INTERNATIONAL JOURNAL OF

INNOVATIONS IN APPLIED SCIENCES

AND ENGINEERING

e-ISSN: 2454-9258; p-ISSN: 2454-809X

MUC16 Protein and the Role of Glycosylation in Developing Antibody Therapies for Ovarian Cancer

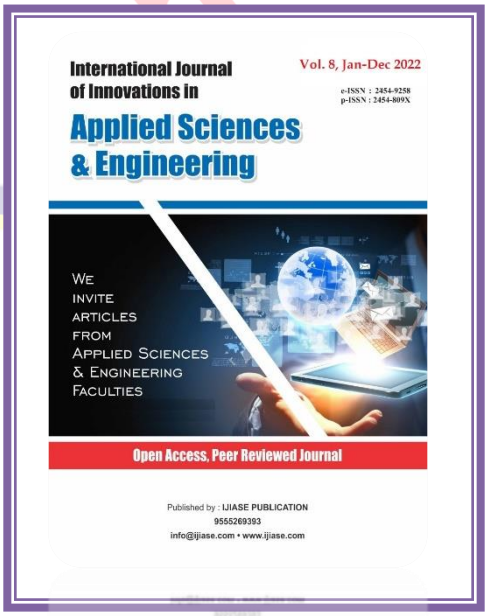
Andrew Manion, Seerat Aujla, Dr. Cory Brooks
Department of Biology, California State University, Fresno

Paper Received: 07th July, 2022; **Paper Accepted:** 30th August, 2022;

Paper Published: 03rd October, 2022

How to cite the article:

Andrew Manion, Seerat Aujla, Dr Cory Brooks, MUC16 Protein and the Role of Glycosylation in Developing Antibody Therapies for Ovarian Cancer, IJIASE, January-December 2022, Vol 8; 104-108



ABSTRACT

To test the competitiveness of our panel of antibodies and determine whether glycosylation affects antibody binding affinity, we examine the aberrant functioning of the MUC16 protein in cancer cells. After identifying the MUC16 protein contains the CA125 biomarker and determining its role in ovarian tumor growth and metastasis, the goal is to determine the impact glycosylation has on the binding affinity on certain antibodies, such as mAR9.6. Due to cleaving and shedding of the extracellular domain of the MUC16 protein, it has been difficult to find a promising region for target therapies; most antibodies cannot detect the remaining MUC16 fragment on the surface of the cell after it has been cleaved off. Additionally, MUC16 can act as a barrier to Natural Killer (NK) cells and monocytes, inhibiting its ability to attack tumor cells (Aithal et. al 6). Therefore, antibodies that target non-tandem repeat domains, such as the SEA5 domain, were used to understand how glycosylation plays a role in antibody binding and analyze the number of epitopes they will bind in the MUC16 protein.

INTRODUCTION

The CA125 antigen, a repeating peptide epitope of MUC16, was found during the development of OA125 monoclonal antibodies in 1981 by Bast et. al as a heavy weight molecular protein expressed on the surface of ovarian cancer cells. MUC16's involvement in tumorigenesis, cancer cell signaling, metastasis, and regulation of immune responses is why it has become a protein of interest for researchers when developing new monoclonal antibodies or anti-cancer drugs (Rauth et. al 2). A key feature of cancer is aberrant glycosylation, which is a process responsible for adding carbohydrates to proteins. There are two types of glycosylation: N-linked and O-linked. N-linked glycosylation is responsible for post translational modification to the protein, whereas O-linked glycosylation is linked to modifications of the hydroxyl group of serine and threonine amino acid residues on proteins (Rauth et. al 3,4). Therefore, abnormalities in glycosylation lead to increased tumor growth by enhancing the expression of E-cadherins, which leads to invasion and metastasis of certain cancers (Thomas et. al 8). Ultimately, the goal is to close gaps in our knowledge on the structure of MUC16 and its influence on molecular interactions, like antibodies, and provide a forum of unanswered questions to be addressed in future studies.

MATERIALS AND METHODS

Protein electrophoresis

Protein electrophoresis was used to determine which proteins are currently present in the gel.

