Identifying How T Cells Utilize Branched-Chain Amino Acids (BCAAs) To Activate mTORC1 or Engage Downstream Mitochondrial Metabolism

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Abstract

Using Jurkat cells, Ramesh and Eleh aim to understand whether mTOR activation using a leucylated LARS occurs in T cells, whether glucose suppresses the metabolism of branched-chain amino acids (BCAA) in T cells, and whether the presence of glucose affects the sensing of leucine in T cells. Previous research proves the activation of mTOR by the LARS-leucine complex solely in 293T mutant cell lines, as well as the direct correlation between the absence of glucose in a cell and the degradation of BCAAs in human kidney, muscle and, heart cells. There has been no thorough study of the aforementioned phenomena in T-cells. In this project, previous literature was used, and qPCR data was generated to study the correlation between glucose in a cell and BCAA degradation and have yet to thoroughly study how a leucylated LARS complex communicates with mTOR. However, the current findings showcase the opposite results from the study of glucose and LARS on heart cells, as the absence of glucose did not lead to the degradation of BCAAs. With further research, Ramesh and Eleh hope to solidify their results on the glucose degradation of BCAAs and move forward in their experiments regarding the LARS-leucine complex and mTOR activation.

Keywords: BCAAs, glucose, *LARS, mTOR1*, cDNA, T cells

Introduction

- Branched-chain amino acids (BCAAs) are essential nutrients and push cell growth
- Amino acids are required for activation of the mammalian target of rapamycin (mTOR) kinase
- Regulates protein synthesis and cell size
- BCAAS also metabolized for TCA cycle, allowing for energy generation for cells

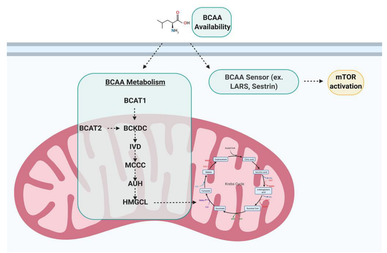


Figure 1. BCAA sensed/metabolized by cells

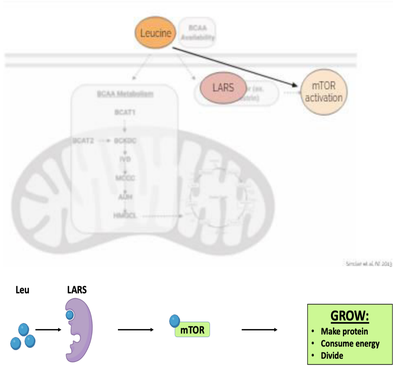


Figure 2. *LARS* role in leucine signaling to mTOR in T cells

- Leucine (a BCAA) serves as a substrate for protein synthesis and protein metabolism
- In T cells, we know Leucine activates mTOR
- However, the amino acid sensor that couples leucine signaling to mTOR is unknown in T cells

- Leucyl tRNA synthetase (*LARS*) was found to be the sensor that communicates with mTOR
- While LARS sensing was demonstrated in HeLa and 293T cells, its function in T cells remains in question

- Glucose has been shown to affect BCAA metabolism
- Glucose supplies metabolites for biosynthesis and is needed for cell proliferation
- An increase in glucose favored mTOR activation
- Decreased gene expression in BCAA metabolism
- Effects of low glucose in T cells unknown

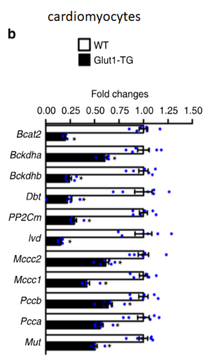


Figure 3. BCAA metabolism gene expression in cardiomyocytes

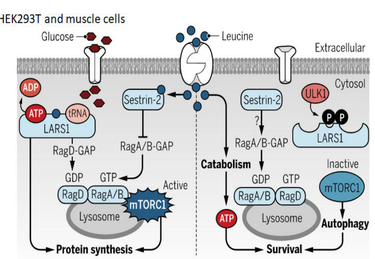
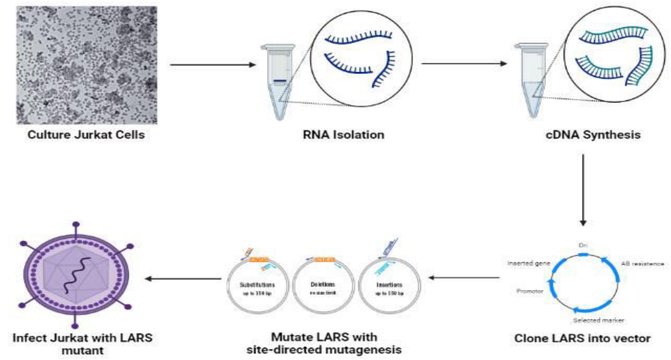


