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mTOR-dependent signalling in Alzheimer's disease

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Abstract

Neurodegeneration and neurofibrillary degeneration are the two main pathological mechanisms of cognitive impairments in Alzheimer's disease (AD). It is not clear what factors determine the fates of neurons during the progress of the disease. Emerging evidence has suggested that mTOR-dependent signalling is involved in the two types of degeneration in AD brains. This review focuses on the roles of mTOR-dependent signalling in the pathogenesis of AD. It summarizes the recent advancements in the understanding of its roles in neurodegeneration and neurofibrillary degeneration, as well as the evidence achieved when mTOR-related signalling components were tested as potential biomarkers of cognitive impairments in the clinical diagnosis of AD.

Keywords: mTOR • Alzheimer's disease • neurodegeneration • neurofibrillary degeneration

Introduction

mTOR is abbreviated from the 'mammalian target of rapamycin'. The lipophilic macrolide rapamycin (or sirolimus) is used as an immunosuppressant and an anti-cancer drug. Rapamycin inhibits cell proliferation and growth through its specific interaction with a cellular protein receptor called FK506-binding protein (FKBP12) [1–3]. Through molecular modifications induced by rapamycin, two genes, TOR1 and TOR2, were found in mammalian cells [1]. Correspondingly, two functionally distinct proteins called mTORC1 and mTORC2, each with a molecular mass of 1.5–2.0 MD, were identified by gel filtration [4–6]. This implies that mTOR associates with many distinct cellular protein complexes. The molecular weight of mTOR itself is approximately 250–290 kD. Only mTORC1 is sensitive to rapamycin [7–9]. The carboxy-terminal region of mTOR contains the FKBP12-rapamycin binding (FRB) domain, the catalytic domain that shows homology to phosphatidylinositol kinases, and the FATC sequence. The amino-terminal region of mTOR contains ~20 tandem HEAT repeats (each HEAT repeat consists of two α helices of $<$40 amino acids, each with a specific pattern of hydrophobic and hydrophilic residues), the so-called FAT domain (for FRAP, ATM, TRAP) [10]. Although mTOR contains a lipid kinase-like domain within its C-terminal region, it does not possess detectable lipid kinase activity, but instead it functions as a serine / threonine (S/T) kinase.

Cell signalling through mTOR is regulated by cellular energy level as well as by mitogens and nutrients [1]. As a main modulator of cell growth and proliferation, mTOR controls the efficiency of protein translation within cells *via* its downstream targets. These translation regulators include the eukaryotic initiation factor 4E binding protein 1 (4E-BP1), the 70 kD ribosomal protein S6 kinase (p70S6K) and the eukaryotic elongation factor 2 (eEF2) (Fig. 1). Although neurons are finally differentiated, the size of the neuronal cell soma in diseased conditions (*e.g.* hypertrophy) is regulated by mTOR [11]. Recent studies indicate a broader role for

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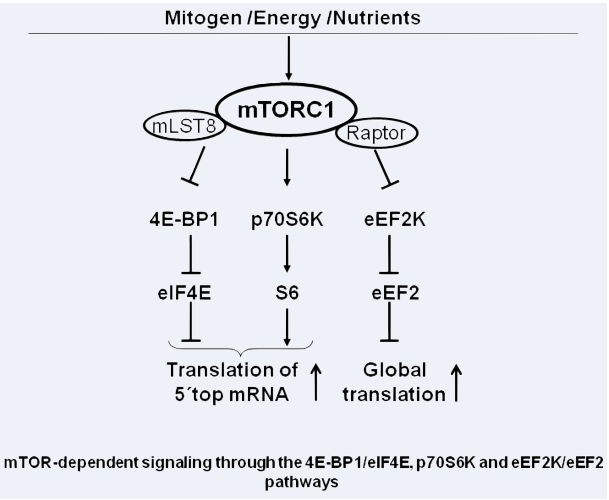

Fig. 1 This figure summarizes the downstream signalling targets of mTORC1 and the consequences of their activation on translation.

mTOR in the physiological processes of neuronal development, as well as in the neuropathological processes of different brain diseases [3, 12].

