Cell Cycle 11:17, 3175-3179; September 1, 2012; © 2012 Landes Bioscience

The role of mTOR signaling pathway in spinal cord injury

Haruo Kanno,* Hiroshi Ozawa, Akira Sekiguchi, Seiji Yamaya, Satoshi Tateda, Kenichiro Yahata and Eiji Itoi
Department of Orthopaedic Surgery; Tohoku University School of Medicine; Sendai, Japan

The mammalian target of rapamycin (mTOR) signaling pathway plays an important role in multiple cellular functions, such as cell metabolism, proliferation and survival. Many previous studies have shown that mTOR regulates both neuroprotective and neuroregenerative functions in trauma and various diseases in the central nervous system (CNS). Recently, we reported that inhibition of mTOR using rapamycin reduces neural tissue damage and locomotor impairment after spinal cord injury (SCI) in mice. Our results demonstrated that the administration of rapamycin at four hours after injury significantly increases the activity of autophagy and reduces neuronal loss and cell death in the injured spinal cord. Furthermore, rapamycin-treated mice show significantly better locomotor function in the hindlimbs following SCI than vehicle-treated mice. These findings indicate that the inhibition of mTOR signaling using rapamycin during the acute phase of SCI produces neuroprotective effects and reduces secondary damage at lesion sites. However, the role of mTOR signaling in injured spinal cords has not yet been fully elucidated. Various functions are regulated by mTOR signaling in the CNS, and multiple pathophysiological processes occur following SCI. Here, we discuss several unresolved issues and review the evidence from related articles regarding the role and mechanisms of the mTOR signaling pathway in neuroprotection and neuroregeneration after SCI.

Keywords: mTOR, rapamycin, autophagy, spinal cord injury, apoptosis

Submitted: 05/30/12

Revised: 06/24/12

Accepted: 06/25/12

http://dx.doi.org/10.4161/cc.21262

*Correspondence to: Haruo Kanno;
Email: kanno-h@isis.ocn.ne.jp

Introduction

The mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase that plays a key role in the regulation of cell metabolism, cell proliferation and cell death and survival and is involved in physiological processes such as transcription, mRNA turnover and translation, ribosomal biogenesis, vesicular trafficking, autophagy and cytoskeletal organization.[1]

The mTOR pathway is one of the most studied signaling pathways and is involved in trauma and various diseases in the CNS. mTOR signaling is affected in a number of neurodegenerative conditions, including Alzheimer disease, Parkinson disease, cerebral stroke and Huntington disease, and inhibition of mTOR activity can reduce the neurodegeneration associated with these conditions.[2-5] In addition, the inhibition of mTOR can reduce neural tissue damage in CNS injuries, such as traumatic brain injury and neonatal hypoxia-ischemia-induced brain injury.[6,7] We recently reported that inhibition of mTOR using rapamycin reduces neural tissue damage and locomotor impairment after spinal cord injury (SCI).[8] Other previous studies have shown that mTOR regulates axonal regeneration in response to SCI[9,10] and acts to limit astrocytic scar formation in the injured spinal cord.[11]

Together, these observations highlight the essential role of mTOR in neuroprotection and neuroregeneration in the CNS. However, the role of mTOR has not yet been fully elucidated. A number of cellular functions are regulated by mTOR signaling, and multiple pathophysiological processes are involved in CNS disease and trauma. In this Extra View, we discuss several unresolved issues and review the evidence from related articles regarding the role and mechanisms of the mTOR

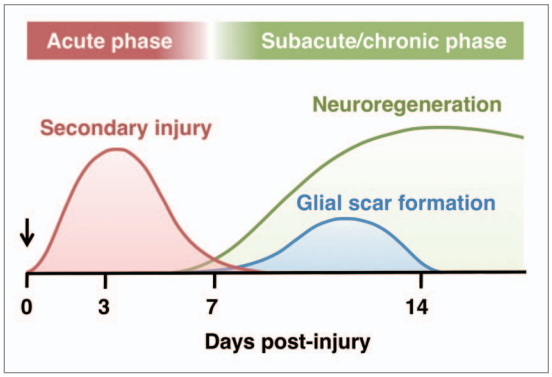

Figure 1. The time phase of the pathophysiological processes and neuroregeneration after SCI. The black arrow indicates the initial onset of SCI (primary injury). Various pathophysiological processes, including apoptosis, inflammation, microglia/macrophage activation, demyelination and axonal degeneration, mainly occur in the secondary injury phase. Glial scar formation (astrogliosis) occurs between seven and 14 d after SCI. Processes of neuroregeneration, such as axonal regeneration and remyelination, primarily start to appear at 7 days after SCI.

signaling pathway in neuroprotection and neuroregeneration after SCI.

