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Review
mTOR Pathway – Novel Modulator of Astrocyte Activity*

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The mammalian target of rapamycin (mTOR) is a serine/threonine kinase that belongs to the phosphoinositide-3-kinase-related family and has a crucial role in the integration of growth factors, energy factors and nutrient signaling. Abnormal activity of mTOR kinase can cause many neuropathologies, including brain tumours and neurodegenerative diseases. The study confirms that the use of a kinase inhibitor – rapamycin, allows to limit proliferation including inhibition of tumor cells and immune responses. The review presents current knowledge about the role of mTOR in the modulation of nervous system activity focusing on astrocytes which are involved in the maintenance of nervous system homeostasis and support neuronal function. Astroglial activity is associated with the pathogenesis of neurodegenerative diseases like Alzheimer's disease (AD) or Parkinson's disease (PD). Effect of mTOR and its inhibitor on central nervous system functions, in particular astrocytes, is still not fully undersood.

Key words: mTOR, astrocytes, central nervous system, rapamycin.

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Characteristics of mTOR

The mammalian target of rapamycin (mTOR) otherwise known as FK506-binding protein 12-rapamycin complex-associated protein 1 (FRAP1) is a 289 kDa multi-domain protein serine/threonine kinase belonging to the family of the phosphoinositide 3-kinase (PI3-K)-related kinase (PIKK). The main characteristic feature of this family is large size (>2,500 amino acids) and a C-terminally located domain structurally related to PI3-K. The N-terminal region of mTOR is mainly composed of tandem repeats of the HEAT motif mediating protein-protein interaction (AN *et al.* 2003; AVRUCH *et al.* 2005; PERYCZ *et al.* 2008).

The kinase was first identified in the yeast *Saccharomyces cerevisiae* (HEITMAN *et al.* 1991) and later in human osteosarcoma, liver, and T cells (BROWN *et al.* 1994), and other eukaryotic cells (AN *et al.* 2003; AVRUCH *et al.* 2005). In mammals, mTOR is encoded by a single gene FRAP1 (rapamycin complex associated protein 1) (BROWN *et al.* 1994; CHONG *et al.* 2010).

mTOR functions as a catalytic subunit in two protein complexes: mTORC1 and mTORC2 (CHONG *et al.* 2010; BENJAMIN *et al.* 2011) (Fig. 1). The mTOR1 complex is composed of Raptor (regulatory associated protein of mTOR), mLST8 (mammalian lethal with Sec13 protein 8) also known as a protein homologous to β subunits of heterotrimeric G proteins (GβL), PRAS40 (praline-rich Akt substrate 40 kDa) and Deptor (DEP domain-containing mTOR-interacting protein) (MAIESE *et al.* 2013).

Similarly to mTORC1 the mTOR2 complex consists of mTOR, mLST8 and Deptor. Raptor is replaced by Rictor protein (rapamycin-insensitive companion of mTOR), to which bind HEAT domains. The complex additionally contains mSIN1 protein (mammalian stress-activated protein kinase interacting protein 1) and Protor-1 (proline-rich repeat protein-5, PRR5) (PERYCZ *et al.* 2008; WANG *et al.* 2012). Phosphorylation of each com-

