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Mammalian target of rapamycin inhibitors for treatment in tuberous sclerosis

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Tuberous sclerosis complex (TSC) is a genetic multisystem disorder that results from mutations in the *TSC1* or *TSC2* genes, and is associated with hamartomas in several organs, including subependymal giant cell tumors. The neurological manifestations of TSC are particularly challenging and include infantile spasms, intractable epilepsy, cognitive disabilities, and autism. The *TSC1*- and *TSC2*-encoded proteins modulate cell function via the mammalian target of rapamycin (mTOR) signaling cascade, and are key factors in the regulation of cell growth and proliferation. The mTOR pathway provides an intersection for an intricate network of protein cascades that respond to cellular nutrition, energy levels, and growth factor stimulation. In the brain, *TSC1* and *TSC2* have been implicated in cell body size, dendritic arborization, axonal outgrowth and targeting, neuronal migration, cortical lamination, and spine formation. The mTOR pathway represents a logical candidate for drug targeting, because mTOR regulates multiple cellular functions that may contribute to epileptogenesis, including protein synthesis, cell growth and proliferation, and synaptic plasticity. Antagonism of the mTOR pathway with rapamycin and related compounds may provide new therapeutic options for TSC patients.

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Introduction

TSC is an autosomal dominant disorder caused by the inactivation of either of 2 tumor suppressor genes, hamartin (*TSC1*) or tuberin (*TSC2*). In the normal state, the hamartin–tuberin complex activates the protein Ras homolog enriched in brain (Rheb), which inhibits mammalian target of rapamycin (mTOR). If a TSC mutation is present, mTOR is constitutively activated, leading to abnormal cellular proliferation, ribosome biogenesis, and mRNA translation. As a consequence, TSC is characterized clinically by the growth of benign tumors in multiple organs, including the brain, the heart, the kidneys, the lungs, and the skin[1]. Its incidence is estimated to be 1 in 6,000 live births[2]. The severity of the disease is highly variable, ranging from mild skin manifestations to intractable epilepsy, mental retardation, and autism[3].

Rapamycin (also called sirolimus) is an immunosuppressive drug that has recently been shown to extend lifespan in multiple species, including mammals[4]. This anti-aging property is presumably related to the mTOR-inhibiting properties of rapamycin. The mTOR pathway is crucial for the coordination of growth in response to

energy status, stress, and nutrient availability[5]. The potential anti-aging properties of rapamycin and other mTOR inhibitors, such as RAD001 (everolimus), and CCI-779 (temsirolimus) are of great interest. Unfortunately, the side effects associated with these drugs preclude research trials to study their impacts on aging in healthy individuals. In view of this obstacle, experts in the field of aging have suggested that these potential anti-aging drugs should be introduced in clinical trials for the treatment of particular diseases, and then, if appropriate, be approved for prevention of all age-related diseases in healthy individuals[6].

mTOR

mTOR is a 290-kDa serine/threonine protein kinase that is highly conserved among mammals and also has closely related analogs in lower eukaryotes, such as *Drosophila* and yeast[7]. mTOR has been implicated in numerous cellular functions, many of which are related to the fundamental processes of cell growth, survival, and homeostasis[8]. A variety of upstream signaling pathways can regulate mTOR activity in response to different extracellular stimuli or intracellular signals, including nutrient and energy status, growth factors, and stress[9]. In turn, mTOR responds to these upstream signals by modulating multiple downstream pathways, which mediate cellular growth, proliferation, metabolism, and survival, usually due to direct changes in the translation of relevant proteins[10]. Thus, during anabolic states in the presence of nutrients, growth factors, or insulin, signaling through specific upstream pathways, such as the phosphatidylinositol-3 kinase (PI3K)/Akt (protein kinase B) pathway, activates mTOR, leading to increased protein synthesis, cellular growth, and proliferation[11]. In catabolic states with nutrient/energy or oxygen deprivation, other upstream regulators, such as AMP-kinase, inhibit mTOR activity, thus decreasing protein translation and cellular growth, proliferation, and metabolism[9]. Activation or inhibition of mTOR by upstream pathways is generally accomplished through opposing effects on the tuberous sclerosis gene products, hamartin and tuberin, and on the small GTPase protein, Rheb.

The cell signaling pathway involving mTOR is further complicated by poorly defined intermediate steps, multiple feedback loops, and the formation of mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). mTORC1 and mTORC2 are functional complexes of mTOR bound to the regulatory proteins raptor and rictor respectively, which differ in their sensitivity to the mTOR inhibitor, rapamycin[12].