Inhibition of mTOR Reduces Secondary Neural Tissue Damage after SCI

Many previous studies have demonstrated the inhibition of mTOR signaling have a neuroprotective effect in the CNS. We recently examined whether the inhibition of mTOR by rapamycin reduces neural tissue damage after acute SCI in mice.[8] Our results demonstrated that the administration of rapamycin significantly decreases the phosphorylation of the p70S6K protein and increases the expression levels of LC3 and Beclin 1 in the injured spinal cord. These findings indicate that rapamycin promotes autophagy by inhibiting the mTOR signaling pathway after SCI. In addition, we found that mTOR inhibition significantly reduces neuronal loss and cell death in the injured spinal cord. Furthermore, the rapamycin-treated mice showed significantly higher levels of locomotor function. Our results support those of previous reports suggesting that neuroprotective effects are produced by mTOR inhibition after CNS injury.[6,7]

The actual molecular mechanisms underlying the neuroprotective effects regulated by the mTOR signaling pathway remain to be elucidated. The distinct mechanisms of interaction between the activation of autophagy and cell death are also unknown. It is therefore important to clarify the potential neuroprotective mechanisms underlying mTOR inhibition following CNS injury.

The Functional Differences in mTOR Signaling between the Acute and Subacute/Chronic Phases Following SCI

SCI involves multiple pathophysiological and regenerative processes. These processes vary depending on the time phase after the initial onset of injury (**Fig. 1**).[12] First, the spinal cord suffers critical damage from the primary mechanical trauma (primary injury) and develops hemorrhagic necrosis. As a result, the tissue damage expands over time due to the activation of secondary injury processes.[13] The secondary injury mainly occurs between 24 h and three days after the initial onset of SCI.[14,15] Numerous studies have reported the presence of multiple cellular and molecular events, such as cell death, inflammation, macrophage/microglia activation, axonal degeneration and demyelination, during the secondary injury.[12,14,16-19] Following the secondary injury, various regenerative processes are observed. Axonal regeneration mainly begins one week after SCI.[18] Remyelination of axons also begins to appear one week after injury.[19] Additionally, the formation of reactive astrogliosis around the lesion site primarily occurs 1 to 2 weeks after injury.[20,21]

Because these various pathophysiological and regenerative processes depend on the time phase following injury, it is important to consider the functional differences in mTOR signaling between the acute phase, including secondary injury, and the subacute/chronic phase, including neuroregeneration.

During the acute phase of SCI, mTOR regulates crucial functions related to secondary injury, such as cell death, inflammation and macrophage/microglia activation. Our previous study showed that inhibition of mTOR reduces cell death in damaged neural tissue following SCI.[8] Another study showed that inhibition of mTOR reduces the levels of pro-inflammatory markers and NO synthase (NOS) activity that are induced by cytokines in microglia.[22] Furthermore, it has been suggested that inhibition of the mTOR signaling pathway reduces inducible NOS expression and microglial activation that causes neuronal injury.[23]

In the subacute/chronic phase, mTOR signaling regulates the regeneration of damaged neural tissue. Previous studies have suggested that the inhibition of mTOR by rapamycin suppresses new protein synthesis and cell proliferation to promote axonal regeneration after SCI.[9,24] mTOR signaling regulates both CNS myelination and oligodendrocyte differentiation.[25,26] Additionally, mTOR inhibition can reduce reactive astrogliosis at lesion sites following spinal cord ischemia.[11]

Together, these observations suggest that the role of mTOR in injured spinal cords may differ depending on the time phase following SCI. Therefore, functional differences based on the time phase should be considered when conducting future studies to clarify the role of the mTOR signaling pathway in neuroprotection and neuroregeneration after SCI.

The Neuroprotective Mechanisms Regulated by mTOR during the Acute Phase Following SCI

Many studies have shown that inhibition of mTOR induces neuroprotective functions following CNS injury.[2,6-8,27] However, the actual cellular and molecular mechanisms underlying the neuroprotective effects regulated by mTOR signaling has not yet been fully elucidated.