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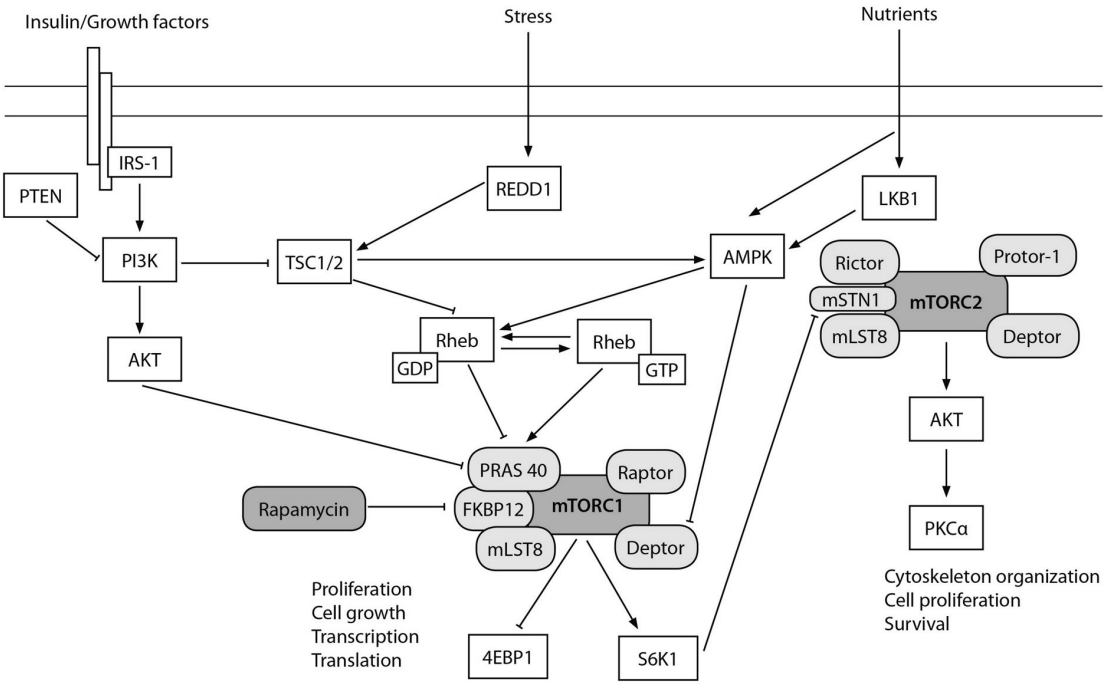

Fig. 1. The overwiew of mTOR signaling pathway. AMPK – AMP-activated protein kinase, AKT – acutely transforming retrovirus AKT8 in rodent T cell lymphoma, 4E-BP1 – eukaryotic initiation factor 4E-binding protein-1, Deptor – DEP-domain containing mTOR-interacting protein, FKBP12 – FK506 binding protein 12, IRS-1 – insulin receptor 1, LKB1 – serine-threonine liver kinase B1, mLST8 – mammalian lethal with Sec13 protein 8, mSIN1 – mammalian stress-activated protein kinase interacting protein 1, mTORC1/mTORC2 – mammalian target of rapamycin complex 1 and 2, PI3K – phosphoinositide 3-kinase, PKCα – protein kinase C alpha, PRAS 40 – proline-rich AKT substrate of 40 kDa, Protor-1 – proline-rich repeat protein-5 (PRR5), PTEN – phosphatase and tensin homolog, Raptor – regulatory-associated protein of mTOR, REDD1 – regulated in development and DNA damage response, Rheb – Ras homolog enriched in brain, Rictor – rapamycin-insensitive companion of mTOR, S6K1 – ribosomal S6 kinase 1.

ponent of the complex influences their activity and function (CHIANG & ABRAHAM 2005; ACOSTA-JAQUEZ *et al.* 2009; MAIESE *et al.* 2013).

mTOR activity is regulated by many factors: metabolic and energy changes, growth factors, mitogens, hormones and amino acids (CHONG *et al.* 2012). mTOR has a significant impact on multiple cellular functions e.g. stem cell development, proliferation and quiescence (MURAKAMI *et al.* 2004; BENJAMIN *et al.* 2011; CHONG *et al.* 2012). mTORC1 is primarily responsible for the control of protein translation by regulating the activity of the eukaryotic initiation factor 4E-binding protein-1 (4EBP1) and the serine/threonine kinase ribosomal protein p70S6K (GINGRAS *et al*. 1998). mTORC2 controls cytoskeleton reorganization through the phosphorylation of PKCα (SARBASSOV *et al.* 2004) and is responsible for contacts between cells (GULHATI *et al.* 2011).

