al., 2014).

3.2.1. Alzheimer disease

AD is the leading cause of dementia in the elderly population and it is estimated that more than 30 million people worldwide are living with AD. Approximately 95% of AD cases are sporadic and of unknown aetiology, while the remaining familial cases are caused by mutations in one of three genes, presenilin 1 and 2, and amyloid precursor protein (APP) (Querfurth and LaFerla, 2010). AD is characterized by the presence of extracellular senile plaques and intracellular neurofibrillary tangles. Senile plaques are mainly composed of amyloid β-peptide (Aβ), proteolytic products of transmembrane APP, whose sequential cleavage by β-secretase and γ-secretase generates Aβ fragments (Tan et al., 2014). The aggregated hyperphosphorylated tau, a microtubule-associated protein, is the main component that drives the intracellular accumulation of neurofibrillary tangles (Querfurth and LaFerla, 2010). According to the amyloid hypothesis, Aβ plays a fundamental pathogenic role in AD, as it initiates a deleterious cascade in brain and ultimately leads to cognitive impairment (Butterfield et al., 2013). Combined evidence from genetic, neurobiological, molecular and behavioural studies suggest that decreased clearance of aberrant conformations (monomers, dimers, oligomers and protofibrils) of Aβ is a significant contributor to synaptic defects, thus supporting the crucial role of the mTOR signalling with AD progression (Holtzman et al., 2011).

In the last decade mTOR signalling has been extensively analysed in AD brain and in AD mouse models demonstrating an aberrant upregulation during the development of the neurodegenerative process (Oddo, 2012; Pei and Hugon, 2008; Richardson et al., 2014). Evidence from post-mortem human AD brains indicates that the levels of phospho-mTOR at Ser-2448 and at Ser-2481, and two of its downstream targets, p70S6K and eIF4E, are increased in hippocampus and in other brain areas (Griffin et al., 2005; Li et al., 2005; Pei and Hugon, 2008; Sun et al., 2014). In addition, mTOR hyperactivity correlated with Braak stages and/or cognitive severity of AD patients. In addition, recent studies on the inferior parietal lobule (IPL) brain region of AD and MCI subjects demonstrated the hyperactivation of mTOR and its downstream signals p70S6K and 4EBP, occurs early in the progression of AD (Tramutola et al., 2015), but no differences between pre-clinical AD (PCAD) subjects compared to controls were observed. Consistent with these results, numerous studies demonstrated that the mTOR upstream signaling pathway, the PI3-K/Akt axis, is impaired in AD brain (Martin et al., 2001; Wei et al., 2002). Increased PI3-K activation, Akt activation and its altered subcellular, and decreased levels, altered subcellular localization and inactivation of PTEN localization, has been described in hippocampal and cortical neurons of AD brain (Kwak et al., 2010; O'Neill, 2013; Pei et al., 2003; Sonoda et al., 2010; Zhang et al., 2006). Another upstream regulator of mTOR, the extracellular signal-regulated protein kinases (ERK1/2), which phosphorylate and inhibit TSC1/TSC2, were activated in brains from AD patients (Pei et al., 2002; Swatton et al., 2004). The sustained activation of neuronal PI3-K/Akt/mTOR signaling in AD brain was reported to cause insulin receptor substrate 1 (IRS1) inhibition, disabling normal activation of PI3-K/Akt by insulin (Gupta and Dey, 2012; O'Neill et al., 2012). Increased IRS-1 inhibitory phosphorylation was demonstrated in the brain of MCI and AD subjects

and animal models of AD providing further insights into the molecular link between AD pathology and insulin resistance (Caccamo et al., 2011; Tramutola et al., 2015).

Autophagy, one of the best-characterized downstream pathways regulated by mTOR, has been largely implicated in AD neurodegeneration (Orr and Oddo, 2013). Several studies suggested that reduced autophagy in AD brain and animal models, leading to the accumulation of protein aggregates is likely caused by the hyperactivation of the PI3-K/Akt/mTOR axis (Di Domenico et al., 2014; O'Neill, 2013; Richardson et al., 2014). Beclin 1, a protein involved in the initiation and execution of autophagy, is reduced in human AD brains at both the mRNA and the protein levels (Pickford et al., 2008; Rohn et al., 2011). Further, other markers of autophagosome formation (e.g., LC3 II/I) and of autophagic flux are reduced in AD brain and its early stages (Hung et al., 2009; Salminen et al., 2013; Tramutola et al., 2015). Pharmacological reduction of mTOR hyperactivity in the brains of AD mice models mice was shown to restore autophagy as indexed by a significant increase in LC3II and other autophagy related proteins, including Atg5, Atg7 and Atg12 (Caccamo et al., 2010, 2011; Halloran et al., 2012; Majumder et al., 2011; Richardson et al., 2014; Spilman et al., 2010).

