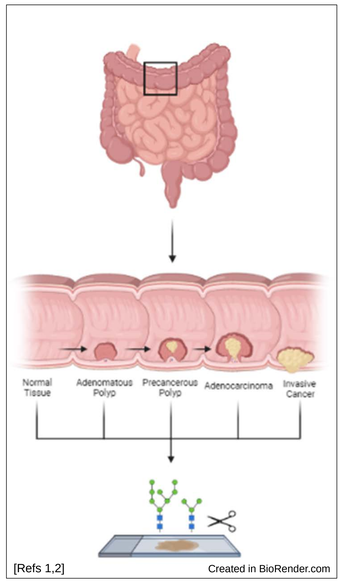
Defining a Human N-glycome Tissue Atlas of the Colon Adenoma to Adenocarcinoma Sequence by N-glycan MALDI-IMS

Rachel Stubler; Grace Grimsley, David Lewin, Kristin Wallace, Richard R. Drake
Medical University of South Carolina, Charleston, SC

Overview



Goal: Identify changes in the structural diversity and number of tissue N-glycans associated with colorectal cancer progression

Colorectal cancer (CRC) is a leading cause of cancer death worldwide, and often develops from defined genetic mutations via pre-cancerous adenomas to adenocarcinomas.

N-glycan MALDI-IMS was applied to a cohort of human colon FFPE tissues from colonoscopies or surgery (n=60). Tissues ranged from normal colon tissue to benign polyps to cancerous tumors.

Tissue Preparation and Analysis Method

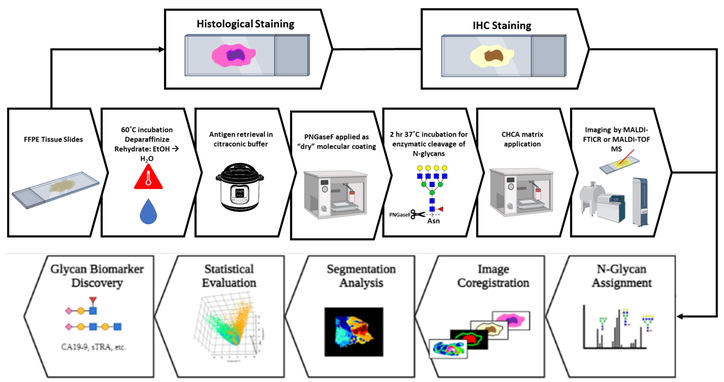


Figure 1. Tissue preparation and analysis workflow. Colon FFPE tissues represented included small normal biopsies from right and left colon (n=25), adenomatous polyps with tubulovillus (n=5) or serrated features (n=3), and adenocarcinomas that were moderately (n=5) or well differentiated (n=5), or mucinous (n=7). Each tissue was processed for N-glycan imaging mass spectrometry using established protocols on a timsTOF fleX MALDI QTOF mass spectrometer. PNGaseF PRIME was used to release N-glycans, and distributions were visualized and quantified using SCiLS Lab software (version 2022a). Created in BioRender.com

References:
1. Drake, R. R., Powers, T. W., Norris-Caneda, K., Mehta, A. S., & Angel, P. M. (2018). In Situ Imaging of N-Glycans by MALDI Imaging Mass Spectrometry of Fresh or Formalin-Fixed Paraffin-Embedded Tissue. *Current protocols in protein science*, *94*(1), e68. https://doi.org/10.1002/cpps.68
2. Holst, S., Wuher, M., & Rombouts, Y. (2015). Glycosylation Characteristics of Colorectal Cancer. In R. R. Drake & L. E. Ball (Eds.), *Advances in cancer research: glycosylation and cancer* (Vol. 126, pp. 203–257). chapter, Elsevier Inc. https://doi.org/10.1016/bs.acr.2014.11.004
3. Sawicki, T., Ruszkowska, M., Danielewicz, A., Niedźwiedzka, E., Arłukowicz, T., & Przybyłowicz, K. E. (2021). A Review of Colorectal Cancer in Terms of Epidemiology, Risk Factors, Development, Symptoms and Diagnosis. *Cancers*, *13*(9), 2025. https://doi.org/10.3390/cancers13092025

Acknowledgements:
This work was supported by CA226086 to KW and P30 DK123704, U01 CA242096 to RRD.