The most common inhibitor of the mTOR pathway is rapamycin, which is already in use as an immunosuppressive and antiproliferative drug (CHIANG & ABRAHAM 2005). mTORC1 is rapidly sensitive to rapamycin (BYFIELD *et al.* 2005; BUTCHER *et al.* 2006), however chronic and prolonged administration of rapamycin also influence mTORC2 function, particularly by preventing the formation of new complexes (CAMMALLERI *et al.* 2003).

mTOR role in CNS physiology

mTOR protein is widely expressed in all crucial systems for the maintenance of homeostasis including the nervous, vascular and immune systems (BROWN *et al.* 1994; CHONG *et al.* 2010). Research conducted over the past few years has shown that mTOR regulates transcription, translation, protein degradation, intracellular vesicle transport actin cytoskeleton organization and some aspects of mitochondrial metabolism (FINGAR & BLENIS 2004; HAY & SONENBERG 2004; MARTIN & HALL 2005). Moreover, mTOR kinase activity is also essential for differentiated cells and non-dividing cells such as neurons, in which mTOR regulates differentiation and survival. In addition, the activity of mTOR is important for the development of axonal and dendritic trees and synaptogenesis (PERYCZ *et al.* 2008). Probably due to translational control processes taking place in close proximity to synapses, mTOR is essential in the phenomena of synaptic plasticity, learning and memory formation (CAMMALLERI *et al.* 2003; CRACCO *et al.* 2005; VICKERS *et al.* 2005; PERYCZ *et al.* 2008). The basis of long-term memory, long-term potentation (LTP) and long-term depression (LTD), is *de novo* mTOR protein synthesis (KELLEHER *et al.* 2004). It has been shown that

in hippocampal slices mTOR activity is essential for the expression of LTP induced by administration of brain derived neurotrophic factor (BDNF) or high frequency stimulation (TANG *et al.* 2002; CAMMALLERI *et al.* 2003). The role of mTOR in the induction of LTP is to control local protein synthesis within the region of synapses (CAMMALLERI *et al*. 2003; CRACCO *et al*. 2005; VICKERS *et al.* 2005). As in the case of LTP some forms of LTD are dependent on protein synthesis and activity of mTOR (HOU & KLANN 2004).

mTOR is involved in the regulation of energy balance of the body by taking part in the regulation of appetite, depending on the availability of nutrients. Hence, the inhibition of appetite and weight loss induced by administration of L-leucine or leptin correlates with increased activity of mTOR in hypothalamic arcuate nucleus neurons. Moreover, intracerebral administration of rapamycin prevents the anorectigenic action of leptin and leucine. In addition, fasted animals showed a decrease in the activity of mTOR in the arcuate nucleus neurons secreting orexigenic substances: neuropeptide Y and AgRP (agouti-related protein) (COTA *et al.* 2006).

A role for mTOR has been found in the regulation of glial cells by influence on oligodendrocyte development and the myelination process (DELLO RUSSO *et al.* 2013).

Astrocyte roles in the CNS

Astrocytes, also known as astroglia, are the most abundant cells in the brain and belong to the group of macroglial cells which is subdivided into other specialized cell types: ependymal cells, Schwann cells and oligodendroglia (GARCIA-SEGURA & MCCARTHY 2004). Two main types of astrocytes have been distinguished: protoplasmic and fibrous (SOFRONIEW & VINTERS 2010). The most frequently used morphological markers of astrocytes activity are glial fibrillary acidic protein (GFAP) (ENG *et al.* 2000), glutamine synthetase and S100β protein (NORENBERG 1979). Astrocytes are present in all areas of the central nervous system (BUSHONG *et al.* 2002) which indicates their commitment to maintaining nervous system homeostasis and supporting neuronal functions (PASTOR *et al.* 2009).

Astrocytes show the ability to modulate synaptic activity and are responsible for preserving neuronal integrity in conditions of disease and injury (PASTOR *et al.* 2009). Together with endothelial cells and pericytes, astroglia form the blood-brain-barrier (BBB) and induce its properties (BALLABH *et al.* 2004; ABBOTT *et al.* 2006). The 'tripartate synapse' hypothesis highlights that astrocytes also take part in processing of information by neural circuits (HALASSA *et al.* 2007; BARRES 2008). Effects on synaptic transmission are possible by regulation of the secretion of synaptically active molecules like glutamate, gamma-aminobutyric acid (GABA) or D-serine (HALASSA *et al.* 2007; NEDERGAARD *et al.* 2003; PEREA *et al.* 2009; SHIGETOMI *et al.* 2008) the regulation of release of these neurotransmitters is possible by increasing intracellular concentration of Ca^{++} (AKAOKA *et al.* 2001; ANDREIUOLO *et al.* 2009). Astrocytes are also responsible for the extracellular homeostasis of K^{+} and extracellular pH (KIRISCHUK *et al.* 2012).

