Glycosylation and Cancer

- Cancer Defined
- N-linked Glycans
- O-linked Glycans
- Poly-N-acetyllactosamine/Galectins
- GSL
- GPI Anchors
- HA and GAGS
- Diagnostics & Therapeutics

Wells, 2013

Hallmarks of Cancer:

Douglas Hanahan¹,²* and Robert A. Weinberg³,*
Cancer is clonal and while it may be influenced by predisposing germline mutations, it is caused primarily by somatic mutations.
Cancer Cells Arise from Adult Stem Cells/Progenitor Cells And/OR From Dedifferentiation (ips)
Tissue Progression (Morphology)

- Normal
- Hyperplasia
- Mild dysplasia
- Carcinoma in situ (severe dysplasia)
- Cancer (invasive)

Metastasis

1. Cells grow as a benign tumor in epithelium
2. Break through basal lamina
3. Invade capillary
4. Travel through bloodstream (less than 1 in 1000 cells will survive to form metastases)
5. Adhere to blood vessel wall in liver
6. Escape from blood vessel (extravasation)
7. Proliferate to form metastasis in liver
Glycosylation and Cancer

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Wells, 2011
Her-2 (RTK) Drives GnT-V Expression
(often upregulated in breast cancer)
Tissue/Cancer Type Specificity

**Overexpression of GlcNAc-TIII in Cells and Mice**

<table>
<thead>
<tr>
<th>Cell-cell interactions</th>
<th>ALTERED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth control</td>
<td>ALTERED</td>
</tr>
</tbody>
</table>

**Absence of GlcNAc-TIII in Mouse**

<table>
<thead>
<tr>
<th>Viability</th>
<th>NORMAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertility</td>
<td>NORMAL</td>
</tr>
<tr>
<td>Liver tumor progression</td>
<td>RETARDED</td>
</tr>
</tbody>
</table>

Glycosylation and Cancer

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- **O-linked Glycans**
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Wells, 2011
Table 1 | Mucin-type O-glycans and alterations in cancer

<table>
<thead>
<tr>
<th>O-glycan</th>
<th>Structure</th>
<th>decreased in cancer*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tn antigen</td>
<td>GalNAcα-Ser/Thr</td>
<td>↑</td>
</tr>
<tr>
<td>STn antigen</td>
<td>Sialylα2-6GalNAcα-Ser/Thr</td>
<td>↑</td>
</tr>
<tr>
<td>Core 1, T antigen</td>
<td>Galβ1-3GalNAcα-Ser/Thr</td>
<td>↑</td>
</tr>
<tr>
<td>Sialyl-T antigen</td>
<td>Sialylα2-3Galβ1-3GalNAcα-Ser/Thr</td>
<td>↑</td>
</tr>
<tr>
<td>Core 2</td>
<td>GlcNAcβ1-6Galβ1-3GalNAcα-Ser/Thr</td>
<td>↓</td>
</tr>
<tr>
<td>Core 3</td>
<td>GlcNAcβ1-3GalNAcα-Ser/Thr</td>
<td>↓</td>
</tr>
<tr>
<td>Core 4</td>
<td>GlcNAcβ1-6(FucNAcβ1-3)GalNAcα-Ser/Thr</td>
<td>↓</td>
</tr>
<tr>
<td>Type 1 chain</td>
<td>[GlcNAcβ1-3 Galβ1-3]_n</td>
<td>↓</td>
</tr>
<tr>
<td>Type 2 chain</td>
<td>[GlcNAcβ1-3 Galβ1-4]_n poly-N-acetyllactosamines</td>
<td>↑</td>
</tr>
<tr>
<td>Sialyl-Lewis*</td>
<td>Sialylα2-3Galβ1-3 (Fucα1-4) GlcNAcβ1-3Galβ1-3</td>
<td>↑</td>
</tr>
<tr>
<td>SLeα</td>
<td>Sialylα2-3Galβ1-4 (Fucα1-3) GlcNAcβ1-3Galβ1-3</td>
<td>↑</td>
</tr>
<tr>
<td>Sialyl-dimeric</td>
<td>Sialylα2-3Galβ1-4 (Fucα1-3) GlcNAcβ1-3</td>
<td>↑</td>
</tr>
<tr>
<td>Lewis*</td>
<td>Galβ1-4 (Fucα1-3) GlcNAcβ1-3Galβ1-3</td>
<td>↑</td>
</tr>
</tbody>
</table>

*The symbol ↑ denotes an increase in cancer, whereas the symbol ↓ denotes a decrease in cancer. Fuc, fucose; Gal, galactose; GalNAc, N-acetylgalactosamine; GlcNAc, N-acetyllactosamine; sialyl, sialic acid; SLeα, Sialyl-Lewis*; STn, Sialyl-Tn.

