# PROGRESS REPORT

**Original aims of the project.**

After traumatic brain injury (TBI), injury-compromised neurons progressively degenerate by necrosis and/or apoptosis. Moreover, axons do not regenerate after injury in the adult mammalian central nervous system because of the extrinsic inhibitory environment and diminished intrinsic regenerative capability of affected neurons. However, emerging evidence has shown that neuronal regeneration can occur by intrinsically activation of the mammalian target of rapamycin (mTOR) in neurons.

The TOR protein is an evolutionarily conserved central regulator of cell growth in response to growth factors, nutrients and stresses. Recent studies have revealed that mTOR plays a crucial role in regulation of neuronal growth, formation of new axonal growth cone, axonal protein synthesis, dendrite development, synaptic plasticity and neuronal viability. Further evidence demonstrates that activation of mTOR signaling can promote axonal regeneration of adult CNS after brain injury and that mTOR plays a role in dendritic branching and synaptic plasticity after TBI. These observations clearly suggest that intrinsic activation of mTOR has a critical role in brain cell regeneration and survival after TBI. However, current knowledge of the underlying mechanism and the downstream substrate of mTOR is still very limited. Historically, mTOR is known as a signaling kinase in the cytoplasm to modulate mRNA translation. Studies of mTOR localization in certain cell types showed that it is predominantly localized in the cytoplasm. In addition, two well-characterized mTOR downstream effectors, S6K1 and 4EBP1, are both responsible for mRNA translation in the cytoplasm. During these decades, substantial effort has been devoted to the cytoplasmic mTOR signaling and functions. In contrast, the specific role of TOR in the nucleus has largely been overlooked, and its nuclear targets and transcriptional regulation are poorly understood. Our recent studies in yeast and non-neuronal mammalian cells have revealed a novel function of mTOR in the nucleus: nuclear mTOR is crucial for regulation of cell growth. We show that mTOR directly binds to certain gene promoters to directly regulate their transcription. Inhibition of mTOR causes its dissociation from the promoters, repression of transcription, and cell growth inhibition. In addition, we identified Maf1 as a major downstream substrate of TOR and a key transcriptional repressor of TOR-regulated gene transcription in the

**varListTask continued**

nucleus. Interestingly, Maf1 is highly expressed in brain and enriched in the cortex and hippocampus, and it is concentrated in the nucleus of these neurons. Our preliminary data further indicated that mTOR is also localized in the nucleus of neuronal cells. It is known that various brain injuries, including those caused by trauma, cause alteration of transcriptional activation, suggesting that transcriptional activation plays a critical role in neuronal survival, regeneration and repair. These findings raise the possibility that mTOR has crucial function in transcriptional regulation in neuronal cells which is important for promoting neuronal regeneration and survival after traumatic brain injury. Here, I propose the following specific aims to test this hypothesis.

Aim 1. To establish the mTOR-mediated transcriptional regulation in neuronal cells

We hypothesize that mTOR in the nucleus of neuronal cells plays a crucial role in transcriptional regulation. According to our recent findings and preliminary results, we will identify the mTOR-regulated genes potentially responsible for promoting neuronal growth, regeneration and survival after traumatic brain injury. We will perform various biochemical and genetic approach to demonstrate the role of mTOR in transcriptional regulation of these genes by transcription assays. We will focus on the neurons of cerebral cortex and hippocampus because they are most vulnerable to traumatic brain injury. To establish the role of mTOR in transcriptional regulation, we will determine the DNA binding activity of mTOR to gene promoters by ChIP analysis. Results from this aim will mark significant advance regarding the novel function of mTOR in transcriptional regulation in neuronal cells. Moreover, the study will lead to the identification of mTOR-regulated transcription of genes which will provide important rationale for further investigation of the molecular mechanism by which mTOR promotes neuronal regeneration and survival after traumatic brain injury.

Aim 2. Determine the mechanism and functional significance of mTOR-regulated

**varListTask continued**

transcription in response to traumatic brain injury
To determine the mechanism of mTOR-mediated transcriptional regulation, we will investigate whether Maf1 is a key downstream substrate of mTOR responsible for its transcriptional regulation in neuronal cells. Genetic and biochemical analyses will be performed to determine the regulation of Maf1 activity by mTOR. Then, we will evaluate the functional significance of Maf1 by determining the neuronal protective function using in vitro assay mimicking traumatic brain injury.

Successfully completion of this pilot research will provide the feasibility and establish the novel transcription-regulatory function of mTOR in promoting neuronal regeneration and survival in response to traumatic brain injury. In addition, this study will provide groundwork for further investigation of the detailed mechanism of mTOR-regulated neuronal regeneration and survival, which will lead to the identification of effective molecular (e.g. Maf1) and gene targets for promoting neuronal survival, regeneration and repair after traumatic brain injury.

## PROGRESS REPORT CONTINUED

**Project successes.**

We have successfully achieved the major goals of this project. Briefly, we have identified the key mTOR-regulated genes potentially responsible for promoting neuronal growth and regeneration. By various biochemical and genetic approaches, we have shown that the role of mTOR in transcriptional regulation of RNA Pol III-dependent genes including the 5S and pre-tRNA genes in the primary cerebral cortex and hippocampal neurons. We also established the role of mTOR in transcriptional regulation, by demonstrating that mTOR physically binds to these gene promoters. Therefore, these results have laid an important foundation on the novel function of mTOR in transcriptional regulation in neuronal cells.

