A New Journey for Cancer Research - Glysite™ Scout Glycan Screening Kit for Comprehensive Detection of Glycan Expression

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Introduction

From the simplest single-celled organisms to humans, all cells are densely covered with layers of glycans attached to surface proteins, lipids, and even RNA. These glycan structures play a critical role in a myriad of cellular functions, including cell-cell interactions, self recognition, cell signaling, and determination of cell fate. Aberrant glycosylation is observed in multiple disease types, ranging from cancer to autoimmune disorders. In cancer, understanding the glycosylation state can help identify malignancies and provide information about tumor microenvironment, as well as disease characteristics such as cancer progression and metastatic behavior.

Lectins are proteins that bind to specific glycans and have been widely adopted as tools for studying glycosylation. These glycan binders empower researchers to profile, characterize, and enrich glycans or glycoproteins in biological systems. Herein, we adapted the **Glysite™ Scout Glycan Screening Kit**, which consists of a curated panel of lectins, to detect potential glycosylation changes between normal and cancer tissues including colon, breast, pancreas and kidney.

Methods

Tissue	Type	Cancer Biomarker*	Lectin
Breast	Invasive Ductal Carcinoma	EGFR, ER, HER2, PGR, p53	AAL, ECL, GNL, Jacalin, MAL-II, PHA-L, WFL, WGA
Colon	Mucinous Adenocarcinoma	CA 19-9, Calretinin, CEA EGFR, Ki67, p53	AAL, ECL, GNL, Jacalin, MAL-II, PHA-L, WFL, WGA
Pancreas	Neuroendocrine	CA 19-9, CEA, EGFR p53, S100P	ECL, GNL, Jacalin, MAL-II, PHA-L, WFL
Kidney	Clear Cell (ccRCC)	AE1/AE3, EGFR, Ki67 PD-1, PD-L1, p53	AAL, ECL, GNL, Jacalin, MAL-II, PHA-L, WFL, WGA

Table 1. *Tissues used in the immunoassays: matched normal and cancer tissue, cancer type, cancer biomarkers*, and lectins from Glysite Scout Glycan Screening Kit. *Resources from National Cancer Institute (NCI).*

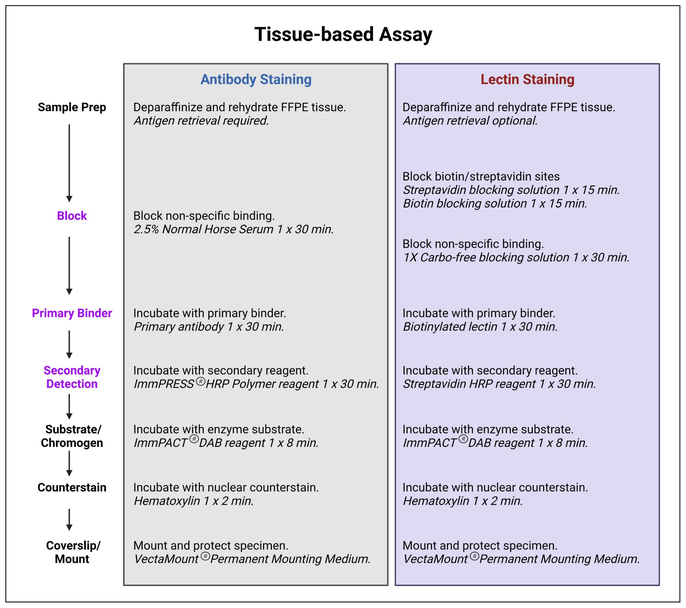


Figure 1. *Workflow of using antibody- versus lectin-based immunoassays. Purple colored text highlights where antibody- and lectin- integrated workflows start to differ.*