Contact:
Rachel Stubler
PhD Student, Drake Lab
Medical University of South Carolina
stubler@musc.edu

DRAKE LAB
MEDICAL UNIVERSITY OF SOUTH CAROLINA

Tissue Histology

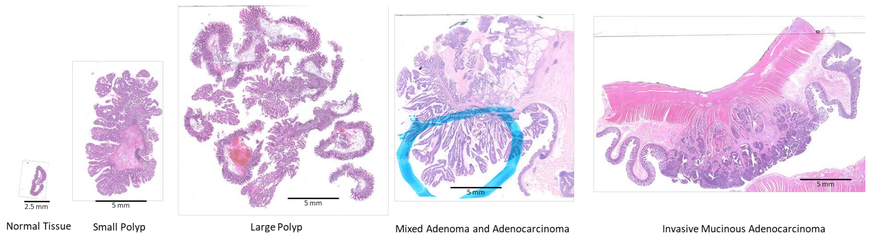


Figure 2. Hematoxylin and eosin stained tissues representing the progression of colorectal cancer.

Normal Tissue

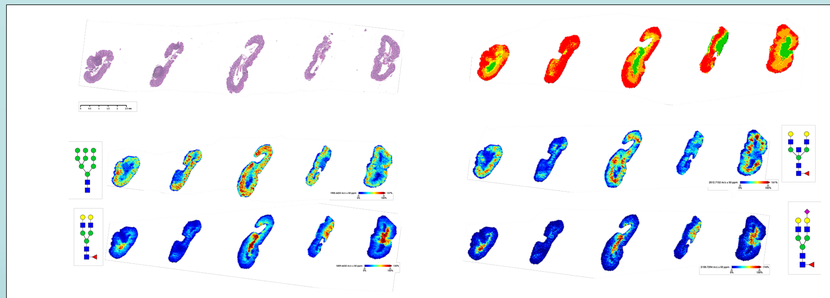


Figure 3. Hematoxylin and eosin stained, segmentation analysis, and MALDI-IMS images of normal tissue biopsies. Highlighted m/z values are 1905.6433, 1809.6432, 2012.7152, and 2100.7294. Glycan images corresponding to m/z are shown.

Adenoma

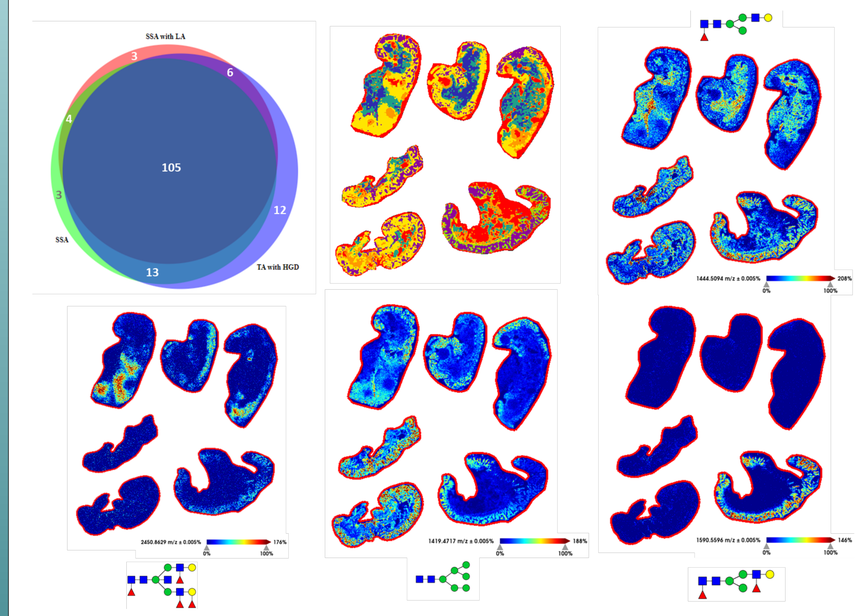


Figure 4. Data from three colon polyps. Venn diagram highlighting similarities and differences among different types of colon polyps (created in BioVenn). Segmentation analysis and MALDI-IMS images of polyps with glycan images corresponding to m/z values shown. Highlighted m/z values are 14.5094, 2450.8629, 1419.4717, and 1590.5596. Representative images highlight glycans more concentrated in specific adenoma types.
TA with HGD- tubular adenoma with high grade dysplasia; SSA- sessile serrated adenoma; LA- lymphoid aggregate