A growing body of evidence indicates that astrocytes contribute to CNS metabolism. Astrocytes have a direct contact with blood vessels, neuronal perikarya, synapses and axons and can take up glucose from blood vessels and deliver it to various neuronal elements. Furthermore, astroglia provide the main store of glycogen granules, especially in the area of high synaptic density (SOFRONIEW & VINTERS 2010), which can have crucial impact on neuronal activity in pathological conditions like hypoglycemia (BROWN & RANSOM 2007).

Astrogliosis

Astrogliosis is an evolutionarily conserved defensive reaction of astrocytes that is characterized by many stages and a heterogeneous profile (SOFRONIEW 2005, 2009; SOFRONIEW & VINTERS 2010; PARPURA *et al.* 2012; VERKRATSKY *et al.* 2012). The main function of this reaction is to increase neuroprotection and trophic support of stressed neurons. Reactive astrocytes provide isolation of the damaged area from the others areas of the CNS, support regeneration of the lesion region, and reconstruction of possible damage to the blood-brain-barrier (SOFRONIEW & VINTERS 2010; ROBEL *et al.* 2011).

There are two types of astrogliosis: isomorphic (preserving morphology) and anisomorphic (changing the morphology). Isomorphic gliosis is a fully reversible state with evident hypertrophy and a series of biochemical and immunological changes which facilitate neuronal growth and synaptogenesis. Anisomorphic gliosis is characterised by cell hypertrophy, proliferative changes and the disappearance of normal domain organization resulting in the formation of permanent glial scars (SOFRONIEW & VINTERS 2010; VERKRATSKY *et al.* 2012).

mTOR and astrocyte activity – is there a connection?

Astrocyte activation has been implicated in the pathogenesis of several neurodegenerative diseases

such as Alzheimer's disease (AD), Parkinson's disease (PD) or Huntington's disease (HD), infections, trauma, ischemia and brain tumors. During these pathological conditions reactive astrocytes are capable of producing a variety of pro-inflammatory mediators, including interleukin-6 (IL-6), IL-1β, tumor necrosis factor-α (TNF-α), neurotrophic factors (DONG & BENVENISTE 2001) and potentially neurotoxic compounds, like nitric oxide (NO). The expression of inducible NO synthetase (iNOS) can be induced in different cell types and tissues by exposure to immunological and inflammatory stimuli (KLEINERT *et al.* 2004). *In vitro* study confirmed that primary astrocyte cultures express iNOS in response to cytokines such as IL-1β (CHASTRE *et al.* 2010), interferon γ (IFNγ), TNF-α, bacterial endotoxin and lipopolysaccharide (LPS) (FEINSTEIN *et al.* 1994; SIMMONS & MURPHY 1994; LISI *et al*. 2011).

Other investigations have shown that rapamycin and its derivative everolimus (RAD001) reduce iNOS expression and activity in microglial (macrophage-like cells in CNS) cultures activated by pro-inflammatory cytokines while no strong effect on astrocytes was observed. These studies demonstrate that mTOR differentially regulates iNOS activity and expression depending on the cell type or activating stimulus, with consequent variable effects in glial cells. In microglial cells rapamycin reduced iNOS mRNA expression and activity but in astrocytes no effect was observed (DELLO RUSSO *et al.* 2009). In astrocytes, a rapid significant increase in iNOS mRNA level was caused by two different pro-inflammatory stimuli. Some researchers have tested the hypothesis that rapamycin increases iNOS mRNA level in a first stage and later modifies iNOS mRNA stability. The obtained results by using primary rat astrocytes supported the hypothesis, and indicated that inhibition of mTOR kinase activity in glial cells results in anti-inflammatory actions (DELLO RUSSO *et al.* 2009). Other findings showed that mTOR controls the rate of iNOS mRNA degradation in astrocytes, which suggests possible beneficial effects of mTOR inhibitors in the treatment of inflammatory-based CNS pathologies (LISI *et al.* 2011). Recent data from DELLO RUSSO and colleagues (2013) also support the notion that mTOR kinase regulates several intracellular processes in astrocytes, such as mRNA degradation of the inducible form of NO synthase (DELLO RUSSO *et al.* 2013).