Neurodegeneration and neurofibrillary degeneration in Alzheimer's disease

Neurodegenerative diseases form a subgroup of neurological disorders in which neurons in the central nervous system are progressively lost. Diseases involving neurodegeneration include Alzheimer's disease (AD), Parkinson's disease, Creutzfeldt-Jakob disease, Huntington disease and amyotrophic lateral sclerosis. The most consistent risk factor for developing neurodegenerative diseases is aging.

The pathogenesis of AD is multi-aetiological, and it is likely because of these various origins that AD has diverse neuropathological changes. The most prominent lesions in AD brains are atrophy, a large number of senile plaques (SPs) formed by amyloid beta (Aβ) between neurons and neurofibrillary tangles (NFTs) made of abnormally hyperphosphorylated tau protein in neurons [13]. The neurodegeneration is hardly visible under the microscope, but it is indeed involved in the dysfunction of memory and intellectual processing in AD patients [14]. Aβ is the major aetiological agent according to the amyloid cascade hypothesis, by initiating a series of events such as synaptic and neuritic injuries, microglial and astrocytic activations (inflammatory response), altered neuronal ionic homeostasis, oxidative damages, changes in the activities of kinases or phosphatases involved in the formation of tau hyperphosphorylation, and cell death [15]. The neurodegeneration and neurofibrillary degeneration in AD brains may also be caused by other mitogenic factors, such as zinc or deficiency of growth factors or estrogens, etc. [16, 17].

AD pathogenesis is complicated by the fact that two different pathways are detected in neurons: neurodegeneration and neurofibrillary degeneration, which share many regulators such as mTOR-dependent signalling components. Neurodegeneration incorporates different abnormal signalling pathways that lead to neuronal loss, including apoptosis and other modes of cell death. Neurofibrillary degeneration is a specific type of neuronal reaction marked by the accumulation of hyperphosphorylated tau protein as paired helical filaments in NFTs, which could also induce abnormal neuronal metabolism and death. The following discussion is focused on the findings and significance of mTOR-dependent signalling in the brains and peripheral tissues of AD patients, and in the cellular and animal models of AD from Dr. Jin-Jing Pei's group at Karolinska Institutet, Sweden and Dr. Jacques Hugon's group at the Institut du Fer à Moulin Inserm, France.

Dysfunction of protein synthesis mediated by mTOR-dependent signalling in Alzheimer's disease brains

To maintain neuronal homeostasis, neurons need to continually synthesize new proteins. Alteration in protein synthesis was detected in AD brains about two decades ago [18, 19]. A few years ago, attention was again paid to protein synthesis in AD research. Together with other groups, studies from both laboratories mainly using phospho-specific antibodies for activated kinases on signals that control protein translation reported an elevation, in brain regions affected in AD, of phosphorylated (p-) double-stranded RNA-dependent protein kinase (PKR) [20–22], p- eukaryotic initiation factor 2 alpha (eIF2α) [22], and p-p70S6K at the T389 site or at the T421/S424 sites [23].

PKR is a pro-apoptotic kinase that attenuates protein translation through the regulation of eIF2α phosphorylation. In 2002, Chang *et al.* [22] reported an increased expression of p-PKR and p-eIF2α in degenerating hippocampal neurons of AD brains. Also in 2002, the same group published another study showing that Aβ peptide was able to activate PKR and that Aβ neurotoxicity was reduced in cultured neurons originating from PKR knock-out mice [24]. Aβ toxicity was able to activate PKR through calcium signalling and caspase 8 activation [25]. Increased PKR expression in AD brains was confirmed by other groups [20, 21], and augmented levels of p-PKR were also found in APP/PS1 knock-in mice [26]. In AD brains, activated PKR was observed in the cytoplasm of hippocampal neurons and in the neuronal nucleus of other cortical areas such as the frontal cortex.

