Learning about human iPSC models for glycosylation-related disease.

Stephen Dalton, Ph.D.
Department of Biochemistry and Molecular Biology
Paul D. Coverdell Center for Biomedical and Health Sciences
Stem cells in early development give rise to all the adult tissues (pluripotent stem cells)
Pluripotent cells differentiate and become more specialized

- neurons, muscle, liver, blood, pancreas cells etc.

2 potential sources of pluripotent stem cells for research

- IVF embryo
  - Embryonic stem cells (ESCs)

- Donor cells
  - Induced pluripotent stem cells (iPSCs)
Early cell fate specification of cultured pluripotent cells

Diverse cell populations: tools for understanding CDG and CMD
propagate cells

ESC/iPSC pluripotent stem cell

Master cell bank

differentiate cells

Transplantable cell

implant into patients

A cell therapy pathway

Cell donor
Reprogramming: taking a specialized cell (from a patient) and converting into a pluripotent stem cell

Shinya Yamanaka
Kyoto University
2012 Nobel Prize for Physiology or Medicine
Modeling CDG and CMD: the strategy of reprogramming

KLF4, SOX2, c-Myc, Nanog, Oct-3/4, LIN-28

CDG or CMD patient iPSCs

Adult Fibroblast Cell

Reprogram Cells

iPS cells

Cardiomyocytes

Adipocytes

Dopaminergic Neurons

Neural Cells

Motoneurons

Pancreatic β-Cells

Hematopoietic Progenitor Cells
Human iPSCs
A pathway to modeling human disease with iPSCs

1. Source patient cells
2. Amplify
3. Reprogram
4. Quality control: genotype
5. Assess differentiation potential
6. Confirm disease phenotype

- Potential drug screens
- Molecular analysis
- Functional analysis (animal models)
Engineering mutations into iPSCs to create human CDG and CMD models

- single or multiple mutations can be introduced into non-patient (normal) cells to model human disease
- patient cells can be genetically corrected and used as research tools
- utility in drug screening (new therapeutics)
- new tools to understand the molecular basis of glycosylation disorders
CRISPR-Cas9 Genome Editing: 1. Gene Disruption

**Guide RNA design**
- 20 base guide RNA
- PAM
  - gRNA 1: GAGGAGCACCAGGAGACGCAACTCATCGGGTAA
  - gRNA 2: CTCTCCTGTTGCCCTGCTGAGTAGATGCCAT

**Genomic locus**
- 300 bp
- 1kb

**Guide RNA plasmids**
- U6
- 20 bp gRNA
- Scaffold
- Terminator

**CRISPR-Cas9 Genome Editing**
1. **Guide RNA design**
   - 20 base guide RNA
   - PAM
2. **Homology directed repair**
3. **Insertion and gene disruption**
2. Introduction or correction of mutations

Guide RNA design

PAM | 20 base guide RNA

ttttcCCTttCtcagaacgtccccacaaatcgg
aaaagGAAaaGagtcttgcaaggtttagcc

sgRNA

ST3Gal5 exon 7

G (994)

ST3Gal5 exon 7

G (994)

CAG | BFP | Zeo

IRES | Poly A

Cas9 | Homology directed repair

Repaired ST3Gal5 gene

CAG | BFP | Zeo

IRES | Poly A

CRE

Repaired ST3Gal5 gene
Modeling human disease with patient-derived induced pluripotent stem cells (iPSCs)

- Cell donor with CDG
  - Skin biopsy
  - Blood

Cells carrying a mutation(s) responsible for congenital disorder of glycosylation

Characterize glycosylation defect at molecular level, use for drug screening etc.

A patient-specific model for human disease
Using iPSCs to understand congenital disorders of glycosylation (CDG) and congenital muscular dystrophy (CMD)

Mike Tiemeyer
Lance Wells
Rich Steet
Glycoconjugates on the cell surface of animal cells
“Salt and Pepper Syndrome” (S&P): applying glycomic technology to investigating human disease mechanism

- identified in 1 African-American family in the southeastern US at the Greenwood Genetic Center, Greenwood, SC
- a missense mutation (p.E332K) was identified in the ST3GAL5 (GM3 synthase) gene of two siblings.
- profound intellectual disability
- failure to thrive
- seizure disorder
- mid-face hypoplasia
- scattered dermal hyper- and hypo-pigmentation

Charles Schwartz, Greenwood Genetic Center
Alteration of glycomic diversity in S&P syndrome

<table>
<thead>
<tr>
<th></th>
<th>GM3</th>
<th>LacCer</th>
<th>Glycoprotein sialylation</th>
<th>High mannose glycans</th>
<th>Apoptosis</th>
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<tr>
<td>Normal</td>
<td>absent</td>
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<td>☹</td>
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<tr>
<td>S&amp;PS Fibroblast</td>
<td>reduced</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>☹</td>
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<td>S&amp;PS Zebrafish</td>
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<td>-</td>
<td>☹</td>
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<tr>
<td>S&amp;PS iPS cells</td>
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<td>-</td>
<td>☹</td>
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<tr>
<td>S&amp;PS NC cells</td>
<td></td>
<td></td>
<td>↑</td>
<td>↑↑↑</td>
<td>☹ège</td>
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</table>
Generation of patient-derived hiPSCs

