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A Systems Approach to Understanding the Role of Glycans in Cancer

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A SYSTEMS APPROACH TO UNDERSTANDING THE ROLE OF GLYCANS IN CANCER

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Abstract

Carbohydrates are a major class of biological macromolecules and are crucially involved in many cellular processes in healthy and diseased cells. The biological importance and vast structurally diversity of the glycome invites the use of high-throughput analytical techniques; however, the inherent similarities of the mono-saccharide building blocks, the complicated regulatory system and template-less biosynthesis has been an impediment to systems level analysis. We have developed a microarray format utilizing naturally-derived, carbohydrate-binding proteins known as lectins in order to probe the glycomes derived from whole cell cultures.

In order to determine how carbohydrate regulation is perturbed by the onset of cancer, we have obtained and analyzed the glycomes of the members of the NCI-60 cell line panel to determine what effect the disease state has on carbohydrate expression. To understand the regulatory role of these perturbations, we have also collected matching mRNA samples from corresponding cell lines and subjected them to array analysis with a custom made, glycogene specific microarray. To correlate these data sets, we performed singular value decomposition analysis to identify trends in gene regulation that are manifested by glycan expression. We present one of the first and most comprehensive attempts to use systems biology techniques to study the synthesis and maintenance of the glycome.

Background

Glycosylation is a dynamic and complicated process

Synthesis and maintenance of the human glycome requires a significant amount of cellular resources. The *N*-linked and *O*-linked glycosylation pathways are not template-driven and require the expression, folding and activation of dozens of glycosyl-transferase and glycosidase enzymes as well as proper trafficking to the correct sub-cellular locale. In addition, synthesis or transport of sugars and sugar donors and expression and localization of substrate proteins and lipids needs to be coordinated.

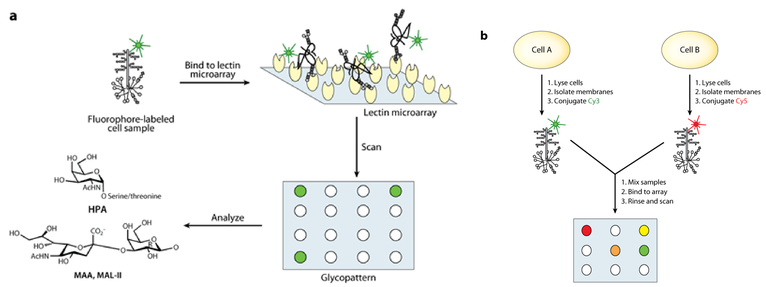
Our lab utilizes lectin microarrays to investigate the glycome

In order to study global effects upon the glycome in a high-throughput manner, our lab developed a microarray-format approach in which lectins (non-immunological, carbo-hydrate-binding proteins of varying specificities) are printed onto hydragel slides and the fluorescently-labeled, membrane-associated glycome is then hybridized to the array. The pattern of lectin binding reveals the expressed carbohydrate epitopes.

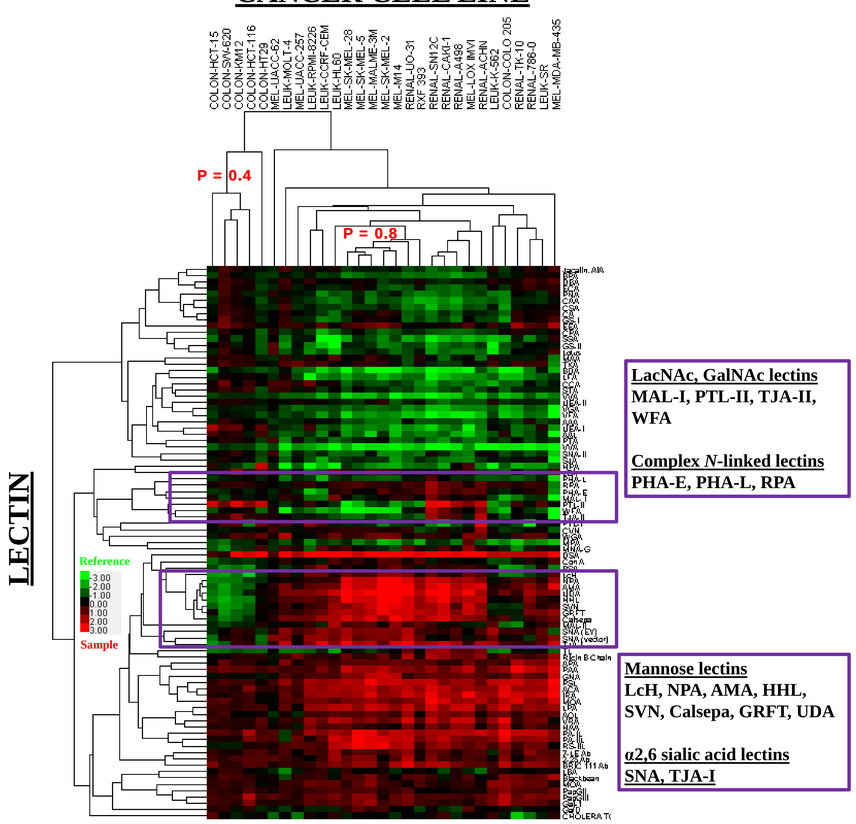
We study glycomic alterations across a broad panel of human cancer cell lines

This project details work in analyzing glycomic changes over the NCI-60 panel of cancer cell lines. This panel covers 60 cell lines across nine tissues and includes samples from shared and disparate donors and cancers of various metastatic potential. These tissues were cultured, mRNA isolated and glycome-containing membranes harvested and analyzed by lectin microarray.

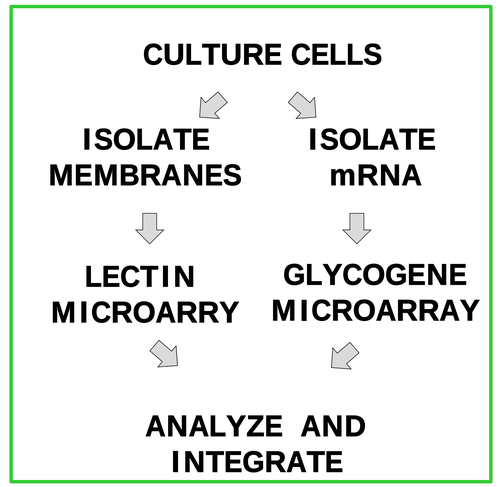
Lectin microarray array approach for high-throughput glycomics



Cells are cultured to 10^7-10^8, sonicated and membranes isolated by ultracentrifugation. Samples are labeled with NHS-Cy3 or -Cy5 and hybridized to lectin microarray at ambient temperature and humidity.



Experimental Flow Chart



Cell-type clustering by glycan features

1. **Nearly all colon lines group together and display a distinctive decrease in mannose and α2,6 sialic acid**
2. **Melanoma and renal cell lines also generally cluster together based on surface carbohydrates and demonstrate expression of mannose and α2,6-sialic acid epitopes**
3. **Renal lines also group and feature increases in mannose, α2,6 sialic acid, type 1 LacNAc and complex *N*-linked glycans**
4. **Leukemia cell lines demonstrate little glycan-based clustering**

Conclusions

1. **The lectin microarray is a viable method for high-throughput glycomic analysis**
2. **We observe tissue-specific carbohydrate profiles**
3. **We observe carbohydrate-depending cell-type clustering**

Future Directions

1. **Complete lectin array analysis of the entire NCI-60**
2. **Complete gene array analysis of the NCI-60 to identify genomic explanations for altered glycosylation**

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