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International Journal of Current Research
Vol. 8, Issue, 10, pp.39574-39578, October, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

REVIEW ARTICLE

mTOR AND ITS ROLE IN CELL SIGNALING

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ARTICLE INFO

Article History:
Received 10th July, 2016
Received in revised form
15th August, 2016
Accepted 28th September, 2016
Published online 30th October, 2016

Key words:

Mammalian target of rapamycin,
Hypertrophy,
Cancer,
Leucine,
Neurodegenerative disease.

ABSTRACT

Many studies have been developed on the mammalian target of rapamycin: mTOR. mTOR is a threonine/kinase protein with major role in cellular growth signaling, regardless the tissue organism. In this study it was shown that mTOR is responsible for signaling both positively and negatively cell growth. In this review we see that the stimulus mTOR pathway allows health benefits, working in protein synthesis, cell growth, immunology, satiety and depression. Studies show that this threonine/kinase protein can be stimulated by various factors such as leucine, high-intensity exercise, omega 3, insulin and phosphatidic acid. Among the biochemical mediators involved in the activation of mTOR, Akt, p70 P13K and P6K1 are the most correlated. On the other hands, there are other studies showing detriments with stimulation of mTOR, as in some types of cancer, neurodegenerative diseases, aging muscle cells and fat tissue formation. However, as the theme covers various topics such as cell anabolism and immunology, it seems to be relevant continuity in research so that we can better understand the biochemical and physiological mechanisms of stimulation of mTOR. Thus, in a not so distant future, we may think of setting up specific cellular therapies, stimulating or inhibiting the mTOR pathway of the patient.

Citation: Henrique Urtassum Ludvig, Igor Brandão and Rafael Longhi, 2016. "mTOR and its role in cell signaling", *International Journal of Current Research*, 8, (10), 39574-39578.

INTRODUCTION

Rapamycin was defined as an antifungal compound produced by Streptomyces hygroscopicus bacterium, primarily found in the soil samples on Easter Island, promising effects in promoting cell life control (Dennis *et al*., 1999). This finding led to the discovery of rapamycin in yeast target (TOR1) and (TOR2) and subsequently homologues in mammals the mTORC1 and mTORC2 (Schmelzle and Hall, 2000). Rapamycin target in mammals (mTOR) is a threonine/kinase protein that integrates environmental stimuli and play an essential role in signaling protein synthesis, as well as in cell growth (Deldicque *et al*., 2005).

Beneficial effects the body through mTOR

According to Deldicque, Theisen and Francaux, (2005), mTOR signaling is mediated by acute resistance exercise. According to these authors, mTOR is a protein kinase complex assimilating cellular energy status signals, as well as environmental stimuli to control cellular protein synthesis. Among the environmental stimuli that influence, one is acute resistance exercise. During this type of exercise mTOR is inhibited by increasing the amount of enzyme dependent AMP (AMPK), which are responsible for controlling the energy metabolism and control the production of adenosine triphosphate (ATP). Therefore, amino acids are recruited for ATP production, rather than being used as raw material for protein synthesis. After the exercise, there is a return to baseline of AMPK. From this moment on, the mTOR returns its activity, reducing autophagy and promoting protein synthesis. Thus, the muscle hypertrophy occur during recovery and rest. According to these authors, mTOR inhibits autophagy and stimulates transcription and translation of mRNA, ribosomes performing the biosynthesis as well as the proliferation and organization of the cellular cytoskeleton (Deldicque *et al*., 2005). Rundqvist, *et al*. (2013) showed that strength exercises and maximum effort is able to activate mTOR, and not the vacuolar protein sorting 34 (Hvps34) as the authors tried to relate. The experiment was conducted in healthy adults aged between 28 ± 5 and BMI 23.5 ± 2.1 kg/m^2.