Different mTOR upstream regulators have been reported to play an important role in astrocyte physiology. Inactivation the tumor suppressor – phosphatase and tensin homolog (PTEN) – promotes astrocyte hypertrophy and proliferation (WULLSCHLEGER *et al.* 2006). Up-regulation of mTOR signaling modulates activity of glutamate transporter 1 in astrocytes (WU *et al.* 2010), whereas down-regulation of the mTOR/p70S6K kinase pathway contributes to astrocyte survival during ischemia (PASTOR *et al.* 2009). A similar pattern of response was obtained in induced epilepsy model with using a phosphatase and tensin homolog (PTEN). PTEN is mutated in autosomal dominant hamartoma and epilepsy-associated glioblastoma. Conditional PTEN knockout mice showed cortical dysplasia, ataxia, a nd seizures (BACKMAN *et al.* 2001). PTEN is a negative regulator of phosphoinositide 3-kinase (PI3K) which is located upstream of mTOR (CULLY *et al*. 2006), and treatment with rapamycin prevents seizures in this animal model (LJUNGBERG *et al.* 2009).

All these examples confirmed that disturbance of mTOR activity disrupts brain functions and the use of a mTOR inhibitor may be involved into prevention of epileptic changes.

Tuberous sclerosis complex and epilepsy

Tuberous sclerosis complex (TSC) is a multi-organ disorder, mainly caused by mutations in TSC1 and TSC2, characterized by the presence of noninvasive, tumor-like lesions (hamartomas) in multiple organ systems including brain, and is highly correlated with mental retardation, autism and epilepsy (CURATOLO *et al.* 2008). Central nervous system is one of the commonly affected target in TSC (CURATOLO & MOAVERO 2012).

Activation of the mTOR pathway with upregulation of p70S6K underlies the pathology of TSC with coincidence of epilepsy in most cases. Mutations in TSC1 and TSC2 that underlie TSC lead to hyperactivation of mTOR and contribute to increased percentage of epileptic cases in experimental models (WALTEREIT *et al*. 2006; HOLMES & STAFSTROM 2007; JOZWIAK *et al.* 2009). Use of an inhibitor of the mTOR pathway (rapamycin) early in the course of TSC can prevent pathological states like astrogliosis and reduced seizure frequency in TSC patients and mouse models of TSC (MEIKLE *et al.* 2007; ZENG *et al.* 2008).

A connection between activation of the mTOR pathway and astrocyte physiology has been noted in TSC. Hyperactivity of the mTOR pathway led to the development of subependymal giant cell astrocytomas (SEGAs) (HALLETT *et al*. 2011). SEGAs are slow-growing glioneuronal tumours, and their continued growth can block cerebrospinal fluid circulation, leading to an increase in intracranial pressure (O'CALLAGHAN *et al.* 1998). The standard treatment for patients with symptomatic SEGA in TSC is surgical resection (KANDT *et al.* 1992; CURATOLO *et al.* 2008). mTOR inhibition has been investigated as a therapeutic strategy in

patients with TSC as an alternative nonsurgical treatment of SEGA (O'CALLAGHAN *et al.* 1998). A clinical study has confirmed that prolonged treatment with mTOR inhibitor reduces the size of astrocytomas (KOENIG *et al*. 2008). After clinical trials on TSC patients, the FDA (Food and Drug Aministration) approved everolimus for the treatment of subependymal giant cell astrocytoma (SEGA) to limit growth of these cells (CURRAN 2012; KRUEGER *et al.* 2010). Currently, everolimus is the only mTOR inhibitor approved for the treatment of TSC (O'CALLAGHAN *et al.* 1998; CURATOLO 2003; CURATOLO & MOAVERO 2012).