In addition to its functions in cellular growth and proliferation, mTOR has other important and complex roles in regulating cell survival and cell death, especially in relation to the processes of autophagy, apoptosis, and immune regulation. Autophagy involves the degradation and recycling of proteins and other macromolecules, and normally promotes cell survival under conditions of bioenergetic stress or in catabolic states where resources are limited. However, in some situations, autophagy may also mediate an alternative (non-apoptotic, autophagic) form of programmed cell death (Type II PCD), thus revealing a dual role of autophagy in promoting both cell survival and death, depending on the cellular context[13]. In anabolic states, in addition to stimulating protein synthesis, mTOR generally inhibits autophagy and thus reduces the degradation of proteins. Conversely, mTOR inhibitors, such as rapamycin, usually stimulate autophagy, with a resultant neuroprotective effect in various models of brain injury[14]. Finally, mTOR plays a critical role in immune responses via regulation of antigen-presenting cells and T-cells, and rapamycin is used clinically as a potent immunosuppressant drug. While the effects of rapamycin on autophagy, apoptosis, and immune regulation may most directly translate into neuromodulatory and neuroprotective properties, these features may also have anti-epileptogenic effects.

The clinical and therapeutic importance of mTOR is wide-reaching and continues to expand. Abnormal mTOR activity, leading to excessive cellular growth and proliferation, has been implicated in the pathophysiology of numerous human cancers, including both sporadic, isolated organ-specific and multiorgan tumors, genetic tumor syndromes. In many of these cases, specific mutations of some component of the mTOR signaling pathway has been documented, resulting in hyperactivation of mTOR or its downstream effectors.

On the basis of the physiological and pathophysiological properties of mTOR, it is reasonable to hypothesize that mTOR signaling could be involved in mechanisms of epileptogenesis[15].

mTOR inhibitors and TSC

The current main clinical complication related to TSC for which treatment with mTOR inhibitors is indicated is subependymal giant cell astrocytoma (SEGA). This complication affects approximately 15% of patients with TSC and it occurs in the pediatric age group[16]. SEGAs tend to lose their propensity to grow in the early twenties.

The traditional management approach is to monitor SEGAs with periodic neuroimaging, and to resect those that exhibit growth and/or are associated with clinical signs of intracranial hypertension. This approach is being challenged by recent observations that suggest that mTOR inhibitors such as rapamycin (sirolimus) and RAD001 can induce partial regression of SEGAs[17,18]. The first report showing clear regression of SEGAs in 5 patients with the use of rapamycin

was published in 2006[17]. Recently, a phase II trial[18] using everolimus to treat SEGAs in 28 patients with TSC showed SEGA reduction of at least 30% in 21 patients (75%) and at least 50% in 9 patients (32%). Everolimus was well tolerated, as only single cases of grade 3 treatment-related sinusitis, pneumonia, viral bronchitis, tooth infection, stomatitis, and leukopenia were reported.

These observations suggest that treatment with mTOR inhibitors could serve as an acceptable alternative to SEGA surgery. Renal angiomyolipomas and lymphangioleimyomatosis are other TSC manifestations against which mTOR inhibitors have proven potential efficacy[19]. In addition, animal models of TSC have suggested that mTOR inhibitors could have beneficial effects on cognitive deficits[20] and on epileptogenesis[15]. Whether similar benefits would be observed in humans with TSC is still unknown. Research trials are ongoing and should soon provide answers to these questions.

The duration of treatment is likely to be prolonged or even lifelong. There is clear evidence that SEGAs grow back after administration of the mTOR inhibitor is stopped[17]. Most experts currently recommend continuation of mTOR inhibitors at the lowest efficacious dose. This cohort of patients, who will experience prolonged exposure to mTOR inhibitors, should be carefully followed longitudinally to better document long-term side effects, but also to compare their longevity with that of similar patients receiving TSCs. These patients represent a unique opportunity to study the potential anti-aging properties of mTOR inhibitors in humans.

In animal models, mTOR inhibitors showed that mTORC1 blockade alone and PI3K-mTOR blockade lead to suppression of tumor development and longer survival of the treated animals[21]. Rapamycin, the first mTOR inhibitor used in individuals with TSC-associated lesions, was able to stimulate regression of subependymal giant cell tumors (SGCTs)[17]. Subsequent studies have confirmed its efficacy in SGCTs, but also in other lesions such as angiomyolipomas[22]. The effects of mTOR inhibitors on the mTOR pathway result in decreased protein synthesis and cell-cycle arrest, as well as decreased angiogenesis. More recently, a new mTOR inhibitor, RAD001, has been used in the treatment of 28 patients with TSC-associated brain lesions but with no symptoms of increased intracranial pressure[18]. In particular, this study reports a reduction in tumor size of at least 30% in 75% of patients and at least 50% in 32% of treated individuals. Varying degrees of reduction of SGCT size have been observed in all the 38 patients treated with mTOR inhibitors (sirolimus or everolimus) to date. Most SGCT reductions occur in the first 3 months of mTOR inhibitor treatment, after which the rate of reduction slows. In recent case reports, a similar anti-tumor efficacy was achieved, even with lower serum levels of everolimus[23]. None of the patients treated with mTOR inhibitors required surgery or developed new SGCTs while receiving treatment[17,18]. Cerebrospinal fluid obstruction was relieved by the reduction in SGCT size[18]. The treatment was also associated with a clinically relevant reduction in the overall frequency of seizures and an improvement in quality of life.

