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Glycosylation Changes in Cancer

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Every Living Cell in Nature is Covered with a Dense and Complex Array of Sugar Chains (Glycans)

Common classes of animal glycans
# FDA-Approved Cancer Biomarkers

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Type</th>
<th>Source</th>
<th>Cancer Type</th>
<th>Clinical Use</th>
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<tbody>
<tr>
<td>α-Fetoprotein</td>
<td>Glycoprotein</td>
<td>Serum</td>
<td>Liver</td>
<td>Monitoring</td>
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<tr>
<td>α-Fetoprotein-L3</td>
<td>Glycoprotein</td>
<td>Serum</td>
<td>Liver</td>
<td>Risk</td>
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<tr>
<td>DCP</td>
<td>Protein</td>
<td>Serum</td>
<td>Liver</td>
<td>Risk</td>
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<td>Human chorionic</td>
<td>Glycoprotein</td>
<td>Serum</td>
<td>Testicular</td>
<td>Staging</td>
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<td>Glycoprotein</td>
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<td>Ovarian</td>
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<td>CA125</td>
<td>Glycoprotein</td>
<td>Cervix</td>
<td>Cervical</td>
<td>Screening</td>
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<td>Pap smear</td>
<td>Cervical smear</td>
<td>Serum</td>
<td>Colon</td>
<td>Monitoring</td>
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<tr>
<td>CEA</td>
<td>Glycoprotein</td>
<td>Serum</td>
<td>Colon</td>
<td>Selection of therapy</td>
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<tr>
<td>EGF receptor</td>
<td>Glycoprotein</td>
<td>Colon</td>
<td>GI stromal</td>
<td>Diagnosis &amp; selection</td>
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<td>KIT</td>
<td>Protein (IHC)</td>
<td>GI tumor</td>
<td>GI tumors</td>
<td>of therapy</td>
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<td>Protein (IHC)</td>
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<td>Breast</td>
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<td>Estrogen &amp; progestrone</td>
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<td>Breast tumor</td>
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<td>Selection of therapy</td>
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<td>receptors</td>
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<td>Prognosis &amp; selection</td>
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<td>HER2/NEU</td>
<td>Glycoprotein (IHC)</td>
<td>Breast tumor</td>
<td>Breast</td>
<td>of therapy</td>
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<tr>
<td>HER2/NEU</td>
<td>DNA (FISH)</td>
<td>Breast tumor</td>
<td>Breast</td>
<td>Prognosis &amp; selection</td>
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<td>Chromosomes 3, 7, 9, and 17</td>
<td>DNA (FISH)</td>
<td>Urine</td>
<td>Bladder</td>
<td>Screening &amp; monitoring</td>
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<td>CEA and mucin</td>
<td>Glycoprotein</td>
<td>Urine</td>
<td>Bladder</td>
<td>Monitoring</td>
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</table>

Adapted from Ludwig, JA & Weinstein, JN Nature Rev. 2005
Glycosylation Changes in Cancer

- Altered glycosylation is a universal feature of cancer cells.
- This is not a random consequence of disordered biology in cancer.
- Of all possible changes, only a very limited subset are frequently correlated with malignant transformation and tumor progression.
- As cancer is a “microevolutionary” process in which only fittest cells in a genetically heterogeneous population survive, specific glycan changes are likely selected for during tumor progression.
- Certain glycan structures are indeed well-known markers for tumor progression and/or biomarkers.
ALTERED GLYCOSYLATION IN CANCER

- Increased β1-6GlcNAc branching of N-glycans
- Changes in the amount, linkage, and acetylation of sialic acids
- O-glycan truncation, generating Tn & sialyl Tn antigens
- Failure of O-glycosylation, with mucin polypeptide exposure
- Expression of immature N-glycans
- Expression of nonhuman sialic acid Neu5Gc, from dietary sources
- Expression of sialylated Lewis structures and selectin ligands
- Altered expression and enhanced shedding of glycosphingolipids
- Increased expression of galectins and poly-N-acetyllactosamines
- Altered expression of ABH(O) blood-group-related structures
- Alterations in sulfation of glycosaminoglycans
- Increased expression of hyaluronan
- Loss of expression of GPI anchors.
The increased size of N-glycans that occurs upon transformation can be explained by an elevation in GlcNAc transferase-V (GNT-V) activity. 