A positive response to an mTOR inhibitor was observed in a rat pilocarpine model of temporal lobe; in this model, chronic infusion of rapamycin into the hippocampus prevented sprouting of mossy fibers (BUCKMASTER *et al.* 2009). Hence, application of rapamycin may be useful in the treatment of acquired forms of epilepsy (ZENG 2008).

One of the causes of acquired and febrile seizure is viral infection of the central nervous system (EEG-OLOFSSON 2003; GETTS *et al.* 2008). Recently, several viral proteins have been shown to interact with the mTOR pathways (BUCHKOVICK *et al*. 2008) e.g. herpes simplex virus type 1 (HSV-1) (HSIEH *et al.* 2007; MISRA *et al.* 2008), adenovirus (O'SHEA *et al*. 2005) or human immunodeficiency virus (HIV) (NARDACCI *et al.* 2005; KELLINGHAUS *et al*. 2008).

mTOR role in CNS pathology

Taking into account the number of mTOR functions in neuronal cells, it is not surprising that the abnormal activity of this kinase signaling pathways correlates with the occurrence of various types of central nervous system pathology.

Alzheimer's disease (AD) and Amyotrophic lateral sclerosis (ALS)

mTOR belongs to the proteins that are necessary for synaptic plasticity and memory consolidation in hippocampus (SLIPCZUK *et al.* 2009), although in Alzheimer's disease (AD) a role for mTOR has not been fully established. It has been noted that during AD the phosphorylation level of mTOR and tau proteins that stabilize microtubules is increased (GRIFFIN *et al.* 2005). Tau proteins and neurofibrillary accumulation can be associated with p70S6K activation (AN *et al.* 2003). In a murine model of AD it has been shown that inhibition of mTOR improves memory and reduces amyloid (Aβ) levels, which may be the result of increased autophagy (SPILMAN *et al.* 2010). Other investigations support the hypothesis that mTOR activation is necessary to prevent induction and development of AD, and reduced mTOR activity promotes the development of the disease (PACCALIN *et al.* 2006).

Reactive astrocytes are involved in the pathogenesis of AD but their exact role has not been fully determined. It is known that astrogliosis is closely related to amyloid plaques or diffuse deposits of amyloid. Reactive astroglia create neuroprotective barriers by forming miniature scars (THAL *et al.* 2000; NAGELE *et al.* 2004) and demonstrate increased expression of presenilin in sporadic form of AD but the explanation for this phenomenon has not yet been elucidated (HUYNH *et al.* 1997; WEGGEN *et al.* 1998). Reactive astrocytes may also contain different forms of amyloid beta protein (THAL *et al.* 2000; NAGELE *et al.* 2004), which indicates that this type of cell takes part in the degradation of amyloid beta deposits (WYSS-CORAY *et al.* 2003). The intensity of reactive astrogliosis is determined by glial fibrillary acidic protein (GFAP) level which is increased in the late stage of AD and simultaneously the level of astrocytes glutamate transporter declines. These factors can increase the sensitivity of local neurons to excitotoxicity (SIMPSON *et al.* 2010). Astrogliosis is additionally characterized by cellular hypertrophy and upregulation of astroglia-specific protein S100β (OLABARRIA *et al.* 2011). Hypertrophic cells are noticed often in senile plaques with activated microglia in an transgenic AD animal model (KUCHIBHOTLA *et al.* 2009). In the early stage of AD, astrocytes demonstrate atrophy as manifested by a decreased expression of GFAP, reduction in the size of the soma and decrease in the number of primary processes (OLABARRIA *et al.* 2010; KULIJEWICZ-NAWROT *et al.* 2012; YEH *et al.* 2012).