One potential mechanism underlying these neuroprotective effects is the blocking of apoptosis by the activation of autophagy.[7,28,29] Interestingly, it has been suggested that mTOR inhibition enhances the clearance of mitochondria by inducing autophagy, thereby reducing cytosolic cytochrome c release and downstream caspase activation.[30] Previous studies have demonstrated that inhibition of mTOR upregulates autophagy and shows cytoprotective functions by reducing apoptosis in various disease models, including the myocardial ischemia-reperfusion model[28] and neonatal hypoxia-ischemia-induced brain injury model.[7]

Recently, the essential role of mTOR in the activation of macrophages and microglia has been clarified, as described above. Macrophages from the peripheral circulation and those derived from resident microglia are the primary effector cells of the inflammatory response that follows SCI.[31] Especially in the acute phase after injury, activated macrophages and microglia produce various proinflammatory cytokines, such as IL-1β and TNFα,[32,33] and contribute to secondary tissue damage, neuronal loss and demyelination.[34-36] Recent studies have demonstrated that inhibition of mTOR suppresses macrophage/microglia activation and reduces neuroinflammation.[22] These findings suggest that inhibition of mTOR can suppress macrophage/microglia activation and reduce the inflammation that causes secondary damage following SCI.

Regulation of Angiogenesis by mTOR in Injured Spinal Cord

The mTOR signaling pathway plays an important role in regulating angiogenesis in both normal tissues and cancers.[37] The mTOR pathway modulates the expression of angiogenic factors, such as vascular endothelial growth factor (VEGF), nitric oxide and angiopoietins.[37,38] Previous studies have shown that inhibition of the mTOR pathway decreases angiogenesis and the secretion of angiogenic factors.[39]

SCI results in the disruption of vascular structures during primary injury and induces secondary pathogenic events in the neuronal environment that collectively define axonal regeneration and the extent of functional recovery.[40] Numerous reports have suggested that stimulation of post-traumatic angiogenesis with angiogenic factors such as VEGF promotes neuroregeneration and improves functional recovery after SCI.[41,42] Optimizing treatments to target vascularization after SCI is a valuable therapeutic strategy.

Therefore, modulating the mTOR signaling pathway to stimulate angiogenesis may be a promising approach to promote neuroregeneration after SCI.

Suppression of Cellular Senescence and Organismal Aging by mTOR Inhibitor after SCI

Both the ability of injured axons to resume growth after SCI and the various neuronal functions associated with plasticity decline with increasing age.[12,43,44] Experimental studies of SCI demonstrated an age-dependent decline in locomotor recovery.[44-46] In addition, the number of motor neurons in the spinal cord has been reported to decrease with increasing age in mammals.[47]

Spinal cord injury induces accumulation of β-amyloid precursor protein and β-amyloid peptide in the injured spinal cord, which is associated with the neurodegenerative processes of aging motor neuron.[47,48] Reactive oxygen species (ROS) that can induce DNA damage and accelerate cellular senescence are upregulated in the damaged neural tissue after SCI.[49-52]

Recent studies revealed that an inhibition of mTOR decelerates cellular senescence and increases the lifespan in diverse organisms.[53-55] mTOR is involved in age-related diseases, such as atherosclerosis, metabolic syndrome, osteoporosis, neurodegeneration and macular degeneration.[56-59] Previous studies have demonstrated that rapamycin treatment reduces the pathology of neurodegenerative disease in the CNS.[60] Rapamycin treatment increases the expression of markers of autophagy in neurons and reduces the levels of β-amyloid peptide in an experimental model of Alzheimer disease.[5,61] In addition, inhibition of TOR by rapamycin may lead to increased antioxidant defenses and reduced DNA damage and tissue aging.[62,63]

Therefore, the inhibition of the mTOR signaling pathway is thought to decelerate cellular senescence and tissue aging in the CNS and thus prevent an age-dependent decline in neuroregeneration ability. Rapamycin treatment may therefore have beneficial effects on the regenerative capacity of an injured spinal cord in order to develop SCI therapies that remain effective for older patients.

The Clinical Applications of mTOR Inhibitors

The inhibition of mTOR produces both immunosuppressive and tumor suppressive effects.[64-66] To date, mTOR inhibitors have been widely used as immunosuppressants in patients who undergo transplantation surgery and as anticancer agents.[67-69] Various pharmacological immunosuppressants that inhibit mTOR have been developed and are used to prevent rejection and graft dysmotility following organ transplantation for renal, hepatic, intestinal and cardiac diseases.[70-72] mTOR inhibitors can also be important clinical treatment options for patients with tumors, including those with advanced renal cell carcinomas, mantle cell lymphomas, pancreatic neuroendocrine tumors, astrocytomas, sarcomas, breast cancer and lung carcinomas.[68,69,73-75] Numerous mTOR inhibitors, including rapamycin and its analogs (e.g., temsirolimus, everolimus, deforolimus and zotarolimus), have been developed and are used clinically for treating various diseases.[76,77]

On the other hand, the clinical applications of mTOR inhibitors for CNS injury have not yet been established. Many previous experimental studies using CNS injury models have demonstrated promising effects of mTOR inhibition to reduce neural tissue damage. Therefore, the pharmacological inhibition of mTOR may have clinical benefits for reducing secondary damage in patients with SCI. Further studies to clarify the neuroprotective and neuroregenerative mechanisms regulated by mTOR signaling are needed in order to approve the clinical use of mTOR inhibitors in patients with acute SCI.

