2018

Characterization of Metabolic Network in T-helper 2 Cells

Bailee Lichter
University of Nebraska-Lincoln, blichter337@gmail.com

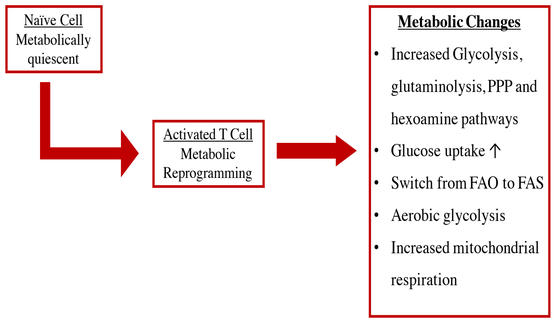
Characterization of the Metabolic Network in T-helper 2 Cells

Bailee Lichter

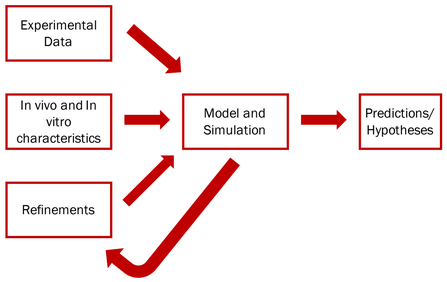
Department of Biochemistry, University of Nebraska-Lincoln

INTRODUCTION

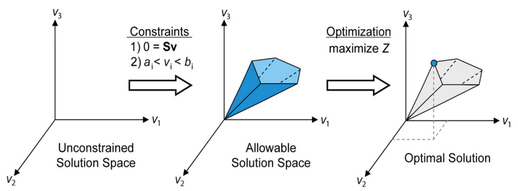
- T-helper 2 (Th2) cells function in the adaptive immune system to coordinate responses to large extracellular pathogens (Luckheerham, 2012).
- Th2 cells are one of the differentiated subtypes cells of CD4+ T cells. CD4+ T cells recognize foreign pathogens and differenitated in specialized subtypes to affectively destroy the pathogen (Luckheerham, 2012).
- Upon CD4+ T cell activation, the cells undergo metabolic reprogaming, resulting in a shift in metabolism (Almedia, 2016).



- Computational modeling combines experimental data and known characteristics of in vivo and in vitro systems to create a model. The model can simulated under different situations.

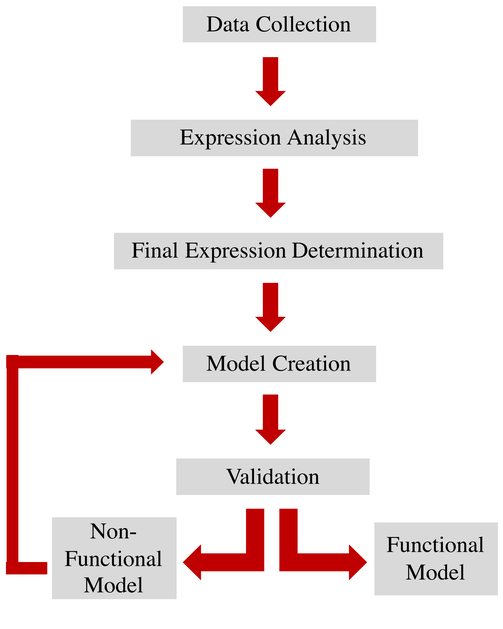


- Constraint based modeling is a mathematical technique in which outputs are based on maximum and minimum limits, or constraints. Flux Balance analysis, a form of constraint based modeling will be used to make the metabolic model. This is a technique that analyzes the flow of metabolites through a metabolic network (Orth, 2010).



Orth, J. D.,(2010).Nature Biotechnology.