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The participants, chosen randomly performed three sprints at full speed on a stationary bike for 30 sec with rest of 20min for each sprint. The groups were separated with and without intake of essential amino acids (EAA) and combined or not with carbohydrates, respecting the rest of one month between sessions. The supplemented groups ingested essential amino acids (EAAs) at dosage 300mg / kg, with and without maltodextrin 1g / kg, and the control group (placebo) ingested only berry juice. After a series of exercises, muscle biopsies were performed, 140min after the last sprint. The researchers found increased phosphorylation of the enzymes ribosomal S6 kinase 1, and (p70 S6K1) and threonine kinase (389Thr389), and 14.7 times the resting level for the supplemented participants (P = 0.007). These ribosomal proteins are closely related to the activation of mTOR. In another experiment Miniaci *et al*. (2014) found samples with higher protein synthesis in myocytes from rats that have undergone glucose deprivation. The authors evaluated mTOR and protein kinases p70 S6K1 activation in muscle cells of rats and mice, as well as in human cells, but these not being muscle tissue. In muscle cells of rodents there was phosphorylation and activation of ribosomal S6K1 and p70 proteins as well as activation of mTOR during the intended period of deprivation of glucose, respectively 30, 60, 120 and 180min. Proteins were measured by immunoblotting analysis (Western Blot). Researchers have shown that abstinence glycidic was able to signal both mTOR, such as ribosomal proteins in the muscle cells of rodents.

Following the same point of view, Jamart *et al*. (2013) showed that there is a greater activation of autophagic mechanisms in rats in fasting state than in fed state to the same protocol of resistance exercise. The test was performed with 36 rats, all females at 12 weeks. The animals were divided into four groups (n = 9 each group): fed and rested, fed and exercised, fasted and rested and fasted and exercised. All groups underwent 90min mat the speed 10m/ min which corresponds to a low level for this type of animal (55% VO_2 max). Through tests such as SDS-PAGE and immunoblotting the researchers found that muscle autophagy process occurred in all groups, by measuring a protein chain marker associated with microtubules in 3 isoform B (LC3b-II). However, animals that have passed through the intentional fasting, showed a more acute state of cellular autophagy, which were related to the fall in plasma insulin concentration, and decreased activation of mTOR pathways, such as the serine / kinase 473 and 308 ($AktSen^{473\ /\ 308}$) threonine / kinase 32 FOXO3 ($FoxO3Thr^{32}$) and serine kinase ULK1 75 ($ULK1Sen^{75}$). Blomstrand *et al*., (2006), comments in their review study about a group of essential amino acids known as branched chain aminoacids (BCAA) are able to activate specific enzyme mechanisms involved in protein synthesis. These amino acids such as leucine, isoleucine and valine indicate the mTOR pathway and p70 ribosomal S6K1 protein, thus stimulating muscle growth. In Wilkinson *et al*., study (2013), the authors compared the production of muscle protein synthesis between the orally intake of leucine and the β-hydroxy-β-methylbutirate (HMB), a leucine metabolite. Subjects were divided into two groups. One group ingested HMB (n: 8, aged 22 ± 1 years and BMI 23 ± 1 kg /m^2), and the other ingested leucine (n: 7, age 21 ± 0.3 years and BMI 25 ± 0.6 kg /m^2). Both solutions were prepared based on water. The leucine group received 3,42g dissolved in 400ml of water and HMB group received 2,42g dissolved in 400ml of water. Both groups did not significantly activated mTOR ($p < 0.05$). However, there was a difference in the intensity of the stimulus. While the effect of HMB produced an approximate 70% increase in protein synthesis, leucine generated an approximately 110% increase. The researchers believe that the difference can be explained by the fact that HMB does not stimulate insulin production and Akt, unlike leucine. Pedroso *et al*., (2015) comments that there is a relationship between leucine and satiety, glucose homeostasis and metabolic energy balance. According to researchers, the amino acid leucine is capable of activating intracellular mechanisms mTOR pathway, more precisely via mTORC1 complex, stimulating protein synthesis. These authors also related leucine with appetite control. But his findings were conclusive only in intravenous administration in rats. In addition, researchers have shown potentially positive effects in regulating the energy balance, as in glucose homeostasis. Thus, leucine would act as stimulator of glucogenesis and consequently inhibiting gluconeogenesis.