References

1. Wullschleger S, Loewith R, Hall MN. TOR signaling in growth and metabolism. Cell 2006; 124:471-84; PMID:16469695; http://dx.doi.org/10.1016/j.cell.2006.01.016.
2. Ravikumar B, Vacher C, Berger Z, Davies JE, Luo S, Oroz LG, et al. Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease. Nat Genet 2004; 36:585-95; PMID:15146184; http://dx.doi.org/10.1038/ng1362.
3. Santini E, Heiman M, Greengard P, Valjent E, Fisone G. Inhibition of mTOR signaling in Parkinson's disease prevents L-DOPA-induced dyskinesia. Sci Signal 2009; 2:ra36; PMID:19622833; http://dx.doi.org/10.1126/scisignal.2000308.
4. Ma T, Hoeffer CA, Capetillo-Zarate E, Yu F, Wong H, Lin MT, et al. Dysregulation of the mTOR pathway mediates impairment of synaptic plasticity in a mouse model of Alzheimer's disease. PLoS ONE 2010; 5:5; PMID:20862226; http://dx.doi.org/10.1371/journal.pone.0012845.
5. Spilman P, Podlutskaya N, Hart MJ, Debnath J, Gorostiza O, Bredesen D, et al. Inhibition of mTOR by rapamycin abolishes cognitive deficits and reduces amyloid-beta levels in a mouse model of Alzheimer's disease. PLoS ONE 2010; 5:e9979; PMID:20376313; http://dx.doi.org/10.1371/journal.pone.0009979.
6. Erlich S, Alexandrovich A, Shohami E, Pinkas-Kramarski R. Rapamycin is a neuroprotective treatment for traumatic brain injury. Neurobiol Dis 2007; 26:86-93; PMID:17270455; http://dx.doi.org/10.1016/j.nbd.2006.12.003.
7. Carloni S, Buonocore G, Balduini W. Protective role of autophagy in neonatal hypoxia-ischemia induced brain injury. Neurobiol Dis 2008; 32:329-39; PMID:18760364; http://dx.doi.org/10.1016/j.nbd.2008.07.022.
8. Sekiguchi A, Kanno H, Ozawa H, Yamaya S, Itoi E. Rapamycin promotes autophagy and reduces neural tissue damage and locomotor impairment after spinal cord injury in mice. J Neurotrauma 2012; 29:946-56; PMID:21806471; http://dx.doi.org/10.1089/neu.2011.1919.
9. Park KK, Liu K, Hu Y, Smith PD, Wang C, Cai B, et al. Promoting axon regeneration in the adult CNS by modulation of the PTEN/mTOR pathway. Science 2008; 322:963-6; PMID:18988856; http://dx.doi.org/10.1126/science.1161566.
10. Liu K, Lu Y, Lee JK, Samara R, Willenberg R, Sears-Kraxberger I, et al. PTEN deletion enhances the regenerative ability of adult corticospinal neurons. Nat Neurosci 2010; 13:1075-81; PMID:20694004; http://dx.doi.org/10.1038/nn.2603.
11. Codeluppi S, Svensson CI, Hefferan MP, Valencia F, Silldorff MD, Oshiro M, et al. The Rheb-mTOR pathway is upregulated in reactive astrocytes of the injured spinal cord. J Neurosci 2009; 29:1093-104; PMID:19176818; http://dx.doi.org/10.1523/JNEUROSCI.4103-08.2009.
12. Schwab ME, Bartholdi D. Degeneration and regeneration of axons in the lesioned spinal cord. Physiol Rev 1996; 76:319-70; PMID:8618960.
13. Ozawa H, Keane RW, Marcillo AE, Diaz PH, Dietrich WD. Therapeutic strategies targeting caspase inhibition following spinal cord injury in rats. Exp Neurol 2002; 177:306-13; PMID:12429232; http://dx.doi.org/10.1006/exnr.2002.7998.
14. Beattie MS, Farooqui AA, Bresnahan JC. Review of current evidence for apoptosis after spinal cord injury. J Neurotrauma 2000; 17:915-25; PMID:11063057; http://dx.doi.org/10.1089/neu.2000.17.915.
15. Yong C, Arnold PM, Zoubine MN, Citron BA, Watanabe I, Berman NE, et al. Apoptosis in cellular compartments of rat spinal cord after severe contusion injury. J Neurotrauma 1998; 15:459-72; PMID:9674550; http://dx.doi.org/10.1089/neu.1998.15.459.
