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A Systems Approach to Understanding the Role of Glycans in Cancer

John F. Rakus  
*Martial University, rakus@marshall.edu*

Kaneolani T. Pilobello

Laura K. Mahal

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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 monosaccharide 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, glycoconjugate 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 glycosyltransferase and glycosidase enzymes as well as proper trafficking to the correct subcellular 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, carbohydrate-binding proteins of varying specificities) are printed onto hydragel slides and the fluorescently-labeled, membrane-associated glycome is then hybridized to the lectin microarray at ambient temperature and humidity.

CANCER CELL LINE

**Synthesis and maintenance of the glycome**

**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, glycoconjugate 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.**

**Cell-type clustering by glycan features**
1. Nearly all colon lines group together and display a distinctive decrease in mannose and α,2,6 sialic acid epitopes
2. Melanoma and renal cell lines also generally cluster together based on surface carbohydrates and demonstrate expression of mannose and α,2,6-sialic acid epitopes
3. Renal lines also group and feature increases in mannose, α,2,6 sialic acid, type I 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-dependent 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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