A chronic deterioration of the neuronal autophagy-lysosome system is likely to be a key event in AD neurodegeneration by allowing Aβ production and aggregation and its lower clearance. Indeed, the autophagy-lysosome pathway is an important regulator of APP processing because it regulates the clearance of amyloidogenic fragments of APP and also the major cellular pathway for the removal of Aβ aggregates (Cai et al., 2012; Di Domenico et al., 2014; Funderburk et al., 2010; Mizushima et al., 2008; Nixon, 2007; Salminen et al., 2013; Shintani and Klionsky, 2004). Accordingly, APP transgenic mice depleted of Beclin-1 show significant accumulation of both intraneuronal and extracellular Aβ deposition with marked neurodegeneration (Pickford et al., 2008). Further, pharmacological increase of autophagy via mTOR inhibition led to a reduction in soluble Aβ and tau levels in cell culture and mice models of AD (Bove et al., 2011; Caccamo et al., 2010, 2011; Majumder et al., 2011; Richardson et al., 2014; Spilman et al., 2010). These data were further supported by research showing that genetically increasing autophagic protein turnover ameliorates Aβ pathology and the associated cognitive decline in mice (Yang et al., 2011).

In turn, several studies have shown that diverse Aβ species including monomers and soluble oligomers, over–activate the PI3-K/Akt/mTOR axis, (Caccamo et al., 2010; Gupta and Dey, 2012). Aβ monomers and soluble oligomers bind to IRs, can induce IR internalization in neurons and remove IR from dendrites, imitating what is seen in AD brain (De Felice et al., 2009; Moloney et al., 2010; Zhao et al., 2008, 2009). Moreover, Aβ oligomers block IR activation in vitro and can also inactivate IRS-1 by phosphorylation at serine residues, which associates mechanistically with Aβ-induced activation of JNK via TNFα (Bomfim et al., 2012; Ma et al., 2009). Furthermore, Aβ can increase PI3-K/ Akt and mTOR activation (Caccamo et al., 2010, 2011; Majumder et al., 2011; Zhao et al., 2008). Indeed, levels of Aβ oligomers in AD brain have been correlated with increased phosphorylation of IRS-1 at its inhibitory residue, and with the increased activation of kinases including Akt and mTOR, that are able to target this residue (O'Neill, 2013; Tramutola et al., 2015). Primary neurons exposed to different concentrations of synthetic Aβ monomers and Aβ oligomers showed the up-regulation of mTOR signalling (Bhaskar et al., 2009). In line with these findings injection of naturally secreted Aβ increases mTOR signalling in brains of wild-type mice, and the hippocampi of 12-month-old APP mice, which are characterized by widespread Aβ accumulation, demonstrate the hyperactivation of mTOR (Caccamo et al., 2011).

Post-mortem analysis from human AD brains and studies of mouse models of the disease indicate a link also between mTOR signalling and tau neuropathology (An et al., 2003; Griffin et al., 2005; Pei et al., 2008; Pei and Hugon, 2008; Tramutola et al., 2015). Indeed, the genetic increase mTOR signalling was sufficient to increase tau levels and phosphorylation (Caccamo et al., 2013). There is evidence that dysfunction of the autophagy-lysosome system contributes to the formation of tau oligomers and insoluble aggregates (Hamano et al., 2008; Tan et al., 2014). It is hypothesized that sustained activation of PI3-K/Akt/mTOR, either independent or as a consequence of Aβ modulation, impact directly on all the major features that makes tau pathological in AD, including hyperphosphorylation, missorting, translation and conformational and functional changes (Di Domenico et al., 2014). Many of the kinases and phosphatases implicated in both the functional and pathological phosphorylation of tau in AD are direct components of or interact with the PI3-K/Akt pathway including Akt, GSK3β and PP2A (Zhu et al., 2013).