Bohé *et al*. (2004) showed muscle protein synthesis is more associated with the extracellular concentration of essential amino acids (EAA) than intracellular. According to the authors, an increase in the concentration of EAAs from 41% to 82% above the baseline plasma concentrations are associated from 30% to 57% increase in muscle protein synthesis. Researchers collected samples of blood plasma between 1 and 3.5 h after intravenous infusion of essential amino acids. The survey was conducted in humans, all young and healthy, aged 25 ± 1.2 years and weight 71.5 ± 3.3kg, separated into three groups according to the concentration of administered essential amino acids (low, n=6, medium, n=4 and higher n= 6), in amounts of 43.5, 87 and 261 mg.kg/hour, respectively. After muscle biopsy was performed. According to researchers, the relationship between the leucine concentration in the plasma and an increase in protein synthesis was positive to the concentration of 261mg/kg (550 nmol / L), which appeared the plateau. In Mobley *et al*. study (2015), the phosphatidic acid derivative of soybean ingestion (PA) is capable of signaling to mTOR and muscle synthesis. The group of researchers used male Wistar rats, and after an overnight fast separated them into four groups, feeding them as follows: the first group (control) only ingested water for breakfast, the second, ingested 29 mg of PA, the third 197mg ingested Whey Protein Concentrated (WPC) and the fourth group ingested PA + WPC. After 3 hours of supplementation, the researchers performed muscle biopsies and analyzed the genetic expressions in the Akt-mTOR through immunoblotting test associated with sunset analysis to measure muscle protein synthesis (MPS). In relation to the control group, rats fed PA increased 67% phosphorylation of S6K1 pathways p70 and Thr389 and 51% in muscle protein synthesis. The group that drank only WPC got the best indicators. In the mixed group with PA + WPC was increased on roads MPS and Akt-mTOR, but MPS index was lower than the other isolated groups, such as PA and WPC. According to Smith *et al*. (2011) intake of polyunsaturated fatty long chain acids from omega 3 family (LCN-3PUFA) enhances the signaling of mTOR. Participants received for 8 weeks 4g/day supplementation of omega 3, containing 1.86g/day and 1.5 g/ day of EPA and DHA, respectively. On the day of the experiment, the participants were on hospital observation, receiving insulin and intravenous amino acids, and in the end, performed muscle biopsy. The researchers found a higher signal when associated LCN-3PUFA to a state of hyperinsulinemia and hyperaminoacidemia, especially in Ser^{2448}, p70S6K1 and Thr^{389} ways.

There is a strong connection between mTOR and the immune system, according to Lee *et al*. (2010), specifically mTORC2 complex, this one is related to the development of the defense cells of the body, specifically the defense cells T helper (Th1) and (Th2). According to the authors lymphocytes are activated and differentiated by different routes, such as mTOR, PKC and Akt-mTOR. Delgoffe *et al*., (2009) showed that mTOR acts directly on the differentiation of immune cells. According to the authors, in the absence of mTOR the body's defense cells fail to differentiate into Th1 and strengthen the adaptive immune system of the body. To reach this conclusion, researchers, first, used the vaccinia virus as Th1 inducers, injecting the antidote in mice. Small animals were divided into two groups: one wild-type and other deficient mTOR and CD4 +. The researchers analyzed the immune response on exposure to the antidote, and confirmed a deficiency in adaptive response in the suppressed group of mTOR. According to the authors, mTOR is a key two protein complexes, mTORC1 and mTORC2. However, both have different mechanisms and activations. While the mTORC1 is dependent on Akt, mTORC2 increases the activation of Akt. Both complexes activate effector pathways for the defense cell growth. Laplante and Sabatini (2012), comment among other important functions of mTOR there is the ability to regulate satiety, reducing the action of leptin and insulin in the central nervous system (CNS). According to the researchers the mTORC1 complex reduces the activation of hunger flags, acting on proteins such as NPY and AgRP in the hypothalamus.