16. Dusart I, Schwab ME. Secondary cell death and the inflammatory reaction after dorsal hemisection of the rat spinal cord. Eur J Neurosci 1994; 6:712-24; PMID:8075816; http://dx.doi.org/10.1111/j.1460-9568.1994.tb00983.x.
17. Citron BA, Arnold PM, Sebastian C, Qin F, Malladi S, Ameenuddin S, et al. Rapid upregulation of caspase-3 in rat spinal cord after injury: mRNA, protein, and cellular localization correlates with apoptotic cell death. Exp Neurol 2000; 166:213-26; PMID:11085887; http://dx.doi.org/10.1006/exnr.2000.7523.
18. Hill CE, Beattie MS, Bresnahan JC. Degeneration and sprouting of identified descending supraspinal axons after contusive spinal cord injury in the rat. Exp Neurol 2001; 171:153-69; PMID:11520130; http://dx.doi.org/10.1006/exnr.2001.7734.
19. Totoiu MO, Keirstead HS. Spinal cord injury is accompanied by chronic progressive demyelination. J Comp Neurol 2005; 486:373-83; PMID:15846782; http://dx.doi.org/10.1002/cne.20517.
20. Barrett CP, Guth L, Donati EJ, Krikorian JG. Astroglial reaction in the gray matter lumbar segments after midthoracic transection of the adult rat spinal cord. Exp Neurol 1981; 73:365-77; PMID:6167460; http://dx.doi.org/10.1016/0014-4886(81)90272-7.
21. Tokunaga S, Ozawa H, Kokubun S. Astrocytic response to spinal cord injury. J East Jpn Orthop Traumatol 2005; 17:550-8.
22. Dello Russo C, Lisi L, Tringali G, Navarra P. Involvement of mTOR kinase in cytokine-dependent microglial activation and cell proliferation. Biochem Pharmacol 2009; 78:1242-51; PMID:19576187; http://dx.doi.org/10.1016/j.bcp.2009.06.097.
23. Lu DY, Liou HC, Tang CH, Fu WM. Hypoxia-induced iNOS expression in microglia is regulated by the PI3-kinase/Akt/mTOR signaling pathway and activation of hypoxia inducible factor-1alpha. Biochem Pharmacol 2006; 72:992-1000; PMID:16919605; http://dx.doi.org/10.1016/j.bcp.2006.06.038.
24. Hu LY, Sun ZG, Wen YM, Cheng GZ, Wang SL, Zhao HB, et al. ATP-mediated protein kinase B Akt/mammalian target of rapamycin mTOR/p70 ribosomal S6 protein p70S6 kinase signaling pathway activation promotes improvement of locomotor function after spinal cord injury in rats. Neuroscience 2010; 169:1046-62; PMID:20678995; http://dx.doi.org/10.1016/j.neuroscience.2010.05.046.
25. Narayanan SP, Flores AI, Wang F, Macklin WB. Akt signals through the mammalian target of rapamycin pathway to regulate CNS myelination. J Neurosci 2009; 29:6860-70; PMID:19474313; http://dx.doi.org/10.1523/JNEUROSCI.0232-09.2009.
26. Tyler WA, Gangoli N, Gokina P, Kim HA, Covey M, Levison SW, et al. Activation of the mammalian target of rapamycin (mTOR) is essential for oligodendrocyte differentiation. J Neurosci 2009; 29:6367-78; PMID:19439614; http://dx.doi.org/10.1523/JNEUROSCI.0234-09.2009.
27. Malagelada C, Jin ZH, Jackson-Lewis V, Przedborski S, Greene LA. Rapamycin protects against neuron death in in vitro and in vivo models of Parkinson's disease. J Neurosci 2010; 30:1166-75; PMID:20089925; http://dx.doi.org/10.1523/JNEUROSCI.3944-09.2010.
28. Khan S, Salloum F, Das A, Xi L, Vetrovec GW, Kukreja RC. Rapamycin confers preconditioning-like protection against ischemia-reperfusion injury in isolated mouse heart and cardiomyocytes. J Mol Cell Cardiol 2006; 41:256-64; PMID:16769083; http://dx.doi.org/10.1016/j.yjmcc.2006.04.014.
