Epigenomics & gene expression

Major Histocompatibility Complex (MHC)

Oct 2015
• Master regulatory complex _NLRC5_
  • Best known for regulating MHCI molecules expressed in almost all nucleated cells
  • Expression of NLRC5: B, T, & NK-cells > naïve (?) monocytes

• Master regulatory complex _CIITA_
  • Best known for regulating MHCII molecules mainly expressed in immune cells
Key molecules under investigation

CIITA enhanceosome

Handunnetthi L; Genes & Immunity (2010)

NLRC5 enhanceosome

KOichi S. Kobayashi and Peter J. van den Elsen
NATURE REVIEWS | IMMUNOLOGY
VOLUME 12 | DECEMBER 2012
I. 8 extended MHC haplotypes have been described (Horton et al., 2008)

<table>
<thead>
<tr>
<th>Haplotypes</th>
<th>Length (bp)</th>
<th>Gaps</th>
<th>HLA-A</th>
<th>HLA-B</th>
<th>HLA-C</th>
<th>HLA-DQA1</th>
<th>HLA-DQB1</th>
<th>HLA-DRB1</th>
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</thead>
<tbody>
<tr>
<td>PGF</td>
<td>4754829</td>
<td>0</td>
<td>A*03010101</td>
<td>B*070201</td>
<td>Cw*07020103</td>
<td>DQA1*010201</td>
<td>DQB1*0602</td>
<td>DRB1*150101</td>
</tr>
<tr>
<td>COX</td>
<td>4731878</td>
<td>0</td>
<td>A*01010101</td>
<td>B*080101</td>
<td>Cw*070101</td>
<td>DQA1*050101</td>
<td>DQB1*020101</td>
<td>DRB1*030101</td>
</tr>
</tbody>
</table>

↑ susceptibility for PGF: Multiple sclerosis, systemic lupus etc

↓ susceptibility for PGF: Type 1 diabetes

↑ susceptibility for COX: HIV, Type 1 diabetes, systemic lupus etc

PGF & COX: identify molecular basis behind phenotypic difference?

II. Where does CIITA “start”, NLRC5 “stop” vice-versa put into context of different cell types and treatments?
II. Where does CIITA “start”, NLRC5 “stop” vice-versa put into context of different cell types and treatments?

Samples prepared using leukocyte cones (LC)

- ChIP-analysis of CIITA & NLRC5 all comparisons based on PGF as reference MHC haplotype

- RNA & genomic DNA

- Naïve/ treated CD14 monocytes and naïve/ activated CD4 & CD8 T-cells

- Composite epigenetic- trans eQTL- gene expression maps of CIITA and NLRC5
Strong “peaks” within input that potentially mask signal of ChIP-samples?
ChIP-seq in primary immune cells

Cross-linking of cells
Lysis and sonication
Immunoprecipitation (IP)
De-crosslinking
Isolation of DNA for sequencing

Genomic DNA bound by transcription factor

Adapted from Park 2009
A modified reverse x-linking procedure was introduced.
Insufficient on its own to resolve issue?
Enriched peaks across various experiments

Number of enriched peaks (MACS2 q<0.05)
Proportion of enriched peaks <10kb from TSS of a gene

% called peaks <10kb TSS

LC7 & 8_CD14 IFN
LC15_CD4 APC
LC16_CD14 IFN
LC17_CD4
LC17_CD4 APC
Biological themes from these genes with CIITA / NLRC5 enriched peak in proximity

**DAVID**
Biological themes from 271 genes associated with a CIITA Bi in treated monocytes

- **Infection**
  - 14 MHC / 11 non-MHC
  - 14 MHC / 27 non-MHC

- **Immune**
  - 19 MHC / 71 non-MHC
  - 15 MHC / 48 non-MHC
  - 18 MHC / 63 non-MHC

**Ingenuity Pathway Analysis**

LC15_CD4 APC_CIITA
- 271 genes

LC16_CD14 IFN_CIITA
- 16 genes

LC16_CD14 IFN_NLRC5
- 109 genes

LC7 & 8_CD14 IFN_CIITA
- 223 genes
DAVID Ingenuity Pathway Analysis

Biological themes from 271 genes associated with a CIITA Bi in treated monocytes

- Infection
  - 14 MHC/11 non-MHC
  - 14 MHC/27 non-MHC

- Immune
  - 19 MHC/71 non-MHC
  - 15 MHC/48 non-MHC
  - 18 MHC/68 non-MHC

LC7 & 8_CD14 IFN_CIITA
271 genes

LC17_CD4_CIITA
1196 genes

LC17_CD4_NLRC5
4657 genes

LC17_CD4 APC_CIITA
1118 genes

LC17_CD4 APC_NLRC5
1979 genes
Examining ChIP and input datasets using alternative method