Western Blots and ELISAs

Once the proteins of interest are separated from the gel, it was treated with primary and secondary antibodies, which were the His-tag proteins and anti-goat mouse IgG. Therefore, it could be concluded if the protein that was isolated binds successfully to the secondary antibody once the Western Blot was imaged. To test glycosylation-dependent antibody binding and competitiveness for the epitope were ELISAs and Western Blots. ELISAs helped us determine whether antibody binding affinity is affected by glycosylation by observing the shade gradient of yellow produced once the known CA125 antigen is added. Unglycosylated and glycosylated SEA5 domains were tested to see which type of SEA5 domain shows a higher rate of antibody binding to CA125, which would be displayed by a darker yellow color.

RESULTS AND DISCUSSION

From the ELISAs ran, a slight color gradient was observed in our results. This means that glycosylation could have played a role in antibody binding affinity according to the testing of the unglycosylated and glycosylated SEA5 domains as shown in Figure 1. Furthermore, Figure 2 showed a linear relationship in AR9.6 antibody and absorbance values by the epitopes on the unglycosylated SEA5 domain. Therefore, a lack of glycosylation increased the binding affinity of AR9.6 to MUC16. I believe further testing may be needed to definitively state whether glycosylation does a play a role in antibody binding, as the results from the ELISA included in this figure show that the treatment of the SEA5 domain did not impact the absorbance rate of the AR9.6.

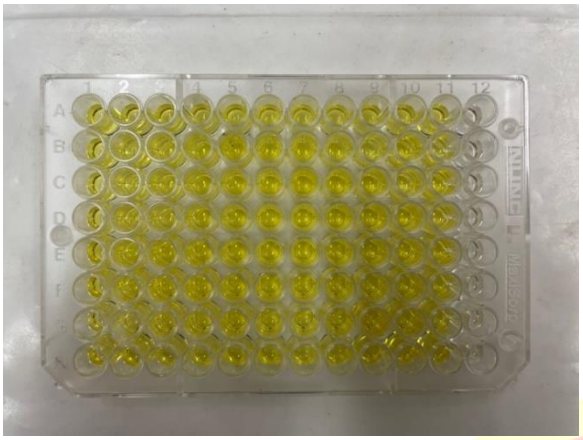


Figure 1: A 96-well plate showing a color gradient throughout all the wells. Rows A-D are unglycosylated and rows E-H are glycosylated.

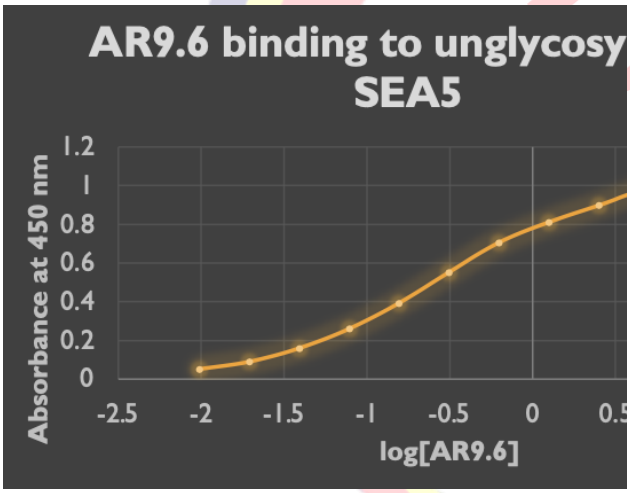


Figure 2: This graph shows an increase of AR9.6 concentration when SEA5 domain is unglycosylated when the 96-well plate was read at 450nm. (Data provided by Andrew M.).

The reason why the color gradient might have been as noticeable was because antibodies conjugate with the circulating ectodomain of MUC16; therefore, this reduces the amount of antibody available to target the cancer cells. Therefore, further investigation can be conducted whether the rate of ectodomain shedding can also affect antibody binding affinity in the MUC16 protein.

The Western Blot showed whether the secondary antibody bonded to the protein of interest. The gel displays in Figure 3 displayed different SEA domains and whether the domain had been tagged by the anti-goat mouse IgG. In Figure 3, the image showed SEA7, SEA9, and SEA13 (unglycosylated) have the protein of interest as marked by the molecular weight from the molecular weight ladder.