We have also successfully uncovered the molecular mechanism of mTOR-mediated transcriptional regulation in neuronal cells, we have identified that Maf1 is a key downstream substrate of mTOR responsible for its transcriptional regulation in neuronal cells. Unexpectedly, during the course of our planned experiments, we discovered that Reelin, an extracellular matrix glycoprotein, can induce Pol III-dependent transcription in the primary neurons. We further showed that PI3K mediates Reelin-induced mTOR-Maf1 signaling for the transcription regulation by Pol III. In terms of the neuronal function of Maf1, we demonstrated that Maf1 is an intrinsic negative regulator of the axonal and dendritic growth and spin development in primary neurons. Therefore, these results have clearly suggested the novel transcription-regulatory function of mTOR in promoting neuronal regeneration in response to traumatic brain injury.

## PROGRESS REPORT CONTINUED

**Project challenges.**

At early period of the project, we had encountered difficulty in isolating the hippocampal neurons for the proposed experiments. With more practice and the technical help of our collaborator Dr. D¶Arcangelo and the members of her lab, our team had been able to isolate this type of neurons and performed the necessary experiments accordingly. In addition, we have originally planned to perform viability assay to examine the effect of Maf1 on protecting the survival of neurons. However, we have not yet been able to demonstrate it because of the problem of antibody and other technical details needed to be optimized. We will continue this part in our future plan.

**PROGRESS REPORT CONTINUED**

**Implications for future research and/or clinical treatment.**

This research has indicated that in primary cortical and hippocampal neurons mTOR plays an important role in transcription of rDNA and tRNA genes. Importantly, these results raise a possibility that mTOR-Maf1 signaling is important for neuroregeneration after TBI, and prevention and treatment of neurodegenerative diseases. Based on these findings, my future research will be focused on the detail role of Maf1 in vivo using animal studies and the examination in TBI models.

The future plan will include the use of mouse models and animal studies to characterize the physiological and pathological roles of mTOR-Maf1 signaling in neuronal repair after brain injury. To this end, I have established collaboration with Dr. Dong-Fu Feng, an expert in using the TBI animal model, to evaluate the functional role of Maf1 in nerve repair after TBI. We will use the virus-mediated in vivo knockdown of Maf1 and the conditional Maf1 knockout mice to evaluate structural and functional recovery in response to traumatic brain injury. He has developed a novel animal model for diffuse axonal injury induced by simultaneous moderate linear and angular head accelerations. By this technique, we can quantitatively evaluate the microscopic injury with diffusion tensor imaging in animal model of diffused axonal injury. In addition to neuronal structural repair, we will evaluate the functional recovery such as memory and cognitive functions by animal behavioral studies. Metabolic and immunological responses will also be examined in this study. This future plan would lead to the development of novel therapeutic strategy for the treatment of TBI.

In this NJBIR project, we have also found that Reelin plays a role in Maf1-mediated transcriptional regulation. In the future, we will continue collaborating with Dr. Gabriella D¶Arcangelo to explore the novel downstream targets of Reelin during neural development and neuronal repair. We will characterize the physiological significance of Maf1 expression pattern in different cortical regions and stages during neural development, in order to explore the mechanisms by which Reelin induces various aspects of neural development. We will use the transgenic mice

**varReportChanges continued**

including the reeler mice (in which Reelin is mutated) and Maf1 knockdown mice to fully characterize the neuronal function of Reelin, mTOR-Maf1 pathway and other signaling pathways during neural development and neuronal repair.

In addition, to have a comprehensive insight into the neuronal mTOR function, I will perform ChIP-sequencing analysis to define the mTOR-regulated transcriptome in neurons. This genomic analysis will provide target genes for mTOR-regulated transcription and neuronal functions. Moreover, I will use proteomic approach such as Mass Spectrometric analysis to identify the mTOR-interacting proteins in neurons. These future research works will not only provide important insight into the molecular mechanisms that regulate various neuronal activities such as development, repair and regeneration, but will also lay an important foundation for development of novel therapeutic strategy for the treatment of TBI.

## PROGRESS REPORT CONTINUED

**Plans to continue the research, including applications submitted to other sources for ongoing support.**

In addition to the planned research as proposed above, I have been preparing a new research grant proposal to continue my research in TBI. The funding sources include NIH National Institute of Neurological Disorders and Stroke (NINDS) and the Department of Defense Traumatic Brain Injury Research Program.

## PROGRESS REPORT CONTINUED

**Explain how you have leveraged NJCBIR funding to obtain additional federal or other support for brain injury research and list the appropriate funding organizations.**

By the NJBIR funding, I have gathered important preliminary data for the proposal of my next round of grant application. I will submit the new proposal to the NIH National Institute of Neurological Disorders and Stroke (NINDS) and the Department of Defense Traumatic Brain Injury Research Program.

## PROGRESS REPORT CONTINUED

**All papers, presentations, chapters, and abstracts should mention that the research was supported by a grant from New Jersey Commission on Brain Injury Research. Copies must be sent to the NJCBIR office, even if you inadvertently forgot to cite NJCBIR support. List and include a copy of all publications emerging from this research, including those in preparation.**

1.      Arachchige Don, A.S., Tsang, C.K., Kazdoba, T.M. D¶Arcangelo, G., Young, W., Zheng, X.F. (2012) Targeting mTOR as a novel therapeutic strategy for traumatic CNS injuries. Drug Discovery Today 17, 861-868.


2.      Miao Chen, Lee G.H., and D¶Arcangelo, G. and Tsang, C.K. mTOR-Maf1 signaling pathway regulates dendritic development induced by Reelin. (Manuscript in preparation).

# PROGRESS REPORT CONTINUED

974425-2012DDT.pdf

974425-Manuscriptinpreparation.pdf