Another neurodegenerative state is amyotrophic lateral sclerosis (ALS, Lou Gehring's disease), in which pathology is based on motor neuron death in the cerebral cortex, brain stem and spinal cord (COZZOLINO *et al.* 2008). Similarly to AD, in ALS astrogliosis and astroglial atrophy are observed. The early stage of ALS is characterized by astroglial degeneration and atrophy while in the later stages reactive astrogliosis is seen. Astrocytes in ALS release neurotoxic factors and initiate microglia activation (ROSSI *et al.* 2008; ROSSI & VOLTERA 2009). Moreover, plasma membrane glutamate transport is reduced, and this can cause excitotoxicity (STAATS & VAN DEN BOSCH 2009). Other studies found that astrocytes can have a deleterious role in ALS via a missense mutation of the gene encoding superoxide dismutase (SOD) (ROWLAND & SHNEIDER 2001). This mutation leads to production by astrocytes of soluble molecules that are toxic to some motor neurons (DI GIORGIO *et al.* 2007; NAGAI *et al.* 2007). Selective silencing of the SOD1 mutant gene in astrocytes

significantly inhibits the progression of ALS in transgenic mice (YAMANAKA *et al.* 2008; WANG *et al.* 2011).

Huntington's disease (HD)

Huntington's disease (HD) is a neurological disorder that is caused by an expansion of a polyglutamine in the N-treminal fragments of huntingtin (Htt). The mutant form of huntingtin is widely expressed in neuronal cells, and is preferentially accumulated in striatal neurons and causes neurodegeneration in the brain (CLABOUGH 2013). Studies indicated that cell-cell interactions between neurons and glial cells play an important role in HD pathology (GU *et al.* 2005; GU *et al.* 2007).

Astroglial pathology in Huntington's disease is characterized by disturbance in glutamate uptake. In astrocytes there are two types of glutamate transporters: glutamate transporter 1 (GLT-1) and glutamate aspartate transporter (GLAST), which have a principal role in reutilization of extracellular excitatory neurotransmitters (ROTHSTEIN *et al.* 1996; FAIDEAU *et al.* 2010), but in HD there is dysfunction of the transporters which leads to excitotoxicity and abnormal production of neurotoxic molecules (MATUTE *et al.* 2005; LOBSIGER & CLEVELAND 2007). In addition, expression of excitatory amino-acid transporter 2 (EAAT2) and ascorbic acid content are reduced in astrocytes in HD (ESTRADA-SÁNCHEZ & REBEC 2012).

Autophagy is a major degradation pathway mainly for stable proteins, and enhanced clearance of mutant Htt by autophagy is a main mechanism in HD that allows increased survival of neurons by lowering excitotoxicity (RAVIKUMAR *et al.* 2004)

In an experimental model of HD the inhibition of mTORC1 does not cause changes in autophagy and huntingtin concentration, but decreases the levels of soluble proteins and aggregates (BERGER *et al.* 2006). Simultaneous inhibition of both mTOR complexes increases authophagy and reduces huntingtin accumulation, which indicates that many components of mTOR signaling pathway may have modulatory effect on HD pathology (ROSCIC *et al.* 2011).

Chen and others (2012) used rapamycin, an autophagy activator, to enhance autophagy in astrocytes and to investigate if the expression of GLT-1 (glutamate transporter 1) could be returned to its initial level. The study carried out on an astrocyte model of Huntington's disease (established in the astrocytes by infection with adenovirus carrying a gene with the N-terminal 552 residues of Huntingtin) showed that rapamycin is able to prevent the suppression of GLT-1 expression and glutamate uptake by mutant Htt-552 in cultured astrocytes (CHEN *et al.* 2012).

Parkinson's disease (PD)

Similarly to AD, an appropriate level of mTOR activation can be crucial for prevention and treatment Parkinson's disease (PD). Experiment in cell culture under oxidative stress conditions revealed that inhibition of mTOR activity may cause increased autophagy and death of dopaminergic neurons (CHOI *et al.* 2010). Chronic activation of the mTOR/4EBP1 pathway can be deleterious by modifying protein translation and causing loss of dopaminergic neurons, but the role of eukaryotic initiation factor 4E-binding protein-1 (4EBP1) in this process is not fully understood (IMAI *et al.* 2008; TAIN *et al.* 2009).

In PD patients upregulation of the regulated in development and DNA damage responses 1 gene (REDD1), which is a component of the stress response, is observed in dopaminergic cells (MALAGELADA *et al.* 2006; ATIYAR *et al.* 2009) and causes inhibition of mTORC1 activity (MALAGELADA *et al.* 2006; REGAZZETTI *et al.* 2012). A related hypothesis proposes that inactivating mTOR and stimulating autophagy (which prevents α-synuclein accumulation) can retain dopaminergic neurons in PD (SPENCER *et al.* 2009).