LC7_CD14 IFN

upstream  TSS  downstream

LC8_CD14 IFN

upstream  TSS  downstream
* LC17_CD4 APC
LC18_CD4 APC
Modified overall procedure to include diagnostic read-out(s)

1. primary immune cells
   - crosslinking
   - lysis, sonication
   - fragmented chromatin

2. Input that has gone through de-crosslinking and clean-up
   - **MI-SEQ for diagnostics**

3. Does sequenced profile of input look desirable?
   - Yes
   - **IP**
   - ChIP-ped chromatin
     - ChIP-ped DNA that has gone through de-crosslinking and clean-up
     - **MI-SEQ for diagnostics as and when necessary**
     - Lib-preps via different kits
     - Selected, matched ChIP & input samples for Hi-SEQ

4. No.
   - A new biological sample is required, re-start whole process.
Collaborative effort with Everest Biotech to obtain Abs for NLRC5

- Only commercial offering from AbCAM (ab105411); non-IP grade antibody

Anti_NLRC5 1:500 (2ug/uL)

- £400 got us two antibodies for further characterisation

- Western blot - NLRC5 antibody (ab105411)

Predicted band size: 205 kDa

<table>
<thead>
<tr>
<th>Lane</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>LC10_CD14</td>
</tr>
<tr>
<td>8</td>
<td>LC10_CD14 +IFN</td>
</tr>
<tr>
<td>9</td>
<td>LC11_CD14</td>
</tr>
<tr>
<td>10</td>
<td>LC11_CD14+IFN</td>
</tr>
</tbody>
</table>
1° Ab rabbit α-NLRC5 2 ug/mL 1/500;  
2° Ab goat α-rabbit HRP 0.04 ug/mL 1/10K

1° Ab rabbit α-NLRC5 1 ug/mL 1/1000;  
2° Ab goat α-rabbit HRP 0.04 ug/mL 1/10K

1° Ab goat [from Zoey] 1/500;  
2° rabbit α-goat HRP [from Zoey] 1/5K

2° donkey α-goat HRP 0.05 ug/mL 1/10K

1, 5 E6.1 lysate equivalent 4.2x10^6 cells

2, 6 E6.1 lysate equivalent 2.1x10^6 cells

3, 7 E6.1 lysate equivalent 0.8x10^6 cells

9, 10 25ul of lysate from Zoey (mouse testes)
1, 3, 5, 7, 9, 10

E6.1 lysate equivalent 3.3x10^6 cells

1° Ab goat α-NLRC5 AB12686 1ug/mL 1/500; 2° Ab donkey α-goat HRP 16ng/mL 1/30K

1° Ab goat α-NLRC5 AB12686 2ug/mL 1/250; 2° Ab donkey α-goat HRP 16ng/mL 1/30K

1° Ab goat α-NLRC5 AB12686 0.4ug/mL 1/1250; 2° Ab donkey α-goat HRP 16ng/mL 1/30K

1° Ab goat α-NLRC5 AB12686 1ug/mL 1/500; 2° Ab donkey α-goat HRP 16ng/mL 1/30K

1° Ab rabbit α-NLRC5 1ug/mL 1/1000; 2° Ab goat α-rabbit HRP 13ng/mL 1/30K
Use CRISPR to alter expression level of CIITA / NLRC5

- Knock-out in Jurkat E6.1 (T-cell model)

CIITA: 44-bp deletion

NLRC5: 41-bp deletion
Strategy #1: Using FACS to get double GFP-dsRED positives

CIITA guide A-pX335 nickase construct

CIITA guide B-pX335 nickase construct

CIITA pES-RGS2 reporter construct

FACS

x3 each CIITA & NLRC5
Strategy #2: Using Miltenyi’s MACS-HKK to enrich for positives

CIITA guide A-pX335 nickase construct

CIITA pMRS-CMV reporter construct with HKK-cassette

CIITA guide B-pX335 nickase construct

Multiple cells into individual wells
No expected RFP or GFP signals with pMRS-constructs

- CRISPR event

+ CRISPR event
Strategy #3: Back to the drawing board...

CIITA guide A-pX335 nickase construct

CIITA pES-RGS2 reporter construct

Multiple cells into individual wells

FACS

CIITA guide B-pX335 nickase construct
Would not have been possible without…
(alphabetical order)

J Knight Team
Current members + *Wanseon

J Knight Alumni
Kat
Peter
Seiko

Ben Davies

Team Monaco
Zoey