Preventing tumor-induced T cell suppression through activating the TLR8 pathway

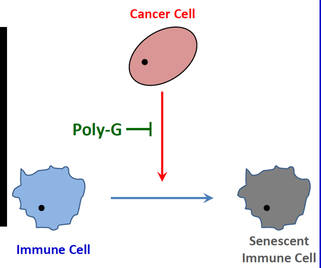
1. Introduction

Obstacle to Cancer Immunotherapy

- Immunotherapy is a breakthrough cancer treatment that utilizes the immune system to target and destroy cancer cells.
- Despite promising, the success rate of cancer immunotherapy is relatively low.
- One main underlying problem is the suppression of immune cells by the tumor microenvironment.
- To boost the efficiency of cancer immunotherapy, it is critical to gain a better understanding of the mechanism by which tumor cells induce immunosuppression and how to prevent it.

T-cell senescence induced by cancer cells

- Cancer cells can induce T cell senescence, a form of immunosuppression.
- Investigating how tumor cells induce T cell senescence can help prevent immunosuppression.
- Poly-G (an activating ligand for TLR8) treatment can prevent tumor-induced T cell senescence, but the underlying mechanism is not well-understood.



2. Research Questions

- **Main Question:** How does TLR8 activation prevent tumor-induced T-cell senescence?
- **Hypothesis:** TLR8 activation down-regulates glucose metabolism in tumor cells, thus decreasing their ability to induce T cell senescence.
- **Rationale:** Cancer cells have very high rates of glucose uptake and metabolism. In an environment with limited glucose availability, T cells are more likely to undergo senescence. Thus, it is possible that TLR8 activation may down-regulate tumor glucose metabolism, making more glucose available to T cells, leading to decreased T cell senescence.
- **Specific Question:** Does TLR8 activation decrease the expression level of key proteins required for glucose metabolism in cancer cells?

3. Research Design, Materials and Methods

Research Design – Use well-established tumor cell lines that were derived from important types of tumor, culture the tumor cells, treat them with or without poly-G for either 8 h (to observe early responses) or 48 h (to observe late responses), and then conduct gene expression analysis.

Tumor cell lines and T cells – The tumor cell lines used in this study are M586, which was derived from melanoma; MCF-7, which was derived from breast cancer; and PC-3, which was derived from prostatic cancer. T cells were isolated from peripheral blood mononuclear cells (PBMC).

Quantitative RT-PCR – The expression level of metabolic enzymes was determined by quantitative RT-PCR, using RNA extracted from indicated cell cultures with or without the treatment of poly-G, a ligand for TLR8.

Statistic Analysis – Detailed information including variables and controls are included in the figure legends.

4. Main Results (all graphs were generated by the finalist unless indicated specifically)

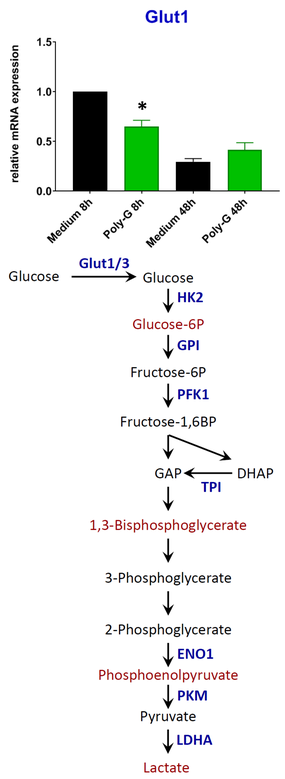


Fig. 1. The pathway of glycolysis. The metabolites highlighted in red have a reduced level upon poly-G treatment in cancer cells. That study was conducted by Ms. Huang, the finalist's graduate student mentor, through mass spectrometry analysis of the metabolites. The metabolites highlighted in blue are the enzymes whose expression level analyzed in this study.