Oct4, Sox2, Klf4, c-myc
Sendai virus

Reprogrammed cells from ‘salt and pepper’ syndrome patient

Differentiated cells (glycan analysis)
iPSC generation and differentiation from S&P fibroblasts

1° Fibroblast (Control or Salt & Pepper)

iPSC

(BIO) Wnt
(Noggin) BMP

activin/nodal (SB435142)

NC

Also, negative for Sox2, Pax6, Oct4, Nanog

Menendez et al. Proc Natl Acad Sci.108:19240
Altered glycolipid profiles in ST3Gal5 cells are more evident in neural crest than in iPSCs.
Differentiation of normal iPSCs to neural crest cells is accompanied by increased GM3 and more complex gangliosides. Normal iPSCs: Neutral GSLs are abundant. Normal NC cells: Complex gangliosides.

K. Aoki, M. Tiemeyer
S&P NC cells lack GM3, but have increased GM1b, GD1c and LacCer

K. Aoki, M. Tiemeyer
N-linked glycans exhibit cell-specific changes in S&P neural crest cells
Analysis of receptor tyrosine kinases in WT and ST3Gal5 deficient neural crest (NC) cells

WT NC

EGFR ErbB3 ErbB4 InsR IGF-1R

ST3Gal5 NC

EGFR ErbB3 ErbB4 InsR IGF-1R

ErbB3

EGFR

WT ST3Gal5 WT ST3Gal5

InsR: insulin receptor
EGFR: epidermal growth factor receptor
IGF-1R: insulin growth factor-1 receptor

M. Dookwah, R. Steet
**PMM2-CDG** and **MPI-CDG**

<table>
<thead>
<tr>
<th>CDG Subtype</th>
<th>Gene Affected</th>
<th>Reaction Affected</th>
<th>Clinical Features</th>
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<tbody>
<tr>
<td><strong>PMM2-CDG</strong></td>
<td><em>PMM2</em></td>
<td>Man-6-P → Man-1-P</td>
<td>hypotonia, psychomotor retardation, epilepsy and ataxia, skeletal defects</td>
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<tr>
<td><strong>MPI-CDG</strong></td>
<td><em>MPI</em></td>
<td>Fru-6-P → Man-6-P</td>
<td>gastrointestinal and liver problems, hypoglycemia, thrombotic events</td>
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</table>
PMM2 CDG

- iPSC lines generated from one patient line
  - currently being characterized

*PMM2*: V231M, R141H mutation
Reduced PMM2 activity in patient human fibroblasts and iPSCs

*PMM2: V231M, R141H mutation*

Deficient glycoenzyme activities can be detected in cell models - validates the approach

Noor Klaver and Rich Steet
# Congenital Disorders of Glycosylation: iPSC Pipeline

<table>
<thead>
<tr>
<th>Gene</th>
<th>Disease</th>
<th>Genotypes</th>
<th>iPSC</th>
<th>Specialized cell types</th>
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<tbody>
<tr>
<td>ST3Gal5</td>
<td>Salt and Pepper</td>
<td>c.994 G&gt;A (E332K) c.862C&gt;T (nonsense)</td>
<td>Yes</td>
<td>Neural Crest Neurons</td>
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<tr>
<td>PMM2</td>
<td>PMM2-CDG</td>
<td>V231M, R141H P113L, R141H F119L, R141H</td>
<td>Yes</td>
<td>Hepatocyte Neuron</td>
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<tr>
<td>POMGnT1</td>
<td>Muscle-Eye-Brain disease</td>
<td>2 diff. genotypes</td>
<td>Yes</td>
<td>Cardiomyocytes Neurons</td>
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<tr>
<td>OGT</td>
<td>X-linked ID</td>
<td>2 diff. genotypes</td>
<td>No</td>
<td>Neurons</td>
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<tr>
<td>COG7</td>
<td>COG7-CDG</td>
<td>2 diff. genotypes</td>
<td>No</td>
<td>Neural Crest</td>
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</table>

### Additional iPSC models under consideration

<table>
<thead>
<tr>
<th>STT3A/B</th>
<th>STT3A/B-CDG</th>
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<tbody>
<tr>
<td>ALG9</td>
<td>ALG9-CDG</td>
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</table>
Other hiPSC lines: Congenital muscular dystrophy (CMD)

<table>
<thead>
<tr>
<th>Patient/disease classification</th>
<th>Origin</th>
<th>Cell source</th>
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<tr>
<td>POMGnT1</td>
<td>Wellstone Center</td>
<td>fibroblast</td>
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<tr>
<td>maturation of $\alpha$-DG</td>
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<tr>
<td>Fukutin CMD</td>
<td>Wellstone Center</td>
<td>fibroblast</td>
</tr>
<tr>
<td>maturation of $\alpha$-DG</td>
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<tr>
<td>FKRP</td>
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<tr>
<td>maturation of $\alpha$-DG</td>
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</tr>
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</table>
Micheal Tiemeyer (CCRC, UGA)
Kazuhiro Aoki
Rich Steet
Michelle Dookwah
Lance Wells
Michael Pierce
Noor Klaver
Dalton Lab (Center for Molecular Medicine, UGA)
Michael Kulik
Ryan Berger