METHODS



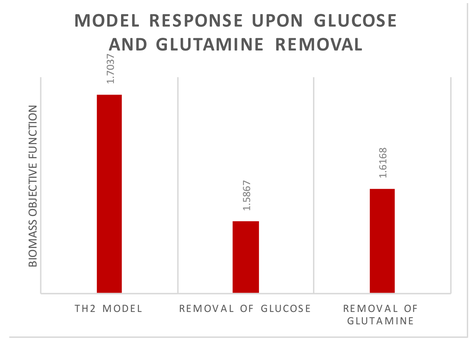
- Data was collected from the public database Gene Expression Omnibus (GEO). Untreated and control samples were used, while knockout and other treated Th2 cells were avoided. 41 samples were used from Affymetrix, Agilent, and Proteomics datasets.
- To determine sample expression, Affymetrix was normalized and designated present/marginal/absent calls. Present and marginal calls represented an active gene. Agilent data was normalized and compared to a control probe. Expression levels greater than the control signified an active gene. For the proteomics data, if the protein count was greater than zero, the gene was active.
- Final expression of genes was determined by combining all datasets. Genes active in microarray and proteomics, were high-confidence active genes and used in the model. Genes were also added from microarray if 90% of the sample were active, and from proteomics data if 75% of genes were active.
- Model was created in MatLab using the COBRA toolbox.
- Models were validated by looking at the biomass objective function, gene essentiality, flux through major metabolic processes, and the model's response to removoal of glucose and glutamine.
- A nonfunctional model was reverted back to the model creation step. Model was edited by changing biomass objective function, adding and removing reactions, adjusting reaction bounds, and fixing errors due to syntax.
- A functional model was created when the validation results corresponded to what was expected.

RESULTS

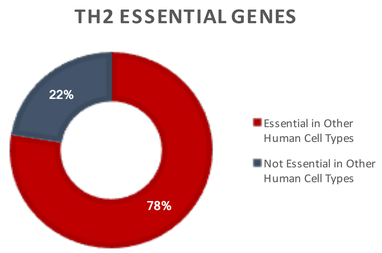
- The following table summarizes the components of the final Th2 metabolic network model

Final Th2 Metabolic Network Model	
Genes	1151
Metabolites	2601
Reactions	4654
Biomass Objective Function	1.7037/hour

- The biomass objective function is the biomass production per hour, which relates to the cell's growth. The biomass objective function was compared to published values and found to be in agreement.
- Upon removal of glucose or glutamine, we see a decrease in the biomass objective function.



- Flux through major metabolic pathways was analyzed. It was determined there is flux through the major metabolic pathways, which include the TCA cycle, glycolysis, fatty acid synthesis, and fatty acid oxidation.
- It was determined there were 49 essential genes specific to this Th2 metabolic network model. 38 of those 49 genes were also essential in other human cell types.



DISCUSSION

- Both glucose and glutamine are important for proper functioning of Th2 cells. We observed a decreased biomass function upon their removal. This indicates slowed growth, agreeing with what would be observed in nature. The reason we didn't see the biomass objective function drop to zero upon removing glucose or glutamine is because there are other carbon sources available that the cell can use for metabolism.
- Flux through major metabolic pathways indicated that the reactions are occurring. While not every reaction in the pathway had flux, every metabolic process had an overall flux signifying that the processes were active. This is what we would expect and serves as further validation of the model.
- Gene essentiality was determine by removing the gene and looking at the biomass objective function. A decrease in this value indicates the gene's importance to the model. We expected a high number of essential genes in our model to be essential in other human cells types. Many of the essential metabolic pathways in other human cell types are also present in our model.

CONCLUSION

- We have successfully created a Th2 metabolic network model.
- The Th2 metabolic network model contains 1151 genes, 2601 metabolites, and 4654 reactions.

REFERENCES

- Almeida, L., Lochner, M., Berod, L., & Sparwasser, T. (2016, November 4). Metabolic pathways in T cell activation and lineage differentiation. Science Direct. doi:https://doi.org/10.1016/j.smim.2016.10.009
- Luckheeram, R. V., Zhou, R., Verma, A. D., & Xia, B. (2012). CD4+T Cells: Differ entiation and Functions. Clinical and Developmental Immunology, 2012, 925135. http://doi.org/10.1155/2012/925135
- Orth, J. D., Thiele, I., & Palsson, B. Ø. (2010). What is flux balance analysis? Nature Biotechnology, 28(3), 245–248. http://doi.org/10.1038/nbt.1614

ACKNOWLEDGMENTS

This project was made possible by the guidence of Tomas Helikar and Bhanwar Lal Puniya. Thank you to the following lab members for their contribution to the project; Alex Ciurej, Robert Moore, Sydney Townsend, and Ab Rauf Shah.