

**UTILIZING A PEPTIDE-BASED APPROACH TO TARGET AND EVALUATE NOVEL BIOMARKER NEU5GC SIALYL LEWIS A IN CANCER**

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**Background** Glycosylation is a post-translation modification that is commonly altered in cancer, due to aberrant expression of glycan-modifying enzymes. Neu5Ac-Sialyl Lewis A (CA 19–9), part of the Lewis blood antigen family, is a well-studied blood biomarker that is FDA approved for the detection of pancreatic cancer. However, its counterpart, N-glycolylneuraminic acid (Neu5Gc)-Sialyl Lewis<sup>A</sup>, is under studied in human malignancies due to the inactivation of the *CMAH* gene and the absence of Neu5Gc-Sialyl Lewis<sup>A</sup> specific targeting molecules. In this study, we have developed a novel Neu5Gc-Sialyl Lewis<sup>A</sup> binding peptide allowing for the evaluation of Neu5Gc-Sialyl Lewis<sup>A</sup> expression across tumor types as well as a method for tumor cell targeting to deliver therapeutics such as small molecules, oligonucleotides, and immunotherapies.

**Methods** FOX Three Molecular Guidance System™ was utilized to identify the MGS5 tumor targeting peptidic ligand. Cancer cell binding and internalization of MGS5 was evaluated using flow cytometry and confocal microscopy. Target identification and validation studies consisted of a Retrogenix Cell Microarray evaluating binding to 6052 full length human plasma membrane proteins, an array comprising 300 different glycans, sialidase treatment of cancer cells followed by internalization analysis via flow cytometry, and use of an anti-Neu5Gc blocking antibody. Formalin-fixed paraffin embedded (FFPE) tissue microarrays (TMA) for an assortment of cancers and normal tissues were used to evaluate Neu5Gc-Sialyl Lewis<sup>A</sup> expression.

**Results** We identified a 15 amino acid peptide, MGS5, as a peptide of interest to bind and internalize into cancer cells. Target identification and validation studies demonstrated MGS5 to be highly specific for the Neu5Gc version of Sialyl Lewis<sup>A</sup>. Utilizing MGS5 for detection, Neu5Gc-Sialyl Lewis<sup>A</sup> was found to be significantly expressed in numerous cancer tissues, with minimal staining observed across normal tissues. Color deconvolution followed by thresholding was applied across tissue samples to provide a nonsubjective analysis of MGS5 staining. Pancreatic ductal adenocarcinoma compared to normal healthy pancreatic tissue showed a 3-fold increase of Neu5Gc Sialyl Lewis<sup>A</sup> expression. Both nodal and non-nodal metastatic lesions maintain elevated expression of Neu5Gc Sialyl Lewis<sup>A</sup>. Tissue samples with low grade and high grade PanIN lesions displayed a 2-fold increase in Neu5Gc Sialyl Lewis<sup>A</sup> expression compared to normal healthy pancreas, suggesting a possible use as an early detection biomarker.

**Conclusions** We have discovered that MGS5 targets the Neu5Gc version of Sialyl Lewis<sup>A</sup> providing us with a novel and therapeutically relevant cancer targeting agent and biomarker.

<http://dx.doi.org/10.1136/jitc-2023-SITC2023.0190>