Intraneuronally, p-p70S6K appeared especially in neurons that are predicted to develop NFTs at later stages [23]. By indirect enzyme-linked immunosorbent assay, p-p70S6K (T389 or T421/S424) studies showed significant increases in homogenates of the medial temporal cortex from 22 AD patients as compared to

13 control brains. In the same set of tissues from AD and control cases, we found a dramatic increase for p-eIF4E, p-mTOR (S2481) and p-4E-BP1 (T70 and S65) in AD brains [27, 28]. The increase of mTOR-dependent signalling was positively and significantly correlated with total tau and p-tau. The mTOR-dependent signalling is thought to be involved in the translation of a special group of proteins including tau with 5'top mRNA. Thus, it is speculated that the decade-long continuous synthesis of tau in degenerating neurons is regulated by up-regulated mTOR-dependent signalling through the p70S6K and 4E-BP1 pathways [29].

The total level of eukaryotic elongation factor 2 (eEF2) is significantly decreased in AD when compared to controls [28]. Elongation factor-2 kinase (eEF2K) is a Ca^{2+}/calmodulin-dependent protein kinase that is highly specific for eEF2. The level of p-eEF2K was significantly increased in AD brains. In contrast to other mTOR-dependent signalling components, eEF2K is inactivated once it is phosphorylated. An acidic pH of 6.5 is regularly achieved in various brain regions during ischaemia and hypoxia, and the hippocampus is the most affected region [30, 31]. Global protein synthesis during tissue acidosis was inhibited as eEF2K is activated in a pH-dependent manner [32]. Although these two pathological conditions showed opposite effects on the level of tau phosphorylation, an increase during ischaemia and a decrease during hypoxia [33, 34], yet acidic brain pH values in AD brains [35] might activate eEF2K, thus causing reduced global translation [28, 36, 37].

Global inhibition of protein synthesis is widely recognized as a response of biological systems to oxidative stress [38, 39]. But the translation of some proteins is not inhibited and the translational control of specific mRNAs is required for survival during cell growth under stress conditions [40, 41]. This mechanism might give the exposed cells an enhanced ability of resistance to the onset of neurodegeneration (as will be discussed later). Ribosomes (polyribosomes) are specialized complexes that are responsible for mediating protein synthesis, and they are composed of nucleic acids and proteins. Specialized nucleic acids, rRNA and tRNA molecules, are essential for ribosomes to translate mRNA into proteins. There is a significant impairment in ribosome function in AD brains [36, 37], and this impairment is very likely associated with the reduced synthesis of global proteins.

Neurodegeneration mediated by mTOR-dependent signalling

An up-regulation of p70S6K phosphorylation at the T389 site is induced in *Drosophila* tauopathy models that overexpress wild-type or mutant tau (tau^{R406W}). Neurodegeneration in the brains of flies, quantified by counting TUNEL-positive neurons, is suppressed by feeding flies mTOR-specific inhibitor rapamycin [42]. mTOR is thought to enhance tau-induced neurodegeneration in a cell cycle-dependent manner, and to drive cell-cycle activation and apoptosis in post-mitotic neurons. In these models, p70S6K activation does not mimic AD anomalies because there is no evidence that tau is genetically changed in AD [43] and because only normal copies of tau mRNA exist in the AD brain [44–46]. In this study, the evaluation of mTOR activation using phosphorylation levels of p70S6K at the T389 site as 'readout' might not be completely accurate, because in addition to p70S6K, the main downstream effectors of mTOR also include eIF4E and eEF2. Moreover, because of the availability of phospho-antibodies to p70S6K, most data generated for p-p70S6K were obtained with antibodies either to the T389 or the T421/S424 sites of p70S6K. Although some authors believe that T389 is the best site phosphorylated by mTOR and the major site with which rapamycin interferes to attenuate p70S6K phosphorylation [47], the T421/S424 sites are more sensitive to rapamycin in human SH-SY5Y neuroblastoma cells pretreated by zinc [48].

In differentiated mouse N2a neuroblastoma cells (N2a cells) treated with Aβ42, we indeed found a dramatic decrease for p-mTOR at the S2448 site and for p-p70S6K at the T389 site [49]. In differentiated human SH-SY5Y neuroblastoma cells (SH-SY5Y cells), a consistent reduction for p-mTOR at the S2448 site and significant increases of p-p70S6K at the T389 and the T421/S424 sites were induced by Aβ42 [15]. A dramatic increase of p-p70S6K was seen in mouse neuroblastoma N2a cells, human neuroblastoma SH-SY5Y cells and cultured rat brain slices treated with zinc [48, 50].

