Fulfilling the Promise of Translational Glycobiology

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Disclosure

In accordance with United States National Institutes of Health (NIH) policies and procedures, the Brigham and Women’s Hospital (BWH) has returned all Rights of Intellectual Property related to HCELL to the Inventor (RS).
Outline of Presentation

Theme:
“Promoting Communication”

- Challenges in terminology of glycoconjugates
- Moving from inductive to deductive reasoning in glycobiology: Postulates for establishing glycoconjugate bioactivity
- Guiding principles for development of “Translational Glycobiologists”
**Why focus on glycoconjugate terminology?**

**Genome-**Proteome-Glycome **Relationship**

- **Genome** implies phenotype
- **Proteome** predicts phenotype
- **Glycome** is phenotype

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Genome  ➔  Nascent Proteome  ➔  Glycome

- Adapted from house figures provided by Dr. Joseph Loscalzo
Terminology

**Glycoconjugate** = glycan + scaffold

**Scaffold** is the core protein or core lipid.

A glycoconjugate is a pertinent glycoprotein or glycolipid, which displays characteristic biochemistry.

A glycoconjugate with a distinct bioactivity _conferred by the glycan_ should bear a unique name.
The Most Potent Ligand for E-Selectin on human cells: Hematopoietic Cell E- and L-selectin Ligand (HCELL)

HCELL

(***CD44 is scaffold protein***)

**Multiantennary Complex-Type N-Glycan**
+ **Sialyl Lewis X Termini**

**Chondroitin Sulfate**

**O-Glycosylation**
CD44

- A rather ubiquitously expressed cell surface glycoprotein.

- The **CD44 protein** is best known for its role as a lectin – it is the principal adhesion receptor for hyaluronic acid.
Hematopoietic Cell E-/L-selectin Ligand (HCELL)

- A sialylated, fucosylated (sLex) glycoform of HUMAN CD44 expressed on human hematopoietic stem cells (HSC), but not on intermediate-stage progenitors or mature blood cells.
- Defined as CD44 reactive to mAb HECA452 and KM93 (anti-sLex), and with E-selectin-Ig (E-Ig), by Western blot.
- On HCELL of human HSC, the sialofucosylations that bind E-selectin are expressed on N-glycans displayed on CD44s.
- HCELL is the most potent native E-selectin ligand expressed on any human cell.
- HCELL is the human “bone marrow homing receptor”
Splice Variations: >700 variants possible

Post-translational Modifications
- GAG substitutions
- Sulfation
- Phosphorylation
- Palmitoylation

Glycosylation (N- or O-linked glycan residues)

For most current review:
{ E-mail me if you want copy – RSackstein@rics.bwh.harvard.edu }
CD44 is not “a selectin ligand”

$\text{HCELL} = s\text{Lex} + \text{CD44}$

$\text{HCELL} \neq \text{CD44}$

The “working end” of CD44 in binding hyaluronic acid is the protein itself, deglycosylated CD44 binds hyaluronic acid better than glycosylated CD44;

the working end of HCELL is the GLYCANS, not the core protein.

Denatured HCELL retains all selectin ligand activity,

CD44 itself is inert in selectin binding.
R. KOCH: POSTULATES FOR ESTABLISHING MICROBIAL PATHOGENICITY (1890)

- The microbe must be found in all organisms suffering from the disease.

- The microbe must be isolated from a diseased organism and grown in pure culture.

  - The cultured microbe should cause disease when introduced into a healthy organism.

- The microbe must be re-isolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.
R. SACKSTEIN:
POSTULATES FOR ESTABLISHING GLYCONJUGATE BIOACTIVITY

· The glycoconjugate (i.e., glycan plus scaffold) must be displayed among cells/organism possessing the relevant bioactivity.
  (Inductive Evidence of Distinct Biologic Effect)

· The glycoconjugate isolated from a given cell/organism expressing the relevant phenotype must be structurally homologous to that same glycoconjugate isolated from other cells/organisms which display the relevant bioactivity.
  (Inductive Evidence of Distinct Structural Biology)

· Following enforced expression on a cell/organism that does not natively express the relevant glycoconjugate, the glycoconjugate should produce the relevant bioactivity.
  (Deductive Evidence of Distinct Biologic Effect)