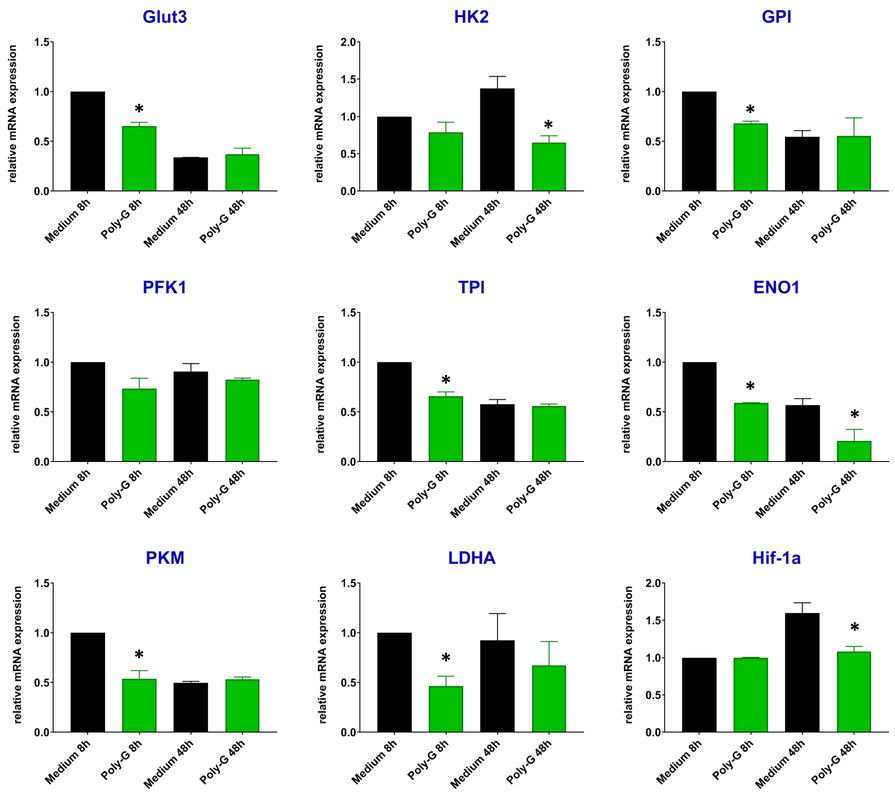


Fig. 2. Activation of TLR8 pathway via poly-G down-regulates the level of expression of glycolytic proteins in tumor cells. Human cancer cells M586 derived from melanoma were grown in medium only or medium plus poly-G treatment for either 8 h or 48 h. RNA were extracted, and the expression levels of key glucose metabolism genes were analyzed by quantitative RT-PCR. The experiments were performed three times. *denotes significant difference between medium and poly-G treatment by the Student's t-test. *Independent variables:* treatment of poly-G on cancer cell culture and length of treatment time. *Dependent variable:* relative mRNA expression in comparison to medium 8 h sample. *Constant*: RT-PCR procedure. Glut1/Glut3: glucose transporter 1/3; HK2: hexokinase 2; GPI: glucose phosphate isomerase; PFK1: phosphofructokinase 1; TPI: triose phosphate isomerase, ENO1: enolase 1, PKM: pyruvate kinase isoform M, LDHA: lactate dehydrogenase A, Hif-1a: hypoxia-inducible factor 1-alpha.

	Glut1	Glut3	HK2	GPI	PFK1	TPI	ENO1	PKM	LDHA	Hif1a
MCF7	—	—		—	—	—	—	—		
PC3	—	—	—			—		—	—	
M586	—	—	—	—		—	—	—	—	—

Table 1. Effects of poly-G on gene expression in other tumor cells, i.e., MCF7 and PC3. Experiments were performed similarly as in Fig. 2 except that different tumor cell lines were used. — denotes significant decrease in expression in poly-G treated cells.

5. Discussion

- Recent research has shown that increasing glucose availability can lead to decreased T cell senescence.
- By down-regulating tumor glucose metabolism, poly-G may be increasing the glucose availability for T cells, decreasing their level of senescence.

6. Conclusion & Implication

- Poly-G diminishes the expression of a variety of glycolytic proteins important for glucose metabolism in cancer cells.
- Altering cancer glucose metabolism could be an effective strategy for preventing tumor-induced immunosuppression.

7. Future Directions

- Determine if specific inhibitors to glycolytic enzymes can lead to similar prevention of tumor-induced immunosuppression.
- Determine whether poly-G treatment leads to an increase in the availability of glucose for T cells.
- Determine how TLR8 activation regulates expression of the proteins critical for glucose metabolism.

8. References

1. Hanahan, D., and Weinberg, R. A. (2011) Hallmarks of cancer: the next generation. Cell 144, 646-674
2. Ribas, A., and Wolchok, J. D. (2018) Cancer immunotherapy using checkpoint blockade. Science 359, 1350-1355
3. Schlosser, H. A., Theurich, S., Shimabukuro-Vornhagen, A., Holtick, U., Stippel, D. L., and von Bergwelt-Baildon, M. (2014) Overcoming tumor-mediated immunosuppression. Immunotherapy 6, 973-988
4. Liu, C., Workman, C. J., and Vignali, D. A. (2016) Targeting regulatory T cells in tumors. FEBS J 283, 2731-2748
5. Ye, J., and Peng, G. (2015) Controlling T cell senescence in the tumor microenvironment for tumor immunotherapy. Oncoimmunology 4, e994398
6. Peng, G., Guo, Z., Kiniwa, Y., Voo, K. S., Peng, W., Fu, T., Wang, D. Y., Li, Y., Wang, H. Y., and Wang, R. F. (2005) Toll-like receptor 8-mediated reversal of CD4+ regulatory T cell function. Science 309, 1380-1384
7. Corbet, C., and Feron, O. (2015) Metabolic and mind shifts: from glucose to glutamine and acetate addictions in cancer. Curr Opin Clin Nutr Metab Care 18, 346-353
8. Li, L., Liu, X., Sanders, K. L., Edwards, J. L., Ye, J., Si, F., Gao, A., Huang, L., Hsueh, E. C., Ford, D. A., Hoft, D. F., and Peng, G. (2019) TLR8-Mediated Metabolic Control of Human Treg Function: A Mechanistic Target for Cancer Immunotherapy. Cell Metab 29, 103-123 e105
9. Effros, R. B. (1996) Insights on immunological aging derived from the T lymphocyte cellular senescence model. Exp Gerontol 31, 21-27
10. Engwerda, C. R., Handwerger, B. S., and Fox, B. S. (1994) Aged T cells are hyporesponsive to costimulation mediated by CD28. J Immunol 152, 3740-3747
11. Debacq-Chainiaux, F., Erusalimsky, J. D., Campisi, J., and Toussaint, O. (2009) Protocols to detect senescence-associated beta-galactosidase (SA-betagal) activity, a biomarker of senescent cells in culture and in vivo. Nat Protoc 4, 1798-1806
12. Dang, C. V. (2012) Links between metabolism and cancer. Genes Dev 26, 877-890