The role of astrocytes in the pathology of Parkinson's disease is not easy to establish. Findings are based mainly on post-mortem studies where reactive astrogliosis in the brain stem has been detected. Additionally in the substantia nigra a lower density of astrocytes was demonstrated compared with other brain regions, which can have pathological relevance in the development of Parkinsons's desease (MCGEER & MCGEER 2008; MENA & GARCIA DE YEBENES 2008).

Tumors in the nervous system

Malignant astrocytic gliomas such as glioblastoma (GBM) are the most common and lethal intracranial tumors. The standard treatments for GBM include surgical resection, chemotherapy and radiotherapy (ARCELLA *et al.* 2013). The occurrence of frequent mutations in regulatory genes of the cell cycle in glioma emphasizes the importance of these genes in cell proliferation and senescence. The retinoblastoma protein (RB) and p53 pathways, which regulate the cell cycle primarily by governing the G1 to S phase transition, are the major targets for inactivating mutations in GBM. The lack of these control points induces tumours by inappropriate cell division driven by phosphoinositide 3-kinase (PI3K) and mitogen activated protein kinase (MAPK) (FURNARI *et al.* 2007).

Moreover, mTOR increases activity of PI3K/Akt which plays an important role in gliomagenesis (ARCELLA *et al.* 2013; FAN & WEISS 2012). Effects of rapamycin have been tested *in vitro* and *in vivo*. Rapamycin inhibits cell growth both in the U87Mg cell line and primary cell cultures derived from GBM patients. *In vivo* study revealed that administration of rapamycin to brain xenografts in nude mice almost doubles the survival time of mice and reduces tumour volume by more than 95% (ARCELLA *et al.* 2013).

mTOR and astrocytes in the spinal cord

The role of the mTOR pathway has been studied in astrocytes in the spinal cord. Astrocytes migrate toward sites of spinal cord injury (FAULKNER *et al.* 2004), where they may have both beneficial and harmful effects on recovery, hence a target is optimising the balance between these effects. An important implication of this finding is that manipulation of the mTORC1 pathway with rapamycin might be beneficial in the treatment of spinal cord injury by limiting the astrogliosis. To determine whether the mTOR pathway is activated in reactive spinal cord astrocytes immunolabeling of spinal cord sections for phosphorylated ribosomal protein S6 (substrate of S6 kinase) has been performed (CODELUPPI *et al.* 2009). Immunolabeling detected elevated levels of phosphorylated S6 ribosomal protein in the white matter of injured spinal cord. The levels of vimentin and GFAP - two intermediate filament proteins that are upregulated in reactive astrocytes, were lowered by rapamycin administration in rats after ischemic spinal cord injury. Furthermore, a mTORC1 inhibitor decreased infiltration of GFAP-positive astrocytes into the epicenter of the injury and reduced astrocyte proliferation and migration (CODELUPPI *et al.* 2009). Hence, blockade of mTORC1 pathway with rapamycin could be beneficial in the treatment of spinal cord injury by reducing astrocyte proliferation and migration. This positive aspect can be reached by the increased Akt activation after rapamycin treatment. Akt kinase enhances astrocyte survival (MANNING & CANTLEY 2007), which could also be beneficial together with inhibition of excessive astrocyte growth and motility. Moderate astrocyte gliosis can be desirable to reduce tissue damage and neuronal cell death following injury (FAULKNER *et al.* 2004), while excessive gliosis may be limited with the use of rapamycin (CODELUPPI *et al.* 2009).

Final remarks: Astrocytes are intimately involved in pathology of most neurodegenerative disorders which reflects that these cells might be a potential therapeutic target for treatment during the development of neurodegenerative diseases. Many findings indicate that mTOR is a key element regulating activity of astrocytes not only in physiological conditions but also in pathology. A future challenge sholud be focused on defining roles of mTOR in glia, and searching for pharmacological agents act on mTOR functions in astroglia.

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