Scheme 3 | Biosynthesis of the Tn antigen and different O-glycan core structures derived from the Tn precursor
Incomplete glycosylation in the O-linked pathway results in expression of the Tn antigen, the sialylated Tn antigen.
Loss of normal topology and polarization of epithelial cells in cancer results in secretion of mucins into the bloodstream.

**NORMAL**

- Mucins
- Basement membrane

**CANCER**

- Altered glycans
- Secretion of mucins into the bloodstream

---

**COSMC - A MOLECULAR CHAPERONE IN THE ENDOPLASMIC RETICULUM REQUIRED FOR EXPRESSION OF FUNCTIONAL T-SYNTHASE TO GENERATE CORE 1 O-GLYCANS**

**Normal cell with wt Cosmc**

- Endoplasmic Reticulum
- Normal Folding
- Active T-synthase
- Golgi Apparatus
- Plasma Membrane
- Normal O-Glycans

**Cell with Mutated Cosmc**

- Endoplasmic Reticulum
- Preteasome
- Loss of T-synthase
- Golgi Apparatus
- Plasma Membrane
- Sialyl Tn and Tn antigens

---

From: Tongzhong Ju and Richard D. Cummings
Mucin Glycosylation in Cancer

Increased Tn and SiaTn is a Hallmark of Many Cancer Cells

Cosmc somatic mutations in Cancer Cells (X-linked)

Target for vaccines (limited by circulating mucins?)

Changes in ECM facilitates Cell Movement (Metastasis)

### Table 1: Summary of the somatic mutations in Cosmc identified in patients with Tn syndrome.

<table>
<thead>
<tr>
<th>Patient (gender)</th>
<th>Tn/SiTn expression</th>
<th>Mutation of Cosmc gene</th>
<th>Change in Cosmc protein</th>
<th>Activity</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.C. (male)</td>
<td>Tn and SiTn</td>
<td>C202T</td>
<td>RG8⁺</td>
<td>2-5 %</td>
<td>[82]</td>
</tr>
<tr>
<td>C.L. (male)</td>
<td>Tn and SiTn</td>
<td>C454A</td>
<td>E152K</td>
<td>0</td>
<td>[82, 208]</td>
</tr>
<tr>
<td>Tr1</td>
<td>Tn (female)</td>
<td>T577C</td>
<td>Si91P</td>
<td>NT(+)</td>
<td>[208]</td>
</tr>
<tr>
<td>Tr2 (male)</td>
<td>Tn and SiTn</td>
<td>G71C</td>
<td>MT1</td>
<td>NT(+)</td>
<td>[208]</td>
</tr>
<tr>
<td>Tr3 (male)</td>
<td>Tn and SiTn</td>
<td>no transcript (n gene C428T)</td>
<td>no protein</td>
<td>0</td>
<td>[208]</td>
</tr>
</tbody>
</table>

*Not tested. ÷ indicates a stop codon.

---

O-Mannosylation and Cancer

LOSS OF ALPHA-DYSTROGLYCAN LAMININ BINDING IN EPITHELIUM-DERIVED CANCERS IS CAUSED BY SILENCING OF LARGE
Glycosylation and Cancer

- Cancer Defined
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- Other links (Neu5Gc, Transcription)
- Diagnostics & Therapeutics

Wells, 2011
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Wells, 2011

Potential interactions that could occur between tumor cells and selectins
Galectin-3 in particular is often upregulated in Cancers

Polylactosamine can be on
N-linked
O-linked
GSL

Cell surface lattice theory: CC

Glycosylation and Cancer

- Cancer Defined
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Wells, 2011
X and A structures overexpressed in cancers