Results

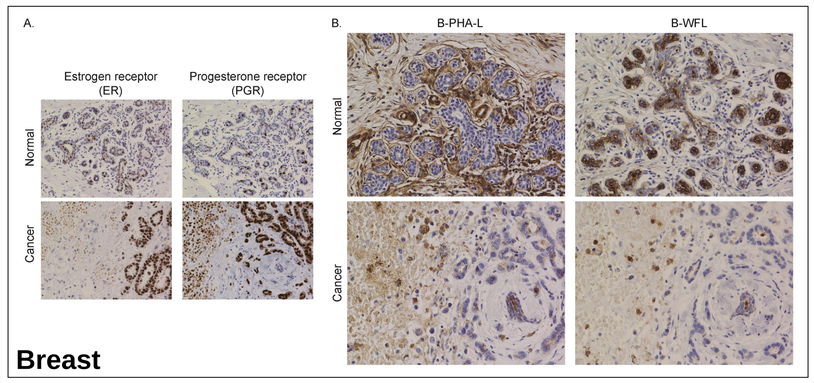


Figure 2. *Staining differences in breast ducts. FFPE human normal breast and invasive ductal carcinoma stained with cancer biomarkers (ER and PGR, A), and biotinylated lectins (B-PHA-L and B-WFL, B). Slides were imaged at 20x.*

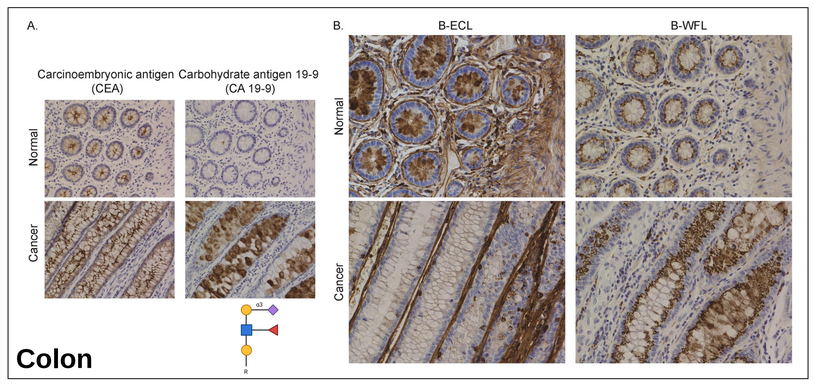


Figure 3. *Staining differences in colon crypts. FFPE human normal colon and colon mucinous adenocarcinoma stained with cancer biomarkers (CEA and CA 19-9, A), and biotinylated lectins (B-ECL and B-WFL, B). Slides were imaged at 20x.*

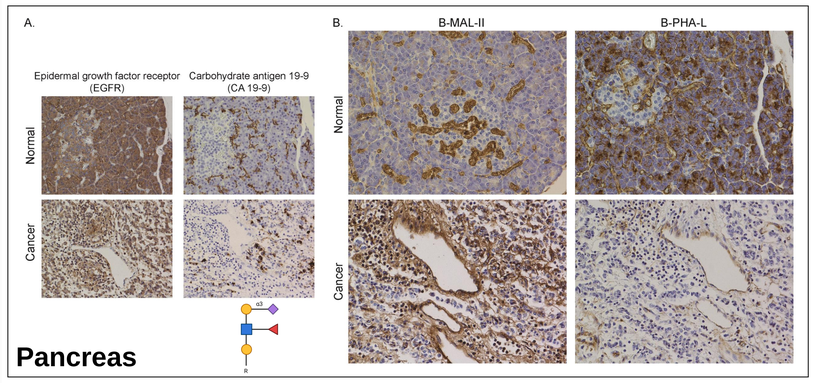


Figure 4. *Staining differences in pancreatic acinar cells and islets. FFPE human normal pancreas and neuroendocrine pancreatic tumor stained with cancer biomarkers (EGFR and CA 19-9, A), and biotinylated lectins (B-MAL-II and B-PHA-L, B). Slides were imaged at 20x.*