In a robust review to Jeon and Kim (2016), the authors showed that mTOR pathways combat the symptoms of depression. The authors believe that the key to the regulation of neuronal circuity is long in the regulation of protein translation. According to the authors, mTOR stimulation activates factors such as ERK1 \ 2 and Akt, both responsible for the initial stages of translation of proteins, play an important role in the pathogenicity of the disease. According to researchers both mTOR pathway, as eEF2 (which is related to the neurotrophic factor BDNF), act in rapid antidepressant mechanisms. In the other way, Cota *et al*., (2008) comments that the excess of lipid in a diet can negatively influence mTOR. Researchers have linked the function of mTOR, more precisely the mTORC1 complex with a high fat diet. The survey was conducted in rats and mice, dividing them into two groups. A group with a high fat diet (4,54kcal/g and 40% butter), and the other with a fat diet (3,81kcal/g and 9% butter) for 4 weeks. The food intake was controlled and offered at the times 1h, 4h and 24h, and the mice had their weight measured 24 hours after the end of the diet. After the experimental period, the rats with high fat diet showed resistance to leptin and weight gain. The researchers have elucidated that the high fat diet was able to inhibit mTOR, desensitizing the action of leptin (hormone active in controlling satiety) in the CNS.

Malefic effects to the body through mTOR

In addition to beneficial effects to mTOR metabolic pathway is also related to harmful effects to health. The experiments from Shao *et al*., (2013) showed mainly two proteins extension activation of the mTOR pathway with Merkell cell carcinoma (MCC), respectively the lactate dehydrogenase B (LDHB) responsible for increasing tumor metabolism and heterogeneous ribonucleoprotein F (hnRNPF) related to the activation of mTOR. The researchers performed a proteomic study of the MCC cells and lung cancer cells, control group, since both are diseases with neuroendocrine activation. For this study, 80 samples MCC cells 16 fresh primary tumors and two human cell lines were used, and tests as qPCR and immunoblotting were used. In order to relate the mTOR pathways with their protein LDHB and hnRNPF expression, the researchers used is suppressing the mTOR pathway as Ku-0063784 and PP242 for 24hrs. The results showed that cells that have undergone deletion mTOR showed low levels of both hnRNPF factor, as in the LDHB RNA level in the cells MCC, showing the relationship between mTOR and proliferation of tumor growth factors.

According to Rozengurt (2014), mTOR is related to the insulin receptor substrate (IRS1-4), as well as G proteins action (ENU) in pancreatic cancer cells (PDCA). The author showed that the P13K / Akt / mTOR route when activated via IRS1-4, promotes the proliferation of cancer cells in the pancreas. According to this author, a specific group of G proteins and act to control the growth of a variety of cancer cells synergistically with insulin and IGF-1. The author still comments that the P13K / Akt pathways, PTG mTORC1 complex and are essential for the development of pancreatic cancer cells, malignant cells more lethal. The study also showed that the use of metformin substance act in decreased activation of the mTOR pathway and is able to interrupt its cycle, preventing the proliferation of cancer cells. In another study to Papadimitrakopoulou (2012), the author related P13K/Akt/mTOR pathway with mutagenic processes in lung cancer. According to this author when this pathway is activated it generates changes in phosphatidylinositol 3-kinase mechanism (P13K). This mechanism is responsible for the activation of Phosphatidylinositol-4,5-bisphosphate and phosphatidylinositol-3,4,5-triphosphate (PIP PIP II and III respectively) and Akt, all involved in the process of growth and cell proliferation. The author also points out, as well as P13K pathway there are numerous other mutations possibilities in P13K / Akt / mTOR pathway. A recent study by Zhang *et al*. (2015) showed the effectiveness of Sorafenid action, a inhibiting Akt medication, in the expression of liver tumor in humans. The authors divided the patients with lung cancer into two groups: a control group and one treated with Sorafenid. Human cells were exposed to 4 mmol / L Sorafenid the following times 0.5, 3, 8, 25 and 48 hours after the collection of tissues. The experiment was performed by immunoblotting method (Western Blot), and showed a sharp decrease in Akt expression compared to time zero. Therefore, there was a decrease in gene expression of P13K and mTOR.