The main difference in these experiments is that zinc was added to the culture media after cells were starved for 1 or 2 days, while Aβ42 was added to differentiated N2a cells and differentiated SH-SY5Y cells without equilibrium time for cells to react to 10% serum deprivation after retinoic acid treatment [15, 49]. The dramatic reduction of p-mTOR seen in differentiated N2a cells should be considered as the combined effects of two cell stresses: serum deprivation and Aβ42 treatment [49]. It is interesting to note that p-p70S6K expression in cells exposed to the sudden serum deprivation and Aβ42 treatment was different from p-mTOR expression in both differentiated N2a cells and differentiated SH-SY5Y cells [15, 49]. The increase in p-p70S6K levels in differentiated SH-SY5Y cells treated with Aβ42 [15] is approximately consistent with the augmentation seen in undifferentiated SH-SY5Y cells treated with 100 μM zinc [48]. Another difference is that when tau phosphorylation was investigated, only the bottom-attached cells were harvested. In other experiments, at the end of the treatment, attached cells and cells collected from the supernatant after centrifugation (apoptotic or necrotic cells) were analysed [15, 49].

Levels of p-mTOR at the S2448 site and of p70S6K at the T389 site are decreased in the cortex but not in the cerebellum (devoid of plaques) of double APP/PS1 transgenic mice (carrying the Swedish and London mutations KM670/671 and V717, thy 1 promoter) compared with control mice [49]. However, in SH-SY5Y cells treated with 20 μM Aβ, p-mTOR at the 2448 site was down-regulated and both p-ERK1/2 and p-p70S6K at the T421/S424 sites (15 min.) and at the T389 site (30 min.) were up-regulated [15]. The reduced mTOR phosphorylation is associated with caspase 3 activation, and the inhibition of mTOR by rapamycin enhances Aβ-induced cell death [15]. A similar response of ERK1/2 and p70S6K was observed in rat brain slices exposed to

the selective inhibition of protein phosphatase (PP) -2A or in human SH-SY5Y cells treated with 100 μM zinc [48, 51], but in these two conditions, tau phosphorylation was increased. Moreover, insulin-like growth factor-1 is able to markedly increase p70S6K phosphorylation (T389) controlled by mTOR and to reduce caspase-3 activity, but its protective effect on Aβ-induced cell death is mediated *via* an mTOR-independent pathway. It is interesting to note that in SH-SY5Y cells, Aβ neurotoxicity induces the activation of ERK1/2 kinase, which can consequently phosphorylate p70S6K at both the T389 and T421 sites. This result could explain the difference between the mTOR and p70S6K activations in this model. These data demonstrate that mTOR plays an important role as a cellular survival factor in Aβ toxicity and could represent a possible target for modulating the toxicity of Aβ or zinc.

Tau phosphorylation mediated by mTOR-dependent signalling

In SH-SY5Y cells and primary cultured neurons from rat cortical cortex that are treated with 100 μM zinc, an increased expression and phosphorylation of tau was observed [23, 52]. Pretreatment of cells with rapamycin attenuated the effects induced by zinc. Recently, it was found that inhibition of mTOR and the phosphoinositide 3-kinase signalling pathway activates PP2A and glycogen synthase kinase 3β (GSK3β), the coordinated regulation of PP2A and GSK3β is thought to ensure a balanced tau phosphorylation [53]. The direct role of p70S6K on tau phosphorylation was observed *in vitro* on S262, S214 and T212 sites [54, 55]. A significant increase of p-p70S6K at the T421/S424 and T389 sites was found in N2a cells carrying human APP with Swedish mutation (APPswe) and in the homogenates from the brain regions of transgenic APPswe/PS1 (A246E) mice where most SPs localize, as compared with respective controls. Levels of these phosphorylation sites of p70S6K correspond to the increase of tau phosphorylation at the S262 site [56]. This parallel increase in p70S6K activation and tau phosphorylation could be demonstrated by treating wild-type N2a cells with Aβ25-35. So far, we do not know if other mTOR-dependent signalling components are directly involved in tau phosphorylation. However, studies from Karolinska Institutet have suggested that both Aβ and zinc can activate mTOR-dependent signalling, resulting in the increase of tau synthesis and phosphorylation.

