A Novel Epigenetic Mark, Histone H1 Fucosylation, Regulates Macrophage Plasticity in Rheumatoid Arthritis

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Part I:
Fucosylation Is a Hallmark of Inflammatory Macrophages and Novel Therapeutic Target for Rheumatoid Arthritis
Fucosyltransferases (Futs)

- Fucosylation is the addition of a fucose residue to O-glycans, N-glycans, and glycolipids.
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Adapted from Bing Ma, Joanne L. Simala-Grant, and Diane E. Taylor, 2006

Asn, asparagine; Gal, galactose; GalNAc, N-acetylgalactosamine; GlcNAc, N-acetylglucosamine; Man, mannose; Neu5Ac, sialic acid; Ser, serine; Thr, threonine.
Terminal, sub-terminal fucosylation might play a role in RA pathogenesis.

Expression is represented as: (copy number/Gapdh) x 10^5
Macrophage Development and Polarization

David M. Mosser and Justin P. Edwards, 2008
Macrophage Heterogeneity in Rheumatoid Arthritis

Current therapeutic targets related to macrophages

**Inflammatory**
- Anti-GM-CSF
- GM-CSF
- LPS
- IFN-γ
- TNF-α
- Anti-TNF-α

**Anti-Inflammatory**
- Tofacitinib
- M-CSF
- IL-4
- IL-13
- IL-10
- Anti-M-CSF

**Futs**
- Tyrosine kinase
- JAK
- Tyrosine phosphatase

**Blocker**
- TRA-8
- DR5 Fas
- TLR2,4
- RANKL

**Fut inhibitors**
- CD200-Fc

**Adapted from J. Li, et al 2012**
Terminal and Sub-terminal *Futs* Are Mainly Expressed in M1 Macrophages Isolated from Human RA Synovial Fluid and Tissues

Expression is represented as:

Th1: CD4+ CD45RA− CCR2+ CCR5− or CD4+ CXCR3− CCR6

Th17: CD4+ CD45RA− CCR2+ CCR5− or CD4+ CXCR3− CCR4+ CCR6+ CD161+

Fibroblasts were isolated from synovial tissues or fragment; all other cells were sorted from synovial fluid.
Correlation between FUTs and TNF in RA/OA Synovial Tissues

A

\[ \alpha(1,2)-\text{Terminal} \]

\[ \alpha(1,3/4)-\text{Sub-terminal} \]

\[ \alpha(1,6)-\text{Core} \]

\[ \text{O-fucosylation} \]

B

\[ \alpha(1,2)-\text{Terminal} \]

\[ \alpha(1,3/4)-\text{Sub-terminal} \]

\[ \alpha(1,6)-\text{Core} \]

\[ \text{O-fucosylation} \]
Expression of Futs from Human RA Synovial Cells

**Key Findings**

1. Expression of most *Futs* (terminal, sub-terminal fucosylation) is highly correlated with *Tnfa*, whereas that of the other *Futs* (core and o-fucosylation) doesn’t correlate with *Tnfa*.

2. Those *Futs* that are correlated with *Tnfa* are restricted in M1 inflammatory macrophages from human RA synovial cells.

3. *Fut* expression is upregulated in the process of macrophage differentiation (>10 fold increase from monocytes to macrophages).
2-D-gal is a Terminal Fut Inhibitor

Fucosylation Pathways

Fucosylation Inhibitor

E. Miyoshi et al. J.Biochem, 143, 725-729
Murrey HE, Hsieh-Wilson LC, PNAS 2006
2-D-gal Inhibited Collagen II-induced Arthritis

2-D-gal (200 mg/kg), fucose: 2 times/week. I.P.

* p<0.01
2-D-gal Inhibited Autoantibodies and Inflammatory Cytokines Production in CIA

* \( P < 0.01 \)
2-D-gal Reduced Inflammatory Macrophages and CD4 T cells in CIA

A

B

C

D

* P < 0.01
2-D-gal Selectively Precludes M1 macrophage Differentiation

Day 0 1 2 3 4 5 6 7

M1: GM-CSF Analysis

Control 2-D-gal (15mM) Fucose (15mM)

Mouse BM-derived M1 MΦ

Human RA Synovial M1 MΦ

Human RA Synovial Fibroblasts

Graph showing viability over time with 2-D-gal and fucose treatments.

 RAW264.7

Viability

Control 2-D-gal Fucose Glucose NaCl Glycerol

5mM 5mM 5mM 5mM 5mM 5mM 5mM

Viability

0 0.2 0.4 0.6 0.8 1 1.2 1.4

BM-M1 MΦ Human RA MΦ Human RA Fibroblasts

** * 
2-D-gal Skews M1 Towards M2 Macrophages

Day 0 1 2 3 4 5 6 7

M1: GM-CSF
M2: M-CSF

Analysis

Viability

2-D-gal (mM)

0 0.01 0.1 1 5 15

M1

2-D-gal (mM) overlay

No LPS

LPS (30mins)

p-ERK1/2

IFN-γ

IL-10

pg/ml

0 200 400 600 800

0 0.01 0.1 1

2-D-gal (mM)

0 0.01 0.1 1

2-D-gal (mM)

p-ERK1/2

p-ERK1/2

68.1 ± 7.5

72.9 ± 10.2

6.7 ± 1.1

2.4 ± 0.3
1. Fucosylation is a hallmark of inflammatory macrophages;

2. Fucosylation inhibitor, 2-D-Gal, prevents the M1 inflammatory macrophage differentiation and skews M1 to M2 macrophages;

3. Fucosylation inhibitor, 2-D-Gal, precludes the development of arthritis in DBA/1J mice whereas fucose facilitates it;

4. In CIA mice, 2-D-gal reduced the inflammatory macrophages and pathogenic CD4 T cells (including IL-17^+, IFN-γ^+ CD4 T cells); it also reduces autoantibody production.

**QUESTION:**
How does fucosylation regulate macrophage differentiation and polarization?

*Adapted from Qiagen*
Part II: A Novel Epigenetic Mark, Histone H1 Fucosylation, Orchestrates Macrophage Differentiation and Plasticity By Remodeling the Enhancer Landscape in Rheumatoid Arthritis

Unpublished data (slides 18-26), only available at the conference.
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