Loss in sulfation
Correlated with Cancers

Enhanced Selectin binding
Provides an Undefined Selective Advantage to Tumor Cells

Loss of P- and E-Selectin inhibit metastasis
N-Acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc)

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Wells, 2011
Loss of GPI Anchors in Cancer

Often result of somatic mutation
In PigA (early step)-X-linked

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Wells, 2011
HA Overexpressed In Some Cancer

**Effects of hyaluronan (HA) on oncogenic signaling**

- HA Overexpressed
- In Some Cancer

**Heparin/Heparan Sulfate**

Very complex interactions with progression of various cancers

1. Cell-cell adhesion
2. Angiogenesis
3. Growth factor presentation

Heparin inhibits metastasis in certain cancer types (therapeutic)
Glycosylation and Cancer

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Wells, 2011

An example of a direct association of glycan alteration and malignant transformation

HTLV-1

sialyl Lewis^x

Essentials of Glycobiology
Second Edition
Chapter 44, Figure 7
Glycosylation and Cancer

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Wells, 2011

Multiple Diagnostic Abs recognize a glycan or glycoprotein

CA 19-9 (pancreatic cancer, mucin glycoepitope?)
CA125 (ovarian carcinoma, mucin glycoepitope?)
Sialyl-Tn & Tn Antibodies (ovarian carcinoma)
Sialyl-Lewis^x (lung and breast cancer)
Neu5Gc (multiple cancer types)
Using decoy substrates for upregulated structures

Figure 3: Disaccharide decoys act as metabolic inhibitors of glycosylation. a) The disaccharide N-acetylglycosamine.
Glycans play significant roles in the “hallmarks of cancer”
Definition and Examples of Biologics

“Class of Medicinal Products that Contains Active Substance(s) Produced from or Extracted from a Biological (living) System”

- Proteins, Nucleic Acids, Sugars, Intact Cells
  - Vaccines (Prevnor - Pneumonia, Gardasil - HPV)
  - Antibodies (Avastin, Humira, Remicade, Herceptin)
  - Fc Fusion Proteins (Enbrel, Orencia)
  - Hormones (Efonvin - long acting FSH, Growth Hormone)
  - Enzymes (Pulmozyme – rHU-DNAase- )
  - Proteins (Clotting factors, Interleukins, Albumin)
  - Gene Therapy and RNAi (Still in development)
  - Cell Therapy (Provenge – Prostate cancer vaccine)
  - Antibody Drug conjugates - Adcetris (Brentuximab vedotin for HL)

<table>
<thead>
<tr>
<th>Product Type</th>
<th>Examples of Targeted Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell therapy</td>
<td>Cancer</td>
</tr>
<tr>
<td>Clotting factor</td>
<td>Hemophilia</td>
</tr>
<tr>
<td>Cytokine or growth factor</td>
<td>Cancer, hepatitis C</td>
</tr>
<tr>
<td>Enzyme</td>
<td>Hereditary deficiencies</td>
</tr>
<tr>
<td>Monoclonal antibody</td>
<td>Arthritis, cancer</td>
</tr>
<tr>
<td>Polyclonal antibody</td>
<td>Immune deficiency</td>
</tr>
<tr>
<td>Toxin</td>
<td>Cosmetic</td>
</tr>
<tr>
<td>Vaccine</td>
<td>Influenza and other viruses</td>
</tr>
<tr>
<td>Other</td>
<td>Hereditary emphysema</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. of Biologic Products</th>
<th>Cell therapy</th>
<th>Clotting factor</th>
<th>Cytokine or growth factor</th>
<th>Enzyme</th>
<th>Monoclonal antibody</th>
<th>Polyclonal antibody</th>
<th>Toxin</th>
<th>Vaccine</th>
<th>Other</th>
</tr>
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<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>
8/10 of the top 10 drugs in 2014 will be Biologics