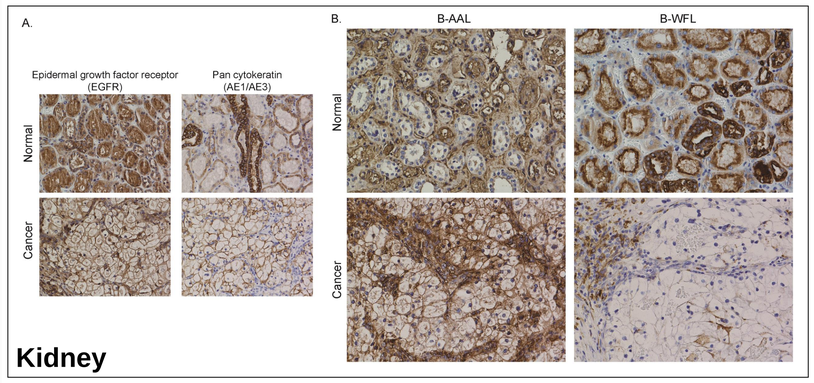
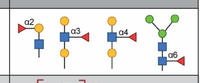
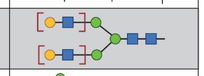
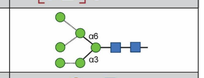
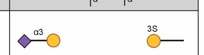
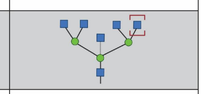


Figure 5. *Staining differences in renal tubules. FFPE human normal kidney and clear cell renal cell carcinoma stained with cancer biomarkers (EGFR and AE1/AE3, A), and biotinylated lectins (B-AAL and B-PHA-L, B). Slides were imaged at 20x.*

Lectin	Name	Binding Specificity	Binding Motif
Aleuria aurantia	AAL	Fucose	
Erythrina cristagalli	ECL	LacNAc	
Galanthus nivalis	GNL	Mannose	
Jacalin	Jacalin	*O*-glycans	
Maackia amurensis lectin II	MAL-II	Sialic acid, sulfation	
Phaseolus vulgaris - Leucoagglutinin	PHA-L	Complex *N*-glycans	
Wistera floribunda	WFL	GalNAc	
Wheat germ	WGA	*N*-acteyl-containing glycans	

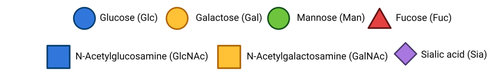


Table 2. *Lectin properties with major binding motifs. National Center for Functional Glycomics (NCFG) analyzes glycan specificity of plant lectins using glycan arrays and provides validation data on NCFG website. Additional resources from Bojar et al., ACS Chem Biol. 2022, 17, 11, 2993-3012.*

Conclusions

Here, we established a standard workflow of lectin-integrated tissue immunoassays. Formalin-fixed, paraffin-embedded (FFPE) tissue sections were prepared from matched pairs of normal and cancer tissue blocks. Our lectin histochemical analysis revealed tissue-dependent glycopatterns (i.e., biotinylated WFL staining varies between colon, breast and kidney). More importantly, our results demonstrate notable changes in glycosylation between the normal and tumor tissues complementing the antibody biomarker data. This indicates that glycans and/or glycoproteins have the potential to be novel cancer biomarker candidates. Currently, FDA-approved glycoprotein antigens as cancer biomarkers are on the rise, including CA 15-3 (mucin 1) as a serum marker for breast cancer, CA-125 (mucin 16) for ovarian cancer and CA 19-9 (sialyl Lewis A, shown in Figure 4A) for pancreatic cancer. **Thus, expanding the view of cancer biology by integrating glycosylation will empower the deconvolution of complex disease biology.**

Acknowledgement

Collaborators
Lectin validation data from NCFG

National Center for Functional Glycomics

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Resources
To learn more about Glysite Scout Glycan Screening Kit, please visit vectorlabs.com.

To learn more about the applications of lectins and glycobiology in cancer research, please check out The Lectins Application and Resource Guide, or scan QR code.