The Expression of mTOR and Trib3 in Oligodendrocyte Lineage Cells

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Abstract

Oligodendrocytes are a type of glial cell in the central nervous system that are responsible for producing the myelin sheath which allows for faster neuronal firing rates. Myelin production is a metabolically expensive process, which requires the oligodendrocytes to be aware of the nutrients are available to them. It has been demonstrated that the Mammalian Target of Rapamycin (mTOR) is required for the later stages of maturation in oligodendrocytes. However, when mTOR is inhibited by Rapamycin in the early stages of oligodendrocyte development, the oligodendrocytes are still able to fully differentiate. Tribbles Homolog 3 (*Trib3*) is a pseudo kinase that has impaired catalytic activity. In previous research, trib3 has been shown to be upregulated by cellular stress and serves as an important regulator of cell death, stress responses, cell differentiation and many other processes. The goal of this research is to analyze the expression levels of mTOR throughout the oligodendrocyte lineage, at day 1 and day 4, and the expression of Trib3 in day 4 cells when they are deprived of leucine. The results indicate that as cells age mTOR expression is increased and as they are deprived of leucine at day 4, trib3 expression decreases.

Introduction

The production of the myelin sheath is a very metabolically expensive process that oligodendrocytes take part in. The myelin sheath is a fatty membrane that wraps around the axons of neurons in the brain and allows for more effective firing of action potentials(1). Oligodendrocytes (OLs) have a very particular maturation process where they go from being an early progenitor, to a late progenitor, to an immature oligodendrocyte, to finally becoming a mature oligodendrocyte with many projections to be able to myelinate neurons (2). It has previously been demonstrated that inhibition of mTOR by Rapamycin at earlier stages in the OL lineage does not prevent the OLs from terminally differentiating, however if mTOR is inhibited at later stages of the lineage, OLs cannot terminally differentiate (2). Oligodendrocytes use many signaling pathways to sense the nutrients in their surroundings, but exact mechanisms are not fully understood. mTOR is protein serine/threonine kinase that is part of the PI3K kinase related family and is a key regulator of cell growth and has been implicated in nutrient sensing (3).

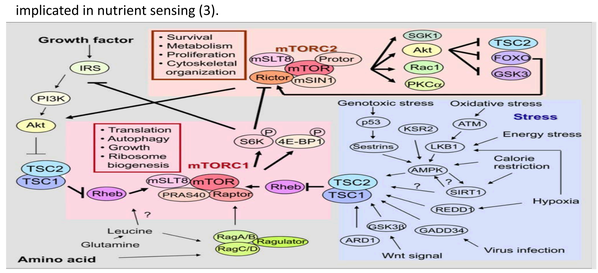

Figure 1. mTOR complex 1 and 2 and their signaling pathways.

Trib3, is a pseudo kinase that has been shown to be involved in regulation of nutrient metabolism during feeding and fasting (4). This pseudo kinase also has the potential to play a role in myelin pathology. Trib3 expression has been shown to be increased in expression preceding the prominent breakdown of myelin in demyelination rats (5). Since trib3 has been shown to respond to nutrients in the environment has the potential to be involved in myelin pathology, this gene is particularly important in OLs which need a plethora of nutrients to mature and eventually produce myelin. The goal of this research is to analyze mTOR expression throughout the lineage of OLs and to analyze trib3 expression during the withdrawal of leucine.

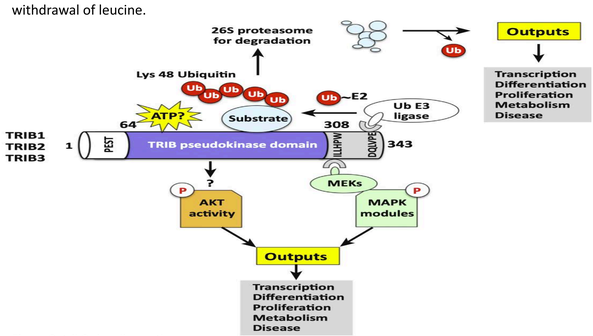

Figure 2. trib3 signaling pathways.

Methods

I. Enriched Oligodendrocyte Cultures

Growth factors and animal serum cause progenitors to increase rapidly in number, but remain undifferentiated. Once these are removed, cells differentiate and grow. Once the growth factors were removed, the oligodendrocyte cultures were grown for various time periods (1 or 4 days). RNA was harvested on day 1, and day 4 of growth to test mTOR expression at different points in the lineage.

II. Starvation of Leucine

Only day 4 cells were used to test trib3 expression during leucine withdrawal. Prior to each RNA harvest on day 4, half of the cells were starved for a duration of 2 or 24 hours. In order to deprive them of leucine, the cells were given N2 Lim, which was a medium lacking leucine. Other cells were given N2 medium which contained all the typical nutrients. After the starvation/feeding period, the RNA was isolated following a Trizol/RNeasy Hybrid protocol.

III. mTOR and Trib3 mRNA Analysis by qPCR

qPCR was performed using iTaqTM Universal SYBR® Green One-Step Kit. GAPDH and HPRT1 were used as reference genes for comparison to the mTOR and Trib3 gene expression. Data analysis of gene expression was conducted using the ΔΔCq method. This determines the fluctuations in expression of the genes of interest relative to reference genes and then compares target samples to control samples.