29. Pan T, Kondo S, Zhu W, Xie W, Jankovic J, Le W. Neuroprotection of rapamycin in lactacystin-induced neurodegeneration via autophagy enhancement. Neurobiol Dis 2008; 32:16-25; PMID:18640276; http://dx.doi.org/10.1016/j.nbd.2008.06.003.
30. Ravikumar B, Berger Z, Vacher C, O'Kane CJ, Rubinsztein DC. Rapamycin pre-treatment protects against apoptosis. Hum Mol Genet 2006; 15:1209-16; PMID:16497721; http://dx.doi.org/10.1093/hmg/ddl036.
31. David S, Kroner A. Repertoire of microglial and macrophage responses after spinal cord injury. Nat Rev Neurosci 2011; 12:388-99; PMID:21673720; http://dx.doi.org/10.1038/nrn3053.
32. Pineau I, Lacroix S. Proinflammatory cytokine synthesis in the injured mouse spinal cord: multiphasic expression pattern and identification of the cell types involved. J Comp Neurol 2007; 500:267-85; PMID:17111361; http://dx.doi.org/10.1002/cne.21149.
33. Yang L, Blumbergs PC, Jones NR, Manavis J, Sarvestani GT, Ghabriel MN. Early expression and cellular localization of proinflammatory cytokines interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha in human traumatic spinal cord injury. Spine (Phila Pa 1976) 2004; 29:966-71; PMID:15105666; http://dx.doi.org/10.1097/00007632-200405010-00004.
34. Popovich PG, Guan Z, Wei P, Huitinga I, van Rooijen N, Stokes BT. Depletion of hematogenous macrophages promotes partial hindlimb recovery and neuroanatomical repair after experimental spinal cord injury. Exp Neurol 1999; 158:351-65; PMID:10415142; http://dx.doi.org/10.1006/exnr.1999.7118.
35. Ferguson AR, Christensen RN, Gensel JC, Miller BA, Sun F, Beattie EC, et al. Cell death after spinal cord injury is exacerbated by rapid TNF alpha-induced trafficking of GluR2-lacking AMPARs to the plasma membrane. J Neurosci 2008; 28:11391-400; PMID:18971481; http://dx.doi.org/10.1523/JNEUROSCI.3708-08.2008.
36. Probert L, Eugster HP, Akassoglou K, Bauer J, Frei K, Lassmann H, et al. TNFR1 signalling is critical for the development of demyelination and the limitation of T-cell responses during immune-mediated CNS disease. Brain 2000; 123:2005-19; PMID:11004118; http://dx.doi.org/10.1093/brain/123.10.2005.
37. Karar J, Maity A. PI3K/AKT/mTOR Pathway in Angiogenesis. Front Mol Neurosci 2011; 4:51; PMID:22144946; http://dx.doi.org/10.3389/fnmol.2011.00051.
38. Zhong H, Chiles K, Feldser D, Laughner E, Hanrahan C, Georgescu MM, et al. Modulation of hypoxia-inducible factor 1alpha expression by the epidermal growth factor/phosphatidylinositol 3-kinase/PTEN/AKT/FRAP pathway in human prostate cancer cells: implications for tumor angiogenesis and therapeutics. Cancer Res 2000; 60:1541-5; PMID:10749120.
39. Guba M, von Breitenbuch P, Steinbauer M, Koehl G, Flegel S, Hornung M, et al. Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor. Nat Med 2002; 8:128-35; PMID:11821896; http://dx.doi.org/10.1038/nm0202-128.
40. Mautes AE, Weinzierl MR, Donovan F, Noble LJ. Vascular events after spinal cord injury: contribution to secondary pathogenesis. Phys Ther 2000; 80:673-87; PMID:10869130.
41. Facchiano F, Fernandez E, Mancarella S, Maira G, Miscusi M, D'Arcangelo D, et al. Promotion of regeneration of corticospinal tract axons in rats with recombinant vascular endothelial growth factor alone and combined with adenovirus coding for this factor. J Neurosurg 2002; 97:161-8; PMID:12134907; http://dx.doi.org/10.3171/jns.2002.97.1.0161.