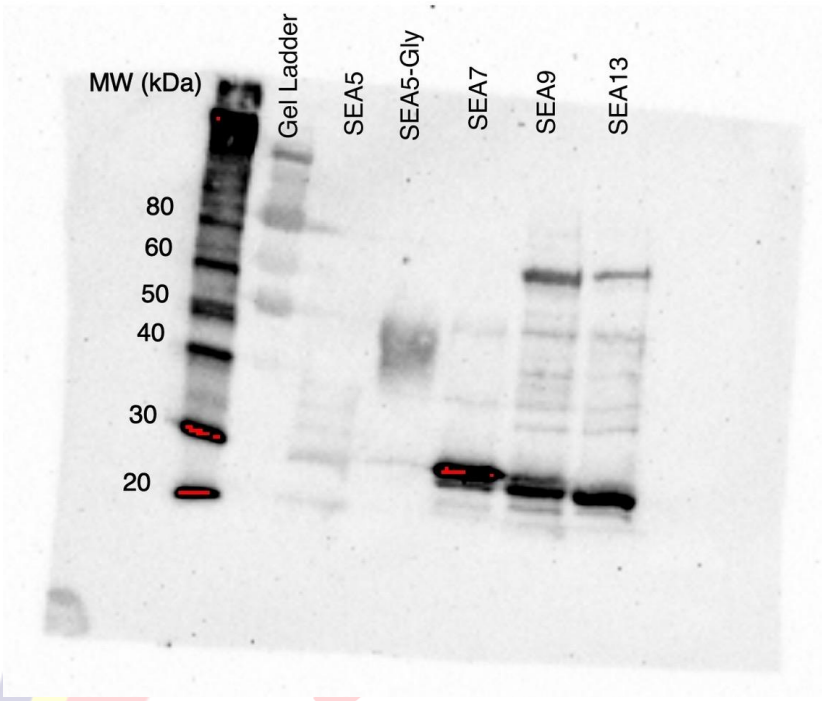


Figure 3: Western Blot was performed on 11/16/2022 and displays which SEA domain were successfully tagged by the secondary antibody.

Overall, the complex structure of this mucin continues to create challenges to efforts being made to improve the CA125 assay and to understand the role of MUC16. The complexity of this antigen does provide different pathways that can be explored to develop anti-cancer therapeutic strategies (Felder et al).

REFERENCES

[1] Aithal A, Rauth S, Kshirsagar P, Shah A, Lakshmanan I, Junker WM, Jain M, Ponnusamy MP, Batra SK. MUC16 as a novel target for cancer therapy. Expert Opin Ther Targets. 2018 Aug;22(8):675-686. Epub 2018 Jul 26. PMID: 29999426; PMCID: PMC6300140. https://doi.org/10.1080/14728222.2018.1498845

[2] Felder, Mildred, Arvinder Kapur, Jesus Gonzalez-Bosquet, Sachi Horibata, Joseph Heintz, Ralph Albrecht, Lucas Fass, et al. "MUC16 (CA125): Tumor Biomarker to Cancer Therapy, a Work in Progress." *Molecular Cancer* 13, no. 1 (May 29, 2014): 129. https://doi.org/10.1186/1476-4598-13-129.

[3] Mereiter, Stefan, Meritxell Balmaña, Diana Campos, Joana Gomes, and Celso A. Reis. "Glycosylation in the Era of Cancer-Targeted Therapy: Where Are We Heading?" *Cancer Cell* 36, no. 1 (July 8, 2019): 6–16. https://doi.org/10.1016/j.ccell.2019.06.006.

[4] Thomas, Divya, Ashok Kumar Rathinavel, and Prakash Radhakrishnan. "Altered Glycosylation in Cancer: A Promising Target for Biomarkers and Therapeutics." *Biochimica et Biophysica Acta. Reviews on Cancer* 1875, no. 1 (January 2021): 188464. https://doi.org/10.1016/j.bbcan.2020.188464