Following enforced expression on a cell/organism which does not natively express the relevant glycoconjugate, the glycoconjugate must be re-isolated from the target cell/organism and must display structural homology with the relevant glycoconjugate as expressed on a native cell/organism.
  (Deductive Evidence of Distinct Structural Biology)
Mesenchymal Stem Cells (MSC) Lack Capacity to Home to Marrow

- Lack HCELL, but express high levels of the CD44 molecule
- Can we glycan engineer CD44 on human MSC to become HCELL?
- Will HCELL+ human MSC home to a tissue bed that expresses E-selectin, e.g., bone marrow?
Glycosyltransferase- Programmed Stereosubstitution (GPS) of Cell Surface Glycans: Deductive Evidence of Glycoconjugate Bioactivity
GPS Engineering to Create HCELL

Human Mesenchymal Stem Cell

(1) N-Sialyl CD44 identified by SACK-1 mAb

(2) α(1,3)-fucosyltransferase [FTVI Treatment Ex Vivo]

(3) HCELL synthesis confirmed by Western blot [CD44 reactive with E-Ig, KM93 and HECA452]
GPS drives homing of human MSC to marrow:

Injection of HCELL-bearing HUMAN MSC into mice results in homing of MSCs to bone marrow, and subsequent production of human bone tissue in mouse bone.

A form of “applied” research (as opposed to “pure” research) wherein the SPECIFIC goal is to alleviate human suffering by hypothesis-driven basic science inquiry, providing mechanistic insights applicable to the patient. Ideally, an inspired process...
13 month old girl with Osteogenesis Imperfecta (Type III)
Research in Glycosciences Inspired by Overarching Medical Necessity: “Translational Glycobiology”

Clinicians
(Physicians, Surgeons, Clinical Researchers, ETC.)

Medical Necessity

Glycochemists
(Organic chemists, Structural chemists, Chemical engineers, Enzymologists, ETC.)

Glycobiochemists
(Immunologists, Cancer biologists, Microbiologists, Cell biologists, ETC.)
THE CURRENT (awful) STATE OF “TRANSLATIONAL GLYCOBIOLOGY”

• There is a general lack of awareness of clinical reality among glycochemists and glycobiologists.

• There is a profound lack of knowledge of glycoscience among clinicians.

• The rapid pace of clinical developments and mounting economic factors leaves little chance that a doctor (in either private or academic settings) will become versed in glycoscientific principles.

How can we overcome these hurdles?
POSSIBLE SOLUTION

(1) Offer glycoscience education in college and medical school
(2) Incorporate glycoscience-related questions on MCATs/Qualifying Exams/Accreditation Boards

However, such knowledge would fade in absence of reinforcement and/or continued Interest. Moreover, in absence of continued learning, progress in the field of glycoscience would be expected to make much of the amassed information obsolete (HOPEFULLY).

(3) Provide relevant clinical and pathologic knowledge to the glycoscientist… provide information on the specific medical entity and expose gaps in understanding of the pathobiology. Create seamless communication(s) between glycoscientist(s) and clinician(s).
Glycobiologists (Immunologists, Cancer biologists, Microbiologists, Cell biologists, ETC.)

Glycochemists (Organic chemists, Structural chemists, Chemical engineers, Enzymologists, ETC.)

Clinicians (Physicians, Surgeons, Clinical Researchers, ETC.)

Translational Glycobiologists

Research in Glycosciences Inspired by Medical Necessity
Biosynthesis & Function of Lactosaminyl Glycans in Hematopoiesis
Sackstein, NHLBLI P01 HL107146

Overarching Clinical Goal: Improving therapeutic outcomes for myelosuppressive conditions, marrow failure states, or myeloproliferative disorders by glycan engineering

Project 1
Robert Sackstein, MD, PhD
Brigham & Women’s Hospital/Harvard Medical School
Structure/Function of Glycans in Early Hematopoiesis

Analytical Core
Vern Reinhold, PhD
U. of New Hampshire
Glycan Structural Analysis

Project 2
Joseph Lau, PhD
Roswell Park Cancer Institute/ SUNY Buffalo
Regulation of Glycan Biosynthesis in Hematopoiesis

Project 3
Karin Hoffmeister, MD
Brigham & Women’s Hospital/Harvard Medical School
Structure/Function of Glycans in Thrombopoiesis

• Addressing clinically relevant problems by glycoscience-based approaches
• Translate discoveries to new diagnostic and clinical applications
• Translation-based training program to develop new generation of “translational glycobiologists”