42. Widenfalk J, Lipson A, Jubran M, Hofstetter C, Ebendal T, Cao Y, et al. Vascular endothelial growth factor improves functional outcome and decreases secondary degeneration in experimental spinal cord contusion injury. Neuroscience 2003; 120:951-60; PMID:12927201; http://dx.doi.org/10.1016/S0306-4522(03)00399-3.
43. Jaerve A, Schiwy N, Schmitz C, Mueller HW. Differential effect of aging on axon sprouting and regenerative growth in spinal cord injury. Exp Neurol 2011; 231:284-94; PMID:21806987; http://dx.doi.org/10.1016/j.expneurol.2011.07.002.
44. Siegenthaler MM, Ammon DL, Keirstead HS. Myelin pathogenesis and functional deficits following SCI are age-associated. Exp Neurol 2008; 213:363-71; PMID:18644369; http://dx.doi.org/10.1016/j.expneurol.2008.06.015.
45. Genovese T, Mazzon E, Di Paola R, Crisafulli C, Muià C, Bramanti P, et al. Increased oxidative-related mechanisms in the spinal cord injury in old rats. Neurosci Lett 2006; 393:141-6; PMID:16236449; http://dx.doi.org/10.1016/j.neulet.2005.09.060.
46. Gwak YS, Hains BC, Johnson KM, Hulsebosch CE. Locomotor recovery and mechanical hyperalgesia following spinal cord injury depend on age at time of injury in rat. Neurosci Lett 2004; 362:232-5; PMID:15158021; http://dx.doi.org/10.1016/j.neulet.2004.03.019.
47. Xie YY, Yao ZB, Wu WT. Survival of motor neurons and expression of beta-amyloid protein in the aged rat spinal cord. Neuroreport 2000; 11:697-700; PMID:10757503; http://dx.doi.org/10.1097/00001756-200003200-00009.
48. Ahlgren S, Li GL, Olsson Y. Accumulation of beta-amyloid precursor protein and ubiquitin in axons after spinal cord trauma in humans: immunohistochemical observations on autopsy material. Acta Neuropathol 1996; 92:49-55; PMID:8811125; http://dx.doi.org/10.1007/s004010050488.
49. Menendez JA, Vellon L, Oliveras-Ferraros C, Cufí S, Vazquez-Martin A. mTOR-regulated senescence and autophagy during reprogramming of somatic cells to pluripotency: a roadmap from energy metabolism to stem cell renewal and aging. Cell Cycle 2011; 10:3658-77; PMID:22052357; http://dx.doi.org/10.4161/cc.10.21.18128.
50. Conti A, Miscusi M, Cardali S, Germanò A, Suzuki H, Cuzzocrea S, et al. Nitric oxide in the injured spinal cord: synthases cross-talk, oxidative stress and inflammation. Brain Res Rev 2007; 54:205-18; PMID:17500094; http://dx.doi.org/10.1016/j.brainresrev.2007.01.013.
51. Vaziri ND, Lee YS, Lin CY, Lin VW, Sindhu RK. NAD(P)H oxidase, superoxide dismutase, catalase, glutathione peroxidase and nitric oxide synthase expression in subacute spinal cord injury. Brain Res 2004; 995:76-83; PMID:14644473; http://dx.doi.org/10.1016/j.brainres.2003.09.056.
52. Yang JY, Kim HS, Lee JK. Changes in nitric oxide synthase expression in young and adult rats after spinal cord injury. Spinal Cord 2007; 45:731-8; PMID:17353913; http://dx.doi.org/10.1038/sj.sc.3102036.
53. Anisimov VN, Zabezhinski MA, Popovich IG, Piskunova TS, Semenchenko AV, Tyndyk ML, et al. Rapamycin increases lifespan and inhibits spontaneous tumorigenesis in inbred female mice. Cell Cycle 2011; 10:4230-6; PMID:22107964; http://dx.doi.org/10.4161/cc.10.24.18486.
54. Harrison DE, Strong R, Sharp ZD, Nelson JF, Astle CM, Flurkey K, et al. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature 2009; 460:392-5; PMID:19587680.
55. Demidenko ZN, Zubova SG, Bukreeva EI, Pospelov VA, Pospelova TV, Blagosklonny MV. Rapamycin decelerates cellular senescence. Cell Cycle 2009; 8:1888-95; PMID:19471117; http://dx.doi.org/10.4161/cc.8.12.8606.
56. Blagosklonny MV. Validation of anti-aging drugs by treating age-related diseases. Aging (Albany NY) 2009; 1:281-8; PMID:20157517.
57. Bové J, Martínez-Vicente M, Vila M. Fighting neurodegeneration with rapamycin: mechanistic insights. Nat Rev Neurosci 2011; 12:437-52; PMID:21772323; http://dx.doi.org/10.1038/nrn3068.
58. Dazert E, Hall MN. mTOR signaling in disease. Curr Opin Cell Biol 2011; 23:744-55; PMID:21963299; http://dx.doi.org/10.1016/j.ceb.2011.09.003.