As well as in cancers, in the neurodegenerative diseases the reduction of the expression of mTOR pathway can have beneficial effects. According to Mendelsohn and Larrick (2016), in elderly muscle satellite cells as well as progenitor cells (SMSCs) suffer from the loss of functionality and capacity for renewal both caused mainly by autophagic decontrol. This dysfunctional mechanism leads to cell senescence remain in state which is correlated with reactive oxygen species, proinflammatory cytokines and activation of p16INK4a. According to the researchers the autophagic control induced by rapamycin, an mTOR inhibitor, can stimulate the renewal, renewing cells.

According to Zheng *et al*. (2016) neurodegenerative diseases and mitochondrial dysfunctions are closely linked. Researchers believe that control mitochondrial bioenergetic efficiency, by reducing their energy waste will make it more efficient and

healthy. For the experiment the researchers used modified cells maternally inherited Leigh syndrome (MILS) respecting the preparation of cells for iPSCs system and using immunoblotting method. It has been used 3 oligomycin drugs, rotenone and antimycin-A and mitochondrial uncoupler (CCCP) with the motive to cause oxidative mitochondrial dysfunction. The results showed that cells treated with rapamycin mTOR inhibitor in the groups with oligomycin, rotenone and antimycin-A inhibited phosphorylation in the mitochondria while maintaining the stable production of energy, and without energy wastage. Other parameters, such as reduction of reactive oxygen species (ROS), as well as improved resistance glutamate toxicity were also found in the samples. A negative and significant effect was discovered by Lu *et al*. (2014) who showed that a relationship between the mTOR pathway and lipogenesis so far unknown. The study was conducted on male mice, aged 8-10 weeks over a 12 week period. The animals were divided into two groups: one group received a high-fat diet with 45% of the diet, and the other the control, received a low-fat with only 4.5% of the diet. Both groups had free access to food and water. However, prior to sacrifice the animals were subjected to fasting 16 hours, followed by their feedback to restore liver glycogen. The researchers found that during the fasting period, the group with the high fat diet produced glycogen levels 3 times higher than the control group. As these authors, the levels of the G protein, which are dependent on the mTORC1 action and SREBP 1 activated the pathway of gluconeogenesis and by extension also lipogenesis pathway.

At last, positive and negative effects have been being shown by the scientific literature through the activation of the mTOR pathway.

Conclusion

mTOR is a threonine / kinase protein rapamycin target in mammalian, and is also responsible for signaling to cells positively and negatively, depending on the metabolic environment in which it is stimulated. mTOR shown to be activated by numerous factors, such as essential amino acids, high-intensity exercise, phosphatidic acid, omega 3, leucine and insulin. The study showed 19 positives articles with stimulation of mTOR, such as in protein synthesis, immune system depression and satiety. However, 8 articles related signaling mTOR with negative aspects, such as cancer, neurodegenerative diseases, lipid tissue formation and aging muscle cells. This review aimed to clarify the effects already found in the scientific literature on the stimulation of mTOR, highlighting its benefits and harms health. However, as the theme cover various topics such as cell anabolism and immunology, it is relevant continuity in research so that we can better understand the biochemical and physiological mechanisms of mTOR stimulation. Thus, in a not so distant future, we may think of setting up specific cellular therapies, stimulating or inhibiting mTOR pathway of the patient.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments/Grant support

This study was supported by Centro Universitário Metodista/IPA and Universidade Federal do Sergipe (UFS).

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