DOI 10.18699/SBB-2022-035

Tocilizumab partially reverses the altered transcriptional regulation of glycolysis in circulating CD8$^+$ T cells of rheumatoid arthritis patients

Harshan S.[1, 2]*, Dey P.[3], Srivatsan R.[1]

[1] *Institute of Bioinformatics and Applied Biotechnology, Biotech Park, Bengaluru, India*

[2] *Manipal Academy of Higher Education, Manipal, Karnataka, India*

[3] *he Edu Bio LLC, Northwest Registered Agent Service Inc., Okemos, Michigan, USA*

* *shilpa.harshan@gmail.com*

Key words: rheumatoid arthritis, tocilizumab, T lymphocyte, RNA seq, gene expression

Background: Peripheral T cells of rheumatoid arthritis (RA) patients show pathological changes. CD4$^+$ T cells use pentose phosphate pathway instead of glycolysis, resulting in a reductive phenotype. These changes drive increased proliferation and tissue invasiveness of these cells. CD8$^+$ T cells also show changes to the glycolysis pathway. However, the transcriptional regulation underlying the altered glycolysis in these cells is poorly understood. The effect of drugs used in the treatment of RA on glycolysis genes and their regulators also remains to be elucidated.

Method: We analysed publicly available RNA sequencing data from circulating CD4$^+$ and CD8$^+$ T lymphocyte subsets of healthy, untreated and treated RA individuals to identify differentially expressed genes (DEGs). Glycolysis related genes and transcription factor genes were selected to create differential co-expression networks for each cell type. Sample-wise edge weights were calculated using for all possible gene-pairs using "linear interpolation to obtain network estimates for single sample" (lionessR) analysis, and the gene-pairs with significantly different edge weights were identified using limma. The networks were annotated using the Gene Transcription Regulation Database (GTRD) and analysed to find high centrality genes.

Results: CD8$^+$ effector memory (Tem) cells and CD8$^+$CD45RA$^+$ effector memory (Temra) cells showed large changes to the transcriptional regulation of glycolysis in untreated RA. GAPDH was upregulated and had high centrality scores in the untreated RA CD8$^+$ Tem cells. Its upregulation in the untreated RA CD8$^+$ Tem cells was reversed in the tocilizumab treated cells. The transcription factors that show opposing differential expression with GAPDH in the untreated and tocilizumab treated CD8$^+$ Tem cells were identified. Tocilizumab treatment partially reversed the RA associated differential expression of genes involved in fatty acid metabolism, citric acid cycle, oxidative phosphorylation complex 1 and ATP synthase. However, PFKFB3, which regulates glycolysis, is not affected in the tocilizumab treated CD8$^+$ cells.

Conclusion: Tocilizumab treatment is associated with a return to a healthy like expression state for several genes. However, tocilizumab does not restore PFKFB3 levels indicating that its effect on glycolytic activity in these cells is limited.

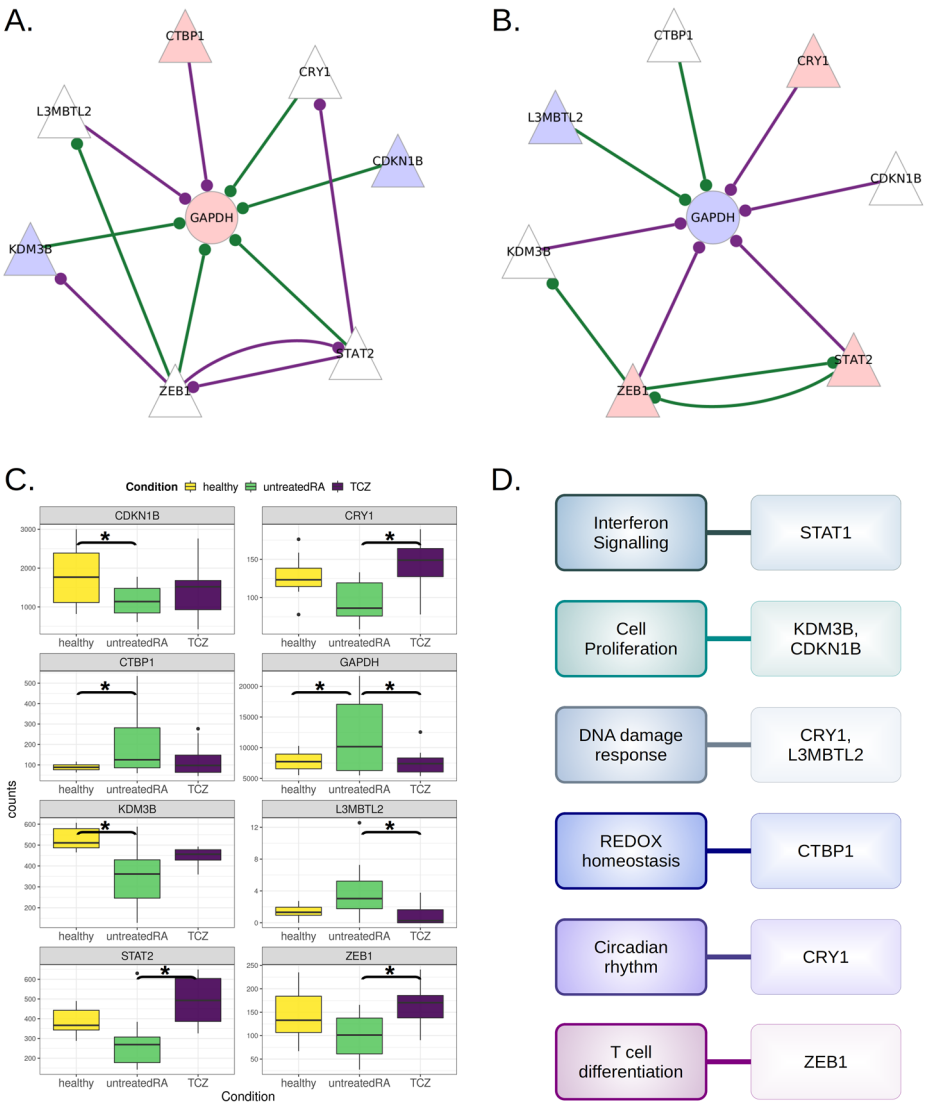

Fig. 1. A. GAPDH and its neighbors in the untreated RA network. Red nodes are upregulated and blue nodes are down regulated. Purple edges show higher mean edge weight in untreated RA samples. Green edges show higher mean edge weight in healthy samples. B. GAPDH and its neighbors in the tocilizumab RA network. Purple edges show higher mean edge weight in the tocilizumab samples. Green edges show higher mean edge weight in the untreated RA samples. C. Expression values of GAPDH and its differentially expressed neighbors in the healthy, untreated RA and tocilizumab treated RA $CD8^+$ Tem cells. Statistically significant differential expression is indicated by an asterisk (*). D. The functions of the differentially expressed transcription factor neighbours of GAPDH in the $CD8^+$ Tem cells

Acknowledgements: This work was fully supported by the Department of Electronics, IT, BT and S&T of the Government of Karnataka. Shilpa Harshan is a PhD student registered under Manipal Academy of Higher Education.