Transcriptomic Analysis of Aortic Dendritic Cells and Macrophages in Mouse Atherosclerosis Model

Jae-Hoon Choi, DVM, PhD

Department of Life Science
Hanyang University, Seoul
Republic of Korea
**Stages in the development of Atherosclerosis**

1. **Nature 2011**

**Cellular Composition of Atherosclerosis**

- **LDLR or ApoE KO mouse**
- **Human**
Lipid accumulation and foam cell formation in atherosclerotic lesion

Lipoproteins affect macrophage phenotype

Circ Res 2016

Cell 2012
Proinflammatory and proresolving mediators balance macrophage phenotype

1. In vitro culture of peritoneal macrophages
2. Laser capture microdissection-mediated microarray of plaque
3. Other artificial system-foamy cell analysis in subcutaneous inserted sponge

So, we need to have a new technique to analyze the characteristics of foam cells in atherosclerotic aorta itself
- Single cell RNA sequencing analysis of whole CD45+ leukocytes from atherosclerotic aortas
- Development of lipid probe-assisted flow cytometry to detect foamy cell from atherosclerotic aortas
- Bulk RNA sequencing analysis of intimal foamy and non-foamy macrophages
Unbiased Single Cell RNA sequencing Analysis of Aortic Leukocytes from Atherosclerotic Aorta

LDLR/- mice: WD for 12 weeks

Sorted cell number: 100,000 cells/100uL

10x Chromium Single cell RNA sequencing

LDLR KO CD45+ aortic cells

Automatically annotated 11 clusters

Representative Enriched Genes

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-2 -1 0 1 2</td>
</tr>
</tbody>
</table>

A

LDLR/- mice
aortic live CD45+ cells
(n = 3781)
Automatically annotated 11 clusters

Limitation of scRNA seq analysis
- Single cell RNA sequencing analysis of whole CD45+ leukocytes from atherosclerotic aortas

- Development of lipid probe-assisted flow cytometry to detect foamy cell from atherosclerotic aortas

- Bulk RNA sequencing analysis of intimal foamy and non-foamy macrophages

**BODIPY493/503-based lipid staining and flow cytometry define foam cells from atherosclerotic aorta**
BODIPY493/503-based lipid staining and flow cytometry define foam cells from atherosclerotic aorta

C
From whole aortic cells

D
Aortic single cell

WT
ApoE
LDLR

BODIPY493/503

SSC

FSC-A

Autoflu + Autoflu

BODIPY493/503-based lipid staining and flow cytometry define foam cells from atherosclerotic aorta

E
Aortic single cells

Normal
Atherosclerosis

Intima
Adventitia

Intima
Adventitia

Foam cell number

---

C
Aortic
SSC
BODIPY

Aortic
SSC
BODIPY

HEMA3
Oil Red O
EM

17

18
Single cell RNA sequencing analysis of whole CD45+ leukocytes from atherosclerotic aortas

Development of lipid probe-assisted flow cytometry to detect foamy cell from atherosclerotic aortas

Bulk RNA sequencing analysis of intimal foamy and non-foamy macrophages

Bulk sequencing of aortic intimal foamy and non foamy MØs shows their distinct gene expression profiles
Bulk sequencing of aortic intimal foamy and non foamy MØs shows their distinct gene expression profiles

Expression profile of top 100 genes of bulk RNA-seq in the clusters of scRNA-seq
Pathway analysis of DEGs in bulk and scRNA seq

Analysis of IL-1β signaling pathway genes in our bulk RNA-seq and scRNA-seq
Analysis of IL-1β signaling pathway genes in our bulk RNA-seq and scRNA-seq

Foamy MØs showed less IL-1β mRNA expression than non-foamy MØs in human and mouse atherosclerotic lesions

A

Human Atheroma

Foamy MØs

CD68

IL1β

(-) control (Dap16)

(+) control (PP16)

Foam cell

IL-1β

B

H&E staining

100μm

20μm

P = 0.057

IL-1β cells % in area

10

5

0
Foamy MØs showed less IL-1β mRNA expression than non-foamy MØs in human and mouse atherosclerotic lesions

C

Mouse Atherosclerotic aortic sinus

<table>
<thead>
<tr>
<th>H&amp;E staining</th>
<th>IL-1β</th>
<th>(-) control (DapB)</th>
<th>(+) control (Ppolb)</th>
</tr>
</thead>
</table>

D

- Foam cell

![Diagram showing IL-1β mRNA levels in different cell types](image)