3.2.2. Down syndrome

Down syndrome (DS), a genetic condition characterized by the triplication of chromosome 21 (HSA21), is the most frequent chromosomal abnormality that causes neurologic deficiencies worldwide. Life expectancy of the DS population increased significantly in the last decades due to improvement in health care, particularly in younger individuals. However, increased lifespan is associated with an increased incidence of AD neuropathology with deposition of senile plaques, containing Aβ peptide, and NFTs, composed of hyperphosphorylated Tau and dementia after the age of 40s (Butterfield et al., 2014; Perluigi et al., 2014a). The neuropathology of AD, in DS, is complex and likely involves impaired mitochondrial function, defects in neurogenesis, increased oxidative stress, and altered proteostasis as result of triplication of HSA21 genes. (Butterfield et al., 2014) However, precise mechanisms by which trisomy 21 lead to the early onset of AD remain to be elucidated.

Our groups investigated the status of the PI3K/Akt/mTOR pathway in the frontal cortex from DS autopsy cases without AD neuropathology (typically under the age of 40 years) and DS with AD neuropathology (Perluigi et al., 2014b). Our results showed a hyperactivation of the PI3K/Akt/mTOR axis in the brains of subjects with DS with or without AD pathology in comparison to healthy individuals. The hyperactivation was demonstrated by increased levels of the phosphorylated forms of the component of the axis PI3-K (p85 subunit at Tyr-508), Akt (at Ser-473), and mTOR (at Ser-2448) and as well as alteration of its downstream signalling target p70S6K, which is increasingly phosphorylated at Thr-389, and autophagy (Perluigi et al., 2014b). In addition, we posed evidences for the hypothesis that the overactivation of PI3-K/Akt/mTOR, directly or indirectly, was able to reduce IRS-1 activity by increasing its inhibitory phosphorylation in DS (Perluigi et al., 2014b). Moreover we suggest that aberrant activation of the PI3-K/Akt/mTOR axis, acting in parallel with other kinases overexpressed in DS (DYRK1A and RCAN1), contributes to the hyperphosphorylation of tau. The analysis of the expression patterns and cellular distribution of the components of the mTORC1 pathway in human hippocampi of DS subjects during prenatal, early postnatal development and in presence of AD pathology was performed (Iyer et al., 2014). The study showed the prenatal upregulation of pS6 and p70S6K, that persisted throughout postnatal development, while also was detected also the upregulation of p4E-BP1 and mTOR in DS hippocampi postnatally (Iyer et al., 2014). This study also confirmed the upregulation of mTORC1 components and downstream signals in DS-AD patients, showing a positive correlation with total tau and p-tau (Iyer et al., 2014). A previous study by our groups reported that DS brain shows an early disturbance of the autophagy pathways (Di Domenico et al., 2013). Indeed, we identified oxidation of the V_0-ATPase pump and cathepsin D together with decreased autophagosome formation (Di Domenico et al., 2013). The hyperactivation of the Akt-mTOR pathway was also demonstrated in the dendrites of hippocampal neurons in Ts1Cje mice, a DS mouse model (Troca-Marin et al., 2014). The authors demonstrated that the levels of p-Akt, p-mTOR (Ser-2448), p-p70S6K (Thr-389), p-S6 (Ser-235/236), and p-4EBP1 (Ser-65) were increased approximately 2-fold in dendrites of Ts1Cje- derived hippocampal neurons (Troca-Marin et al., 2014). The increased hippocampal levels of BDNF and pro-BDNF in Ts1Cje mice suggested that the hyperactivation of the Akt-mTOR cascade might be due to the increased BDNF

signalling (Troca-Marin et al., 2011). In addition increased p-Akt (Ser473) that might lead to a possible dysregulation of mTOR levels have been reported in Ts65Dn hippocampal extracts (Siarey et al., 2006).