Figure 4. *LARS* function in the presence and absence of glucose

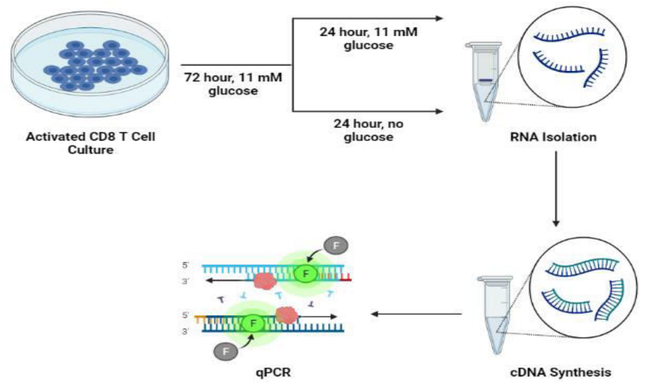
- Glucose has been implicated in *LARS* function
- Normally, ATP leucylates protein in the presence of glucose; mTOR activated
- With no glucose, *ULK1* phosphorylates *LARS*; mTOR1-induced protein synthesis decreases

Methods

Approach I: Confirm LARS Sensing in T Cells



Approach II: Glucose Effects on BCAA Metabolism



Results

Part I: Amplification of LARS cDNA

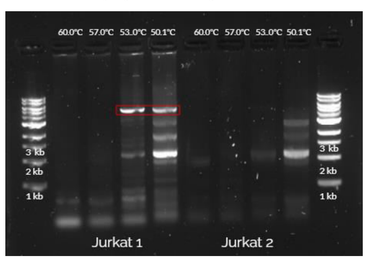


Figure 5. Gel depicting cDNA derived from Jurkat samples

- Two Jurkat samples were used for cDNA synthesis
- Annealing temperature gradient PCR
  - *LARS* size around 4.8 kb

Part II: Glucose and BCAA Metabolism

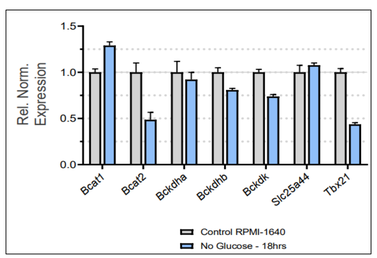


Figure 6. BCAA gene expression in low glucose condition

- Unlike cardiomyocytes, gene expression in BCAA metabolism was not completely increased
  - *Bcat1* saw expression increase
  - *Bckdk* inhibits BCAA metabolism, saw decrease

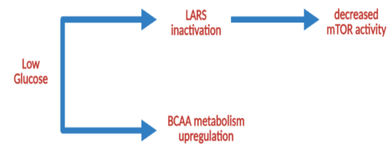
Conclusions

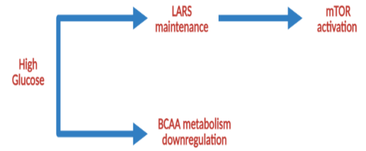
Part I: LARS Sensing of Leucine for mTOR Activation

- *LARS* cDNA was identified at two gradient annealing temperatures from first Jurkat sample
  - cDNA was combined and PCR product is to be subsequently sequenced

Part II: Glucose and BCAA Metabolism

- Results of qPCR in T cells not consistent with those seen in mouse cardiomyocytes
- Bcat1 saw expression increase, but other three BCAA degradation genes saw a decrease
  - Unlike cardiomyocyte study, GLUT-1 was not overexpressed, and rather glucose in media was deprived
- *Bckdk* inhibits BCAA metabolism: predicted decrease in expression
- *Slc25a44* (transporter in and out mitochondria BCAAs)
- *Tbet/Tbx21*: control transcriptional factor important in effector T cell function
- Predicted hypothesis:

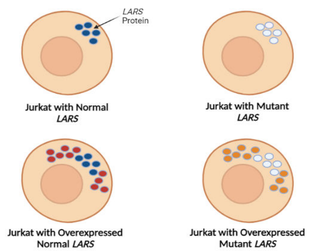




Future Research and Challenges

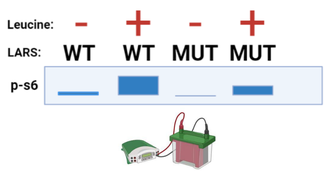
Part I: LARS Sensing of Leucine for mTOR Activation

- Cloning of LARs from cDNA
  - Many variations of LARS (isoforms)
  - Issues with the annealing temperature; optimal temp was 10-13 degrees lower than expected
- Mutate LARS to disrupt leucine sensing/mTOR activation (eventually mimic patient condition)
  - Through site-directed mutagenesis
- LARS sensing of leucine being regulated by glucose



Part II: Glucose and BCAA Metabolism

- Repeat qPCR to affirm results of BCAA degradation gene expression
  - Begin glucose swap earlier in activated CD8 T Cell culture
  - Higher glucose concentration (used 11 mM)
- KLF15 repressive transcription factor: involvement in downregulation of BCAA metabolism



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