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 that requires the oligodendrocytes to be aware of the nutrients available to them. It has been demonstrated that the mammalian target of rapamycin (mTOR) is critical for this process. The results of these studies have shown that once mTOR is inhibited by Rapamycin at earlier stages in the lineage, the oligodendrocytes are still able to fully differentiate. Tribbles Homolog 3 (Trib3) is a pseudokinase that has been shown to be upregulated in many forms of cancer. In previous research, Trib3 has been found to be upregulated in many forms of cancer and is involved in regulation of nutrient sensing. In this study, the production of the myelin sheath is a very metabolically expensive process that requires the oligodendrocytes to be aware of the nutrients available to them. It has been demonstrated that the mammalian target of rapamycin (mTOR) is critical for this process. The results of these studies have shown that once mTOR is inhibited by Rapamycin at earlier stages in the lineage, the oligodendrocytes are still able to fully differentiate. Tribbles Homolog 3 (Trib3) is a pseudokinase that has been shown to be upregulated in many forms of cancer. In previous research, Trib3 has been found to be upregulated in many forms of cancer and is involved in regulation of nutrient sensing.

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 are removed, the oligodendrocyte cultures were grown for various time periods (1-4 days). RNA was harvested on day 1, and day 4 of growth to test mTOR expression at different points in the lineage.

II. Expression of mTOR as a Function of OL Age

The expression of mTOR was determined by qPCR in control samples. GAPDH as the internal reference. Samples 1/3 were from one culture and samples 5/7 were from another.

Results (Cont.)

III. Expression of Trib3 as a Function of Leucine Withdrawal

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 protocol.

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

References


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