Unfortunately, regrowth of SGCTs occurred a few months after drug discontinuation in all but one of the reported patients[24]. Therefore, mTOR inhibition may need to be continuous for the benefits to persist, and the benefits and hazards of long-term treatment with low-dosage mTOR inhibitors should be evaluated.

Conclusions and future perspectives

An early diagnosis of SGCT in neurologically asymptomatic children with TSC may allow prompt surgical removal of the tumor before the appearance of signs of increased intracranial pressure, and this approach is being progressively adopted to lessen the morbidity/mortality rate. Surgical treatment is obviously mandatory in case of life-threatening symptoms. However, the dramatic response of TSC-associated SGCTs to mTOR inhibitors suggests that these drugs could be a potential alternative to surgery in many cases.

mTOR inhibitors could be recommended when an asymptomatic SGCT shows growth in 2 consecutive magnetic resonance imaging evaluations following diagnosis. mTOR inhibitors could also be used as an initial treatment to facilitate subsequent surgery in individuals with bilateral lesions. Medical therapy may also have a role when SGCTs present in an atypical location or exhibit aggressive growth. Furthermore, in case of regrowth after a first resection, considering the higher risk of further surgery, pharmacotherapy could provide an alternative method to keep lesion size under control. Little is known about the long-term efficacy and safety of low dosage use of mTOR inhibitors and whether regrowth could be prevented by a more prolonged treatment course. In animal models, rapamycin dosing comparison studies indicated that the duration of rapamycin treatment is more important than dose intensity in terms of efficacy; prolonged treatment with low doses of mTOR inhibitors resulted in more complete and durable tumor responses[25,26]. Our current understanding of the effects of continuous mTOR inactivation in individuals with TSC is still poor. mTOR inhibitors may also activate pathways that should not be activated, and this issue will need to be taken into account when a long-term treatment is proposed.

The feasibility and timeline for discontinuation of mTOR inhibitor-based pharmacotherapy also remains unclear, and further studies are required to explore the optimal duration of treatment. Since it is known that the growth of SGCTs tends to slow in early adulthood, mTOR inhibitor treatment should theoretically be undertaken

until the patient reaches around 20 years of age. Strategies for future clinical trials with mTOR inhibitors may include the investigation of longer treatment durations with minimum dosage.

When choosing between surgical and/or medical intervention, clinicians should take the risks and benefits of each option into account. There are several issues to be considered, and every decision should be discussed thoroughly with the parents and tailored to the individual case. Depending on the age of the patient, one option may be more valid than the other. For example, pharmacotherapy might be preferred when a growing SGCT is discovered in adolescents, as the therapy may only be required for a few years. On the other hand, in childhood, a single surgical removal could be preferred to many years of pharmacotherapy. The positive effect that mTOR inhibitors have on several manifestations of TSC is an important factor in favor of pharmacotherapy, and should be considered in patients presenting with problems such as renal angiomyolipomas, pulmonary lymphangioleiomyomatosis, and/or intractable epilepsy, in addition to SGCTs. Since the activation of the mTOR pathway has been implicated in epileptogenesis, mTOR inhibition could have antiepileptic effects in patients with *TSC*[18,27].

Inhibition of the mTOR pathway may provide a biologically targeted therapy that has the potential to change current clinical practice regarding management of SGCTs. Currently, it is still unclear whether pharmacotherapy is able to prevent or merely delay the need for surgical resection of SGCTs. In the coming years, medical treatment will certainly play a larger role in the management of children with TSC, as our understanding of the pathogenesis of this disorder at the molecular level improves.

In conclusion, a new treatment era has begun in the field of TSC since the discovery of the potential beneficial effects of mTOR inhibitors. Although the use of mTOR inhibitors is becoming increasingly accepted, especially for the treatment of SEGAs in TSC, questions remain concerning the duration of treatment and long-term side effects. Whether mTOR inhibitors will have a significant impact on longevity in TSC is unknown, but warrants attention, as mTOR inhibitors are increasingly recognized as anti-aging drugs in animal models. Long-term prospective studies in patients with TSC will provide information on the potential anti-aging properties of mTOR inhibitors in humans.

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