Results

I. Expression of mTOR as a Function of OL Age

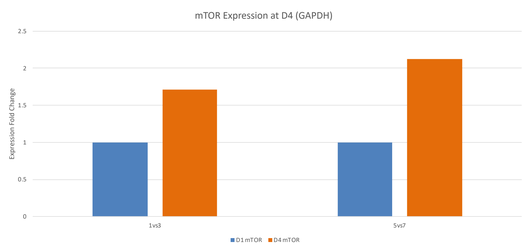

Figure 3. In comparison to day 1 cells, day 4 cells have an increased expression of mTOR when using GAPDH as the internal reference. Sample 1/3 were from one culture and sample 5/7 were from another. Expression fold change values depicted are 1.71119005 for samples 1/3, and 2.12137548 for samples 5/7. The blue bars represent the reference sample expression fold change, which is always 1.

Figure 4. In comparison to day 1 cells the RNA sample 3 showed an increase in mTOR expression fold change and sample 7 showed a slight decrease in mTOR expression fold change using HPRT1 as the internal reference. Sample 1/3 were from one culture and sample 5/7 were from another. Expression fold change values depicted are 4.29452926 for samples 1/3 and 0.75654615 for sample 5/7. The blue bars represent the reference sample expression fold change, which is always 1.

Based on the results, there was an increase in mTOR expression at day 4 of the OL lineage compared to day 1 in 3 out of 4 of the analyses. However, when using HPRT1 as an internal reference, there was some inconsistency where one analysis showed a slight decrease in expression of mTOR at day 4. Significance of the results was not determined.

Results (Cont.)

II. Expression of Trib3 as a Function of Leucine Withdrawal

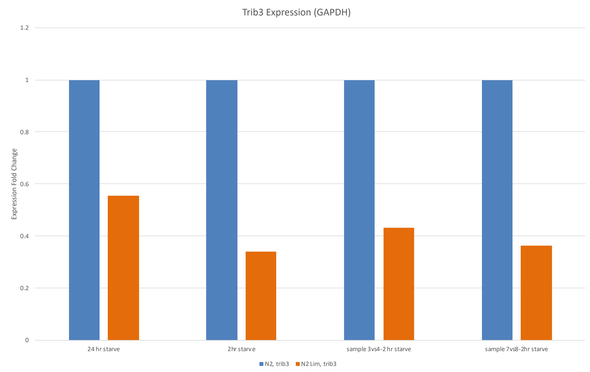

Figure 5. When cells were deprived of leucine using N2 Lim media, there was a decrease in Trib3 expression. The 24 hr starve and 2 hr starve samples were RNA samples that were from the same culture and were exposed to different withdrawal periods. Samples 3/4 and 7/8 were from two separate cultures. The orange bars depict the gene fold expression change when cells lacked leucine and are values of 0.43301833, 0.36286379,0.34073804, and 0.5540264. Expression fold change values depicted by the blue bars is the reference sample expression fold change, which is 1.

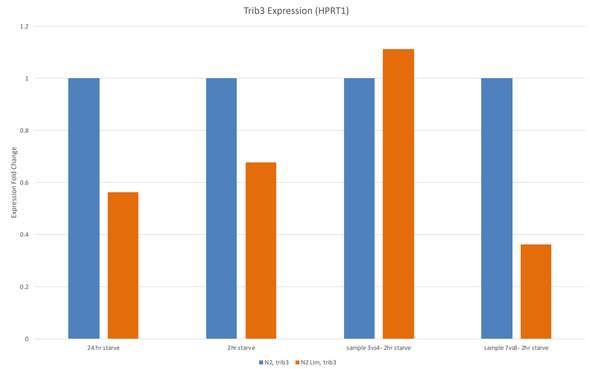

Figure 6. When cells were deprived of leucine using N2 Lim media, there was a decrease in Trib3 expression for 3 out of 4 experiments. The 24 hr starve and 2 hr starve samples were RNA samples that were from the same culture and were exposed to different withdrawal periods. Samples 3/4 and 7/8 were from two separate cultures. The orange bars depict the gene fold expression change when cells lacked leucine and are values of 0.56372495, 0.67706443, 1.11149388 and 0.24316374. Expression fold change values depicted by the blue bars is the reference sample expression fold change, which is 1.

Based on these results, majority of the analyses demonstrated a decrease in expression of trib3 when day 4 cells were deprived of leucine. However, there was some inconsistency when HPRT1 was used as the internal reference and one experiment demonstrated a slight increase when cells were deprived of leucine. Significance of the results was not determined.

Discussion

- mTOR expression at day 4 of the OL lineage showed an increase in expression
- When day 4 cells were deprived of leucine, trib3 showed a decrease in expression
- Since mTOR appears to be crucial for the differentiation of OLs at later stages in the lineage, this could be why there is increased expression of mTOR at day 4
- The decreased expression of trib3 during leucine deprivation conflicts with previous research, and could be due to the experimental conditions
  - There is limited research on trib3 expression in OLs and many of the current studies have only looked at cell lines or cancer cells.
- Significance of the results was not determined, which is a limitation in drawing conclusions about the results
- Future directions:
  - Run more experiments to see reproducibility of these results & determine significance
  - Alter the starvation durations for the trib3 experiment

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