mTOR-dependent signalling in lymphocytes of AD patients: potential biomarkers for AD diagnosis

The control of protein translation involving mTOR, p70S6K, eIFα and PKR modulates cell survival and cell death, and these proteins are increased in AD brains [20–23, 27, 28]. Studies from Hugon and colleagues demonstrated that expressions of p-p70S6K, p-mTOR, p-eIFα and p-PKR are significantly modified in the lymphocytes of AD patients [49, 57]. For example, the p-p70S6K/total p70S6K ratio was reduced in AD patients compared to control individuals, and the p-PKR/total PKR ratio was increased in AD patients and their levels statistically were correlated (positively or negatively) with Mini Mental Status Examination scores or scores of cued recall tests [58, 59]. It is unknown why the lymphocytes of AD patients display modified levels of activated kinases that are implicated in the control of translation and cell survival. Previous reports have emphasized the altered characteristics of lymphocytes in AD patients, such as increased sensitivity to apoptosis [57, 59]. These cellular abnormalities could be linked either to toxic factors released by the brain into the blood or to a general cellular dysfunction that includes neurons and lymphocytes (such as a diffuse reaction to inflammation) and that could be expressed in immune cells by these modified signalling pathways. Further studies that analyse the levels of inflammatory cytokines in the brain, cerebrospinal fluid (CSF) and blood of AD patients could help to decipher such general mechanisms that affect various cell types. These results suggest that the phosphorylated forms of mTOR, p70S6K, eIF2α, and PKR might represent putative biological indicators of cognitive impairments in AD and the assessment of their levels in lymphocytes could complement existing biological tests, such as the levels of Aβ42 and tau protein in the CSF.

mTOR-dependent signalling and other neurodegenerative diseases

mTOR-dependent signalling has been implicated in other neurodegenerative diseases such as amyotophic lateral sclerosis, Parkinson's disease and Huntington disease. A recent report has shown that there is an increased autophagy in transgenic mice with a G93A mutant SOD1 gene, a model for amyotophic lateral sclerosis. mTOR was shown to reduce autophagy. The ratio of p-mTOR immunopositive motor neurons over total motor neurons was decreased in the SOD1G93A transgenic mice compared to control mice, suggesting that mTOR could contribute to autophagy enhancement [60]. An increased expression of RTP801 protein that is transcripted by a hypoxia inducing factor 1 responsive gene was detected in the neuromelanin-containing neurons of the substantia nigra in patients with Parkinson's disease [61]. In cellular and animal models of Parkinson's disease, RTP801 can modulate mTOR activity, leading to cell death. In contrast, in transgenic models of Huntington disease, the application of an mTOR inhibitor can induce autophagy and reduce toxicity of polyglutamine expansion [62]. To clarify the exact anti-apoptotic or pro-apoptotic roles of mTOR and to advocate this kinase as a suitable target for neuroprotection, further studies are needed in different models of neurodegenerative diseases. For example,

mTOR can contribute to cadmium-induced neuronal apoptosis [63], but it is also an important factor in insulin-induced prevention of neuronal apoptosis in cultures exposed to serum deprivation [64].

