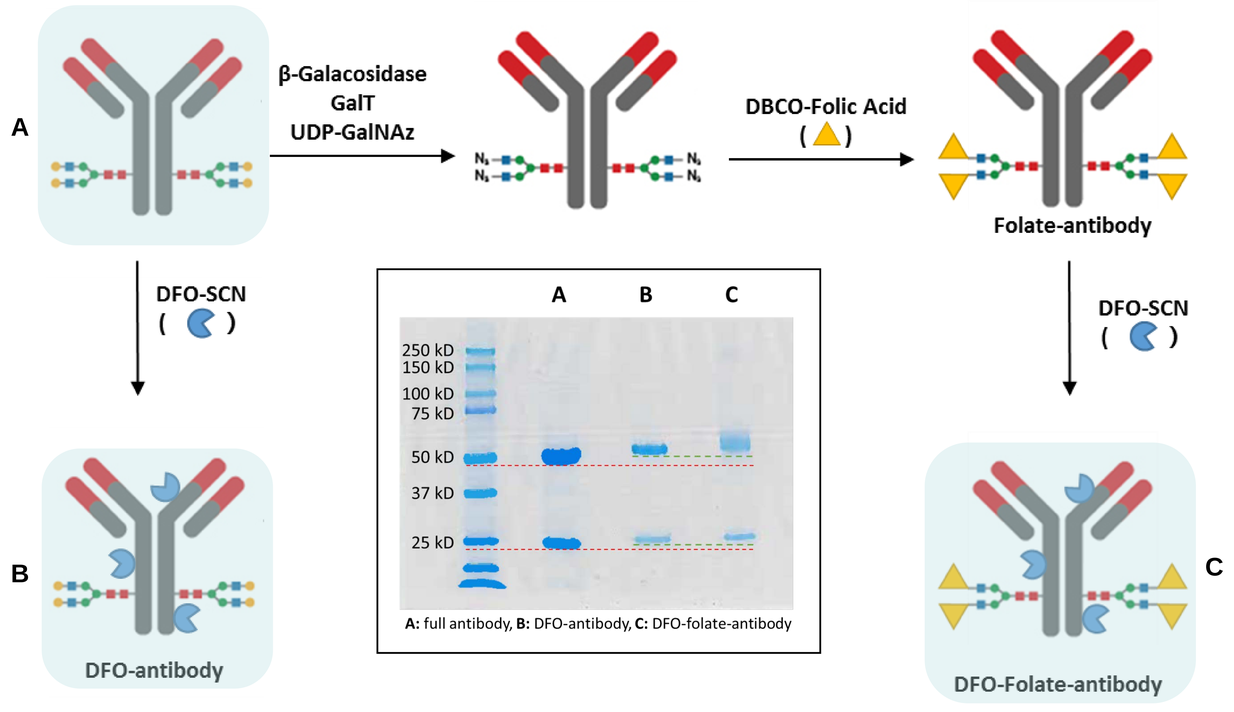
Site-specific Modification for Enhancement of Tumor Microenvironment Delivery of Anti-PD-L1 Antibody

Sydney Nicole Fischer[1], Liang Liu, M.D.[2], Haixun Guo, Ph.D.[2,3]
Department of Biology[1], Department of Radiology[2], and Center for Predictive Medicine[3]
University of Louisville

Introduction

Throughout the past few years, immune checkpoint inhibitor therapies for cancer treatment have been utilized more frequently due to their effectiveness, one of these being the targeting of the PD-1/PD-L1 pathway. The goal of this therapy is to deliver the inhibitory antibody to the tumor microenvironment (TME) to stop the PD-1 and PD-L1 interaction on tumor and T cells. However, the infused antibodies do not just accumulate in tumor foci, but also accumulate in other normal organs, resulting in a relatively low concentration of antibodies in the tumors themselves. The objective of this study is to increase concentration of the antibody in the TME and therefore enhance the therapeutic efficacy. This will be accomplished via antibody engineering. More specifically, the glycosylation site on the Fc portion of the antibody will be modified using beta-galactosidase. The terminal glucose will be cleaved, and replaced with an azide using specific enzymes and substrates, and Click Chemistry will then used to conjugate four molecules of folic acid to the antibody. Folic acid has been shown to be expressed on many tumor cells and will bind and internalize folic acid and its derivatives into the cells. The hypothesis is that the folic acid-modified anti-PD-L1 antibodies will have more tumor accumulation due to folate receptor-targeting delivery. The folate receptors will attract the folic acid on the antibodies, increasing the number of antibodies in the tumor. The increased presence of the antibodies due to the folic acid will ultimately increase the concentration of the antibodies that are capable of binding to the PD-L1 receptors.



Methods

A SiteClick™ Antibody Labeling Kit (ThermoFisher) was used for site-specific modification of the antibody. In the first step of the aforementioned kit, the buffer in the antibody of immunoglobulin G was exchanged to the provided antibody preparation buffer. Following this, beta-galactosidase was used to remove the terminal galactose residues on the N-linked sugars in the Fc region, also known as the glycosylation site. The substrate GAlNAz was transferred onto the modified carbohydrate domain of the antibody using GalT enzyme. The antibody was then conjugated to DBCO-PEG-Folic Acid at four different locations on the antibody (Az site). DFO chelator was conjugated onto the folic acid modified antibody for future radiolabeling and in vivo PET imaging. The concentration of the antibody and its derivatives were determined using the NanoDrop 2000 and the final products were characterized by reducing SDS gel electrophoresis.

Results

After running the gel electrophoresis, it is apparent that the desired antibody conjugate was formed. The antibody itself weighs 150 kDa, but the folic acid has a molecular weight of approximately 1000 Da, which is a small difference when comparing a modified antibody versus an unmodified antibody. However, the reducing SDS Page Gel showed differences between the unmodified antibody, the folate-DFO conjugated antibody, and the DFO conjugated antibody. Between the unmodified antibody, the folate-DFO conjugated antibody, and the DFO conjugated antibody, it is apparent that the desired materials were conjugated to the antibody. With a reducing gel, the small portion of the cleaved antibody is visible at 25 kDa and the larger portion of the antibody is visible at 50 kDa. The smaller portion of the reduced IgG with DFO is slightly higher than the pure reduced IgG, due to the conjugation of the DFO. The smaller portion of the reduced IgG with DFO and Folic Acid is the same weight as the reduced IgG with DFO, showing that nothing else was conjugated to the small portion. With the larger portion, the reduced IgG with DFO is higher than the pure reduced IgG, signifying that DFO was also conjugated to the larger portion. The reduced IgG with DFO and folic acid was higher than both the pure reduced IgG and the reduced IgG with DFO, signifying that the folic acid was conjugated to the antibody as desired.

Conclusions

We've successfully engineered the anti-PD-L1 antibody via the specific glycosylation site on the Fc portion of the antibody, and prepared the folic acid- and DFO-conjugated derivatives. The future work will focus on radiolabeling this antibody derivatives and test their PD-L1 and folate receptor specificity, and in vivo PET imaging characters.

Acknowledgements

Research supported by a grant from the Jewish Heritage Fund for Excellence (Guo) and Louisville Cancer Education Program NIH/NCI (R25-CA134283).