4. mTOR as a therapeutic target

Considering that mTOR signalling pathway is involved in multiple processes regulating neuronal functions, it is an attractive candidate to study age-related cognitive decline (Godoy et al., 2014). In the last decade, several studies focused on the effect of mTOR on lifespan and experimental evidence showed that mutations in mTOR increased the lifespan of yeast (Kaeberlein et al., 2005), *C. elegans* (Vellai et al., 2003), and *Drosophila* (Kapahi et al., 2004). These data were the first evidence to demonstrate that increased longevity could be achieved through reduction of mTOR signaling and suggested that rapamycin, a specific TOR inhibitor, might slow aging in different species. Rapamycin is a metabolite produced by the soil bacterium *Streptomyces hygroscopicus* and was first classified as an antifungal drug (Loewith, 2011; Vezina et al., 1975). Later, additional pharmacological properties were characterized including immunosuppressive effects and antitumor activity. Although use as a fungicide is outdated, rapamycin analogs (rapalogs) are currently used as an adjunctive therapy in clinics to prevent host rejection in transplants, as a monotherapy for cancer treatments, and in drug-eluting stents to prevent re-stenosis of cardiac vessels (Camardo, 2003). Because of the powerful and multifaceted therapeutic effects, much effort has been devoted toward understanding the most important mechanisms of action. The finding that rapamycin is able to extend lifespan in mice and delay many age-related deficits has led to speculation that rapamycin is an 'anti-aging' drug. Several studies have examined the effect of rapamycin on various parameters of healthspan, showing its ability to improve selected physiological functions - normally affected during aging- while other functions showed no changes (Richardson, 2013).

Based on the fact that one of the major consequences of aging is the occurrence of a wide variety of diseases, it is conceivably that rapamycin might have a broad protection against age-related neurodegenerative diseases. The first evidence demonstrating the effect of orally administered rapamycin on memory was performed in a Tg mouse model of AD, 3xTg-AD and hAPP (J20) mice, by the laboratories of Oddo and Galvan, respectively (Oddo, 2012; Spilman et al., 2010). Results from the two groups showed that rapamycin treatment prevented the loss of memory/cognition in two different transgenic mouse models of AD (Caccamo et al., 2010; Spilman et al., 2010). Overall, collected results showed that the loss of memory in Tg-AD mice could be prevented by rapamycin if given shortly after the onset of memory impairment, but not if given at late stages of the disease after robust plaque and tangle deposition. Memory improvement was associated with reduced Aβ levels and fibrillar aggregates in the Tg AD mice (Lin et al., 2013; Majumder et al., 2011). In addition, rapamycin reduced tau-aggregation as well as microglia activation.

Furthermore, rapamycin can reduce the burden of accumulating misfolded proteins through the induction of autophagy (Cortes et al., 2012). This is a likely mechanism through which reduction of plaques and tangles in the brains of the AD-Tg mice is achieved. The success of rapamycin treatment is based on its ability to modulate pathways fundamental to aging, the strongest risk factor for AD.

Rapamycin also appears to have very broad effects on the CNS that help to maintain vascular function and prevent inflammatory events. The FDA has approved rapamycin and several "rapalogs" for the treatment of renal cancer, allograft rejection, subependymal giant cell astrocytoma associated with tuberous sclerosis, and neuroendocrine pancreatic tumors, as well as the prevention of vascular re-stenosis (Maiese et al., 2013). Further, the toxicity profiles of rapamycin and its rapalogues are well characterized (Soefje et al., 2011).

However, the treatment paradigms for mTOR signaling also require further investigation. Studies suggest that the degree and duration of mTOR signaling may be an essential factor for neuroprotection. Further studies are needed to investigate time and duration of treatment as well as new administration route that may by-pass immunosuppressive side effects.

5. Conclusions

There is compelling evidence that cellular mechanisms and signaling pathways regulating aging and age-related neurodegenerative disorders are controlled by mTOR. This signaling network is complex, with many downstream physiological outputs, and thus the mechanisms underlying its age-related effects have not been fully elucidated. In the current review the central role played by mTOR signaling in energy metabolism and autophagy, both of which are finely regulated by mitochondria were highlighted. These pathways are intimately connected within a complex network of signals that are essential for a healthy longevity. It is likely that alteration of one or more of the components of the PI3-K/Akt/mTOR pathway has effects throughout the entire system. Upregulation of the mTOR signaling pathway is thought to play an important role in major pathological processes of AD. Targeting mTOR in the CNS can offer new opportunities for drug discovery; however, further studies are needed for a comprehensive understanding of mTOR, which lies at the crossroads of multiple metabolic and signaling pathways involved in AD etiology and pathogenesis.

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