59. Zhao C, Vollrath D. mTOR pathway activation in age-related retinal disease. Aging (Albany NY) 2011; 3:346-7; PMID:21483039.
60. Garelick MG, Kennedy BK. TOR on the brain. Exp Gerontol 2011; 46:155-63; PMID:20849946; http://dx.doi.org/10.1016/j.exger.2010.08.030.
61. Caccamo A, Majumder S, Richardson A, Strong R, Oddo S. Molecular interplay between mammalian target of rapamycin (mTOR), amyloid-beta, and Tau: effects on cognitive impairments. J Biol Chem 2010; 285:13107-20; PMID:20178983; http://dx.doi.org/10.1074/jbc.M110.100420.
62. Pani G. P66SHC and ageing: ROS and TOR? Aging (Albany NY) 2010; 2:514-8; PMID:20689155.
63. Bjedov I, Toivonen JM, Kerr F, Slack C, Jacobson J, Foley A, et al. Mechanisms of lifespan extension by rapamycin in the fruit fly Drosophila melanogaster. Cell Metab 2010; 11:35-46; PMID:20074526; http://dx.doi.org/10.1016/j.cmet.2009.11.010.
64. Araki K, Ellebedy AH, Ahmed R. TOR in the immune system. Curr Opin Cell Biol 2011; 23:707-15; PMID:21925855; http://dx.doi.org/10.1016/j.ceb.2011.08.006.
65. Dancey J. mTOR signaling and drug development in cancer. Nat Rev Clin Oncol 2010; 7:209-19; PMID:20234352; http://dx.doi.org/10.1038/nrclinonc.2010.21.
66. Delgoffe GM, Powell JD. mTOR: taking cues from the immune microenvironment. Immunology 2009; 127:459-65; PMID:19604300; http://dx.doi.org/10.1111/j.1365-2567.2009.03125.x.
67. Thomson AW, Turnquist HR, Raimondi G. Immunoregulatory functions of mTOR inhibition. Nat Rev Immunol 2009; 9:324-37; PMID:19390566; http://dx.doi.org/10.1038/nri2546.
68. Pópulo H, Lopes JM, Soares P. The mTOR Signalling Pathway in Human Cancer. Int J Mol Sci 2012; 13:1886-918; PMID:22408430; http://dx.doi.org/10.3390/ijms13021886.
69. Khokhar NZ, Altman JK, Platanias LC. Emerging roles for mammalian target of rapamycin inhibitors in the treatment of solid tumors and hematological malignancies. Curr Opin Oncol 2011; 23:578-86; PMID:21892085; http://dx.doi.org/10.1097/CCO.0b013e32834b892d.
70. De Simone P, Metselaar HJ, Fischer L, Dumortier J, Boudjema K, Hardwigsen J, et al. Conversion from a calcineurin inhibitor to everolimus therapy in maintenance liver transplant recipients: a prospective, randomized, multicenter trial. Liver Transpl 2009; 15:1262-9; PMID:19790150; http://dx.doi.org/10.1002/lt.21827.
71. Fishbein TM, Florman S, Gondolesi G, Schiano T, LeLeiko N, Tschernia A, et al. Intestinal transplantation before and after the introduction of sirolimus. Transplantation 2002; 73:1538-42; PMID:12042637; http://dx.doi.org/10.1097/00007890-200205270-00004.
72. Raichlin E, Khalpey Z, Kremers W, Frantz RP, Rodeheffer RJ, Clavell AL, et al. Replacement of calcineurin-inhibitors with sirolimus as primary immunosuppression in stable cardiac transplant recipients. Transplantation 2007; 84:467-74; PMID:17713429; http://dx.doi.org/10.1097/01.tp.0000276959.56959.69.
73. Fleming GF, Ma CX, Huo D, Sattar H, Tretiakova M, Lin L, et al. Phase II trial of temsirolimus in patients with metastatic breast cancer. Breast Cancer Res Treat 2012; PMID:22245973; http://dx.doi.org/10.1007/s10549-011-1910-7.
74. Pavel ME, Hainsworth JD, Baudin E, Peeters M, Hörsch D, Winkler RE, et al.; RADIANT-2 Study Group. Everolimus plus octreotide long-acting repeatable for the treatment of advanced neuroendocrine tumours associated with carcinoid syndrome (RADIANT-2): a randomised, placebo-controlled, phase 3 study. Lancet 2011; 378:2005-12; PMID:22119496; http://dx.doi.org/10.1016/S0140-6736(11)61742-X.
75. Motzer RJ, Escudier B, Oudard S, Hutson TE, Porta C, Bracarda S, et al.; RECORD-1 Study Group. Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. Lancet 2008; 372:449-56; PMID:18653228; http://dx.doi.org/10.1016/S0140-6736(08)61039-9.
76. Zhou H, Luo Y, Huang S. Updates of mTOR inhibitors. Anticancer Agents Med Chem 2010; 10:571-81; PMID:20812900.
77. Fasolo A, Sessa C. Targeting mTOR pathways in human malignancies. Curr Pharm Des 2012; 18:2766-77; PMID:22475451.