Upregulated in Cancer
Loss of normal topology and polarization of epithelial cells in cancer results in secretion of mucins into the bloodstream.

- Selectin binding
- Tumor Antigens

- Biomarkers
- Clotting
Incomplete glycosylation in the O-linked pathway results in expression of the Tn antigen, the sialylated Tn antigen.

Mutated in many Carcinomas. Single hit on X Chromosome
Potential interactions that could occur between tumor cells and selectins
*(All shown in vitro. Most shown in vivo)*
Some pathways for expression of gangliosides in human neuroectodermal tumors

Enriched in Melanomas
Two Major Kinds of Sialic Acids on Mammalian Cells

- Neu5Ac
- Neu5Gc

1 Oxygen atom difference

- Present In Human Cancers and Fetuses?!
- Anti-Neu5Gc Antibodies In Human Cancer?!

- Missing/Immunogenic in Humans?

Contamination of Humans and Biotherapeutic Products by Incorporation of “Gc” (a.k.a., NGNA/Neu5Gc) Despite Anti-Gc Antibody Responses: Implications and Mechanisms

Human Genetic Mutation
Loss of Gc

Gc = Neu5Gc
(N-Glycolyneuraminic acid)

Gc in Food


Increased risk of Cancer
Heart Attacks?

Induced Anti-Gc Antibodies

Human Body
No Intrinsic Gc

Drug Reactions?
Transplant Rejection?

Gc incorporated into Biotherapeutic Products & Cells: Antibodies, Cytokines, Enzymes, Hormones, Stem Cells, etc.

Gc Incorporation

Chronic Inflammation

Milk Products

Serum
Substitutes

Animal
Cell Lines

Stabilizers

Contamination of Humans by Dietary Gc* in the Face of Anti-Gc Antibody Responses: Opportunities for Early Detection, Diagnosis, Prognosis and Therapy of Cancer

*Gc = Neu5Gc
(N-Glycolylneuraminic acid)

Genetic Mutation
Loss of Gc

Gc in Food

Gc Incorporation

Epithelium of Prostate, Colon, Breast, etc.

Increased risk of carcinomas of these organs?

Induced Anti-Gc Antibodies

QUANTITATE Gc in URINE
QUANTITATE ANTI-Gc ANTIBODIES IN BLOOD
PREDICT CANCER RISK
EARLY DETECTION
PROGNOSIS AND MONITORING
ELIMINATE Gc FROM THE BODY

Chronic Inflammation

QUANTITATE Gc in URINE
PREDICT CANCER RISK
EARLY DETECTION
PROGNOSIS AND MONITORING
ELIMINATE Gc FROM THE BODY
Each Neu5Gc-Containing Glycan Represents a Distinct Immune Epitope

A Novel Sialoglycan Array that Allows Detection of Neu5Gc-Specific Antibodies

Affinity-purified Chicken Anti-Neu5Gc IgY Antibodies

Human serum positive for anti-Neu5Gc Antibodies
Incomplete O-linked glycosylation results in expression of sialylated Tn antigen in cancer.

Incorporation of Dietary Neu5Gc generates Neu5Gc-Sialyl Tn and antibodies against it.

- Antibodies Against Neu5Gc Sialyl Tn
- Neu5Gc Sialyl Tn
- Somatic Inactivation of X-linked Cosmc Gene
- Neu5Ac
- Neu5Gc

Dietary Neu5Gc generates Neu5Gc-Sialyl Tn and antibodies against it.

Sialyl Tn

Incorporation of Dietary Neu5Gc generates Neu5Gc-Sialyl Tn and antibodies against it.

Slide Modified from Varki et al. *Essentials of Glycobiology*, Chapter 44, Figure 3
ALTERED GLYCOSYLATION IN CANCER:
POTENTIAL FOR BIOMARKER DISCOVERY

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