Top 10 Drugs: 2010E

1. Lipitor (cholesterol) $11.7B
2. Plavix (anticlotting) $9.6B
3. Advair (asthma) $9.0B
4. Remicade (inflam.) $7.4B
5. Enbrel (inflam.) $7.1B
6. Humira (inflam.) $6.8B
7. Avastin (cancer) $6.7B
8. Rituxan (cancer) $6.1B
9. Diovan (hypertension) $6.0B
10. Crestor (cholesterol) $5.8B

Top 10 Drugs: 2014 (Predicted)

1. Avastin (cancer) $8.9B
2. Humira (inflam.) $8.5B
3. Enbrel (inflam.) $8.0B
4. Crestor (cholesterol) $7.7B
5. Remicade (inflam.) $7.6B
6. Rituxan (cancer) $7.4B
7. Lantus (diabetes) $7.1B
8. Advair (asthma) $6.8B
9. Herceptin (cancer) $6.4B
10. Novolog (diabetes) $5.7B

Doesn’t include EPO and Heparin >10B
The heparan sulfate chain consists of different domains that vary in the extent of modification by sulfation and epimerization.
The synthetic influenza neuraminidase inhibitors Relenza™ and Tamiflu™

Examples of natural products that possess glycan components
Disclosed are novel polypeptides possessing part or all of the primary structural conformation and one or more of the biological properties of mammalian erythropoietin ("EPO") which are characterized in preferred forms by being the product of procaryotic or eucaryotic host expression of an exogenous DNA sequence. Illustratively, genomic DNA, cDNA and manufactured DNA sequences coding for part or all of the sequence of amino acid residues of EPO or for analogs thereof are incorporated into autonomously replicating plasmid or viral vectors employed to transform or transfect suitable procaryotic or eucaryotic host cells such as bacteria, yeast or vertebrate cells in culture. Upon isolation from culture media or cellular lysates or fragments, products of expression of the DNA sequences display, e.g., the immunological properties and in vitro and in vivo biological activities of EPO of human or monkey species origins. Disclosed also are chemically synthesized polypeptides sharing the biochemical and immunological properties of EPO. Also disclosed are improved methods for the detection of specific single stranded polynucleotides in a heterologous cellular or viral sample prepared from, e.g., DNA present in a plasmid or viral-borne cDNA or genomic DNA "library".

- United States Patent 5,441,868
- Lin August 15, 1995
- Production of recombinant erythropoietin
- Abstract

- Inventors: Lin; Fu-Kuen (Thousand Oaks, CA)
- Assignee: Kirin-Amgen, Inc. (Thousand Oaks, CA)
- Appl. No.: 113179
- Filed: October 23, 1987
Darbepoetin alfa has a longer circulating half-life and greater in vivo potency than recombinant human erythropoietin

Joan C. Egrie, Erik Dwyer, Jeffrey K. Browne, Anna Hitz and Michele A. Lykos

Amgen Inc., Thousand Oaks, Calif., USA

Conclusions. Increasing the sialic acid-containing carbohydrate content beyond the maximum found in EPO leads to a molecule with a longer circulating half-life and thereby an increased in vivo potency that can be administered less frequently.

Development and characterization of novel erythropoiesis stimulating protein (NESP)

JC Egrie and JK Browne

Amgen Inc.

Summary: Studies on human erythropoietin (EPO) demonstrated that there is a direct relationship between the sialic acid-containing carbohydrate content of the molecule and its serum half-life and in vivo biological activity, but an inverse relationship with its receptor-binding affinity. These observations led to the hypothesis that increasing the carbohydrate content, beyond that found naturally, would lead to a molecule with enhanced biological activity. Hyperglycosylated recombinant human EPO (rHuEPO) analogues were developed to test this hypothesis. Darbepoetin alfa (novel erythropoiesis stimulating protein, NESP, ARANESP™, Amgen Inc, Thousand Oaks, CA), which was engineered to contain 5 N-linked carbohydrate chains (two more than rHuEPO), has been evaluated in preclinical animal studies. Due to its increased sialic acid-containing carbohydrate content, NESP is biochemically distinct from rHuEPO, having an increased molecular weight and greater negative charge. Compared with rHuEPO, it has an approximate 3-fold longer serum half-life, greater in vivo potency, and can be administered less frequently to obtain the same biological response. NESP is currently being evaluated in human clinical trials for treatment of anaemia and reduction in its incidence.