Adenocarcinoma

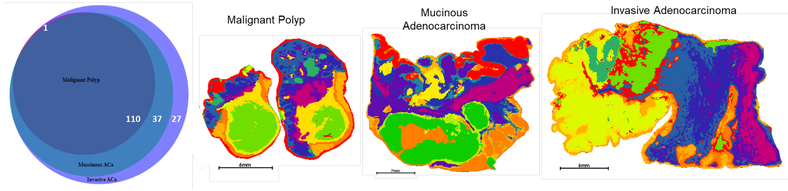


Figure 5. Venn diagram illustrating increasing number of glycans during progression from a malignant polyp to invasive mucinous adenocarcinoma and segmentation analysis of tissue samples (created in BioVenn).

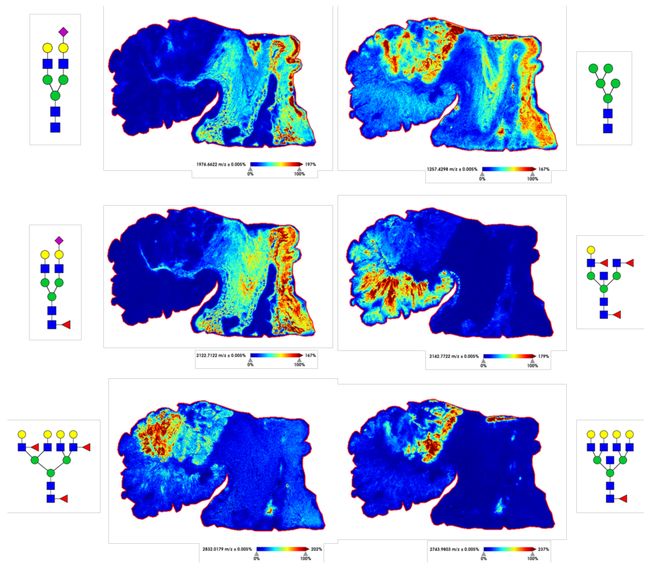


Figure 6. MALDI-IMS images of a tissue containing regions of tubulovillous adenoma and invasive mucinous adeno-carcinoma. Highlighted m/z values are 1976.6622, 1257.4298, 2122.7122, 2142.7722, 2832.0179, and 2743.9803. Glycan images correspond-ing to m/z are shown.

Conclusions

- **High mannose and non-sialylated bi-antennary structures with a bisecting N-acetylglucosamine were most abundant in normal colon biopsies**
- **All tissues analyzed contained high mannose N-glycans.**
- **For adenomas, larger branched N-glycans with multiple fucoses were detected along with sialylated bi-antennary glycans in regions of collagen networks (n=50-60 N-glycans per tissue).**
- **Differences in adenoma N-glycans were detected that were specific to tubular or serrated pathologies.**
- **Samples of mixed adenoma/adenocarcinomas and adenocarcinomas contained areas of epithelium, stroma, and smooth muscle, each with their own distinct set of N-glycans.**
- **Mucinous adenocarcinomas were the most diverse adenocarcinomas and contained tetra-antennary glycans with multiple fucoses (n=4=9) and a bisecting N-acetylglucosamine (n=180-200 N-glycans detected per tissue).**
- **Ongoing characterizations are centered on defining the N-glycans present in immune aggregates in some tissues, correlating with immunohistochemistry data and other histology stains.**

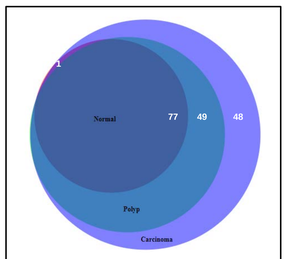


Figure 7. Venn diagram depicting increasing number of glycans as CRC progresses (created in BioVenn).