Perspectives

mTOR-dependent signalling seems to be involved in AD brains as well as in AD models. However, evidence achieved so far tends to implicate an augmented activation of this pathway linked to tau phosphorylation and NFTs. One example of this evidence is the finding of enhanced levels of mTOR-dependent signalling in the neurons of AD brains, specifically in those neurons brains that express increased p-tau protein. Another example is the finding of a reduced activity of this kinase associated with the induction of neuronal apoptosis and Aβ neurotoxicity such as reduced mTOR-dependent signalling in neural cell cultures exposed to Aβ, in the brains of APP/PS1 knock-in mice and in the lymphocytes of AD patients. How is it possible to reconcile these diverging results? If we consider the results in AD brains, it is noticeable that NFTs are associated with increased immunostainings of p-mTOR and p-p70S6K and that p70S6K can phosphorylate tau protein *in vitro* [23, 28, 54]. But a previous study has shown that there is a weak correlation between the number of NFTs and the laminar pattern of neurodegeneration [65]. It is also interesting to note that tau can be dephosphorylated in the course of neuronal apoptosis. Experimental studies in neural cell cultures and *in vivo* have shown that neurons with p-tau protein are more resistant to apoptosis induced by NMDA toxicity or by serum deprivation [66, 67] and that neuronal apoptosis, linked to MK801 administration in rats, is associated with tau de-phosphorylation [68]. Another report has demonstrated that tau de-phosphorylation occurs at the onset of the execution phase of apoptosis and is due to the activation of PP2A-like phosphatase in cerebellar neurons [69]. It is plausible to assume that in the human brain, neurons have a different susceptibility to various cell stresses before the onset of AD pathologies (AD stresses). Aβ or Aβ oligomers could comprise a part of the culprit molecules that lead to neurodegeneration or to neurofibrillary degeneration according to the amyloid cascade hypothesis. Aβ is known to induce oxidative stress, endoplasmic reticulum (ER) stress, calcium stress, tau phosphorylation and to sensitize neurons to excitotoxicity. One group of neurons might react to these AD stresses by increasing the activity of protective pathways such as mTOR and p70S6K, whereas another group of neurons could be more sensitive to cell death triggers such as Aβ and could activate pro-apoptotic kinases such as PKR and phosphatases such as PP2A, leading to tau dephosphorylation. To understand the role of mTOR-dependent signalling in the disease processes of neurodegeneration or of neurofibrillary degeneration, it will be important to identify which group of neurons in AD brains has up-regulated or down-regulated mTOR-dependent signalling and to investigate the underlying mechanisms.

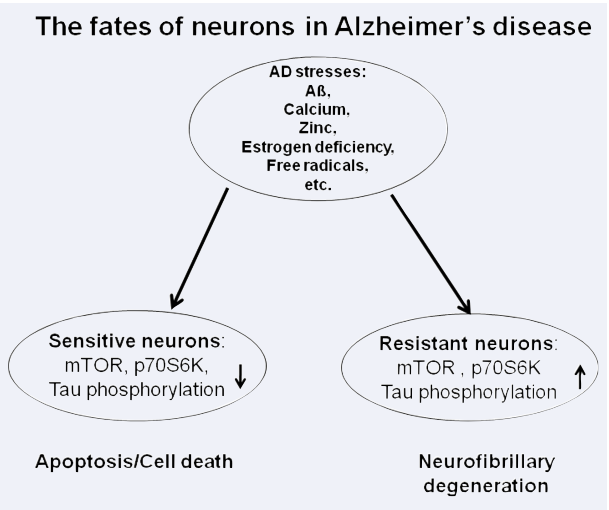

Fig. 2 This figure summarizes the possible effects of cellular stresses in neurons observed in Alzheimer's disease. In sensitive neurons to stress, it is proposed that mTOR and p70S6K activations as well as tau phosphorylation are reduced whereas in more resistant neurons, these signalling pathway are maintained.

Conclusions

The role of mTOR activation in neurodegenerative diseases, especially in AD, seems to be very complex. mTOR is mainly a trophic and protective kinase for neurons but could also be activated sometimes during the progress of neuronal apoptosis. Similarly, an mTOR inhibitor was reported to be a pro-apoptotic agent, but could alleviate the pathological consequences in a murine model of Huntington disease. These seemingly conflicting results were made more complex by the involvement of mTOR in neurofibrillary degeneration. mTOR is also implicated in the molecular mechanisms of learning and memory [12] that are impaired in AD. It is believed that mTOR is involved in the regulation of neuronal reactions, dependent on the exposure dose of stress, duration of stress, and the ability of the neurons to tolerate the stresses (Fig. 2). The understanding of these differences in neuronal sensitivities could open new avenues of research for neuroprotection.

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