Circ Res. 2016
**scRNA seq analysis defines SMC-derived foam cells**

Different gene enrichment in each foam cell cluster
Proliferating foam cells in atherosclerotic lesion

A single-cell RNA-seq approach was used to reveal the transcriptional landscape and heterogeneity of aortic macrophages in murine atherosclerosis. The study compared immune cell compositions among different atherosclerosis models:

- **Cochain et al., 2018**:
  - Macrophages: 12%
  - Others (IL, Granulocytes, NKs): 16%
  - CX3CR1+ T cells: 9%
  - CD8+ T cells: 10%
  - DCs: 15%
  - NK cells: 2%

- **Winkels et al., 2018**:
  - Macrophages: 21%
  - Others (IL, Granulocytes, NKs): 16%
  - CX3CR1+ T cells: 9%
  - CD8+ T cells: 10%
  - DCs: 15%
  - NK cells: 0%

- **Kim et al., 2018**:
  - Macrophages: 22%
  - Others (IL, Granulocytes, NKs): 16%
  - CX3CR1+ T cells: 9%
  - CD8+ T cells: 10%
  - DCs: 15%
  - NK cells: 7%

The study also highlighted the role of T cells and DCs in the immune response against atherosclerosis.
Consideration in scRNA seq analysis

Separation of adventitia from aorta
(KIM et al)
(A) Resident MØs were mostly located at the aortic adventitia of LysM-Cre and ROSA26tdTomato mice (upper; red) as well as CX3CR1-GFP (lower; green) mice, but yet poorly detected in the intima-media portion.
(B) H&E-stained cross-sections of aortas with or without adventitia from chow- or WD- fed Ldlr-/- mice. (C) Immunofluorescence staining of CD206 (red) on cross-sections of aortas with or without adventitia from chow- or WD- fed Ldlr-/- mice. DAPI (blue) staining for nuclei. Adventitial CD206+ MØs were clearly eliminated by the removal of adventitial tissue.
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mice</strong></td>
<td>Ldlr&lt;sup&gt;−/−&lt;/sup&gt; (8-wk male)</td>
<td>Apoe&lt;sup&gt;−/−&lt;/sup&gt; (8-wk female)</td>
<td>Ldlr&lt;sup&gt;−/−&lt;/sup&gt; (6-8-wk male)</td>
</tr>
<tr>
<td><strong>Diet</strong></td>
<td>Western diet (test diet, AIN-76A), 12 wks</td>
<td>Chow or Western diet (Envigo, TD.88137), 12 wks</td>
<td>Chow or high-fat diet (Altromin, 15% milk fat, 1.25% cholesterol), 11 wks</td>
</tr>
<tr>
<td><strong>Enzyme mix</strong></td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt;Mg&lt;sup&gt;2+&lt;/sup&gt;PBSCollagenase I (675 U/mL), Collagenase XI (187.5 U/mL), Dnase I (90 U/mL), Hyaluronidase (90 U/mL)</td>
<td>HBSSCollagenase I (450 U/mL), Collagenase XI (250 U/mL), Dnase I (120 U/mL)</td>
<td>RPMICollagenase I (450 U/mL), Collagenase XI (125 U/mL), Hyaluronidase (60 U/mL)</td>
</tr>
<tr>
<td><strong>Incubation time</strong></td>
<td>37°C, 70 min</td>
<td>37°C, 60 min</td>
<td>37°C, 40 min</td>
</tr>
<tr>
<td><strong>Mesh size</strong></td>
<td>100 µm</td>
<td>50 µm</td>
<td>40 µm</td>
</tr>
<tr>
<td><strong>Tissue</strong></td>
<td>Intact aorta and Adv removed aorta</td>
<td>Intact aorta</td>
<td>Intact aorta</td>
</tr>
<tr>
<td><strong>Platform</strong></td>
<td>10X Genomics</td>
<td>10X Genomics</td>
<td>Drop-seq</td>
</tr>
<tr>
<td><strong>No. of CD45&lt;sup&gt;+&lt;/sup&gt; cells analyzed after QC filtering</strong></td>
<td>3781 (Western diet)</td>
<td>909 (chow)2077 (Western diet)</td>
<td>372 (chow/1854 (Western diet)</td>
</tr>
<tr>
<td><strong>scRNA-seq replication</strong></td>
<td>Total foam cells (SSC&lt;sup&gt;+&lt;/sup&gt;BODIPY&lt;sup&gt;+&lt;/sup&gt;) from Apoe&lt;sup&gt;−/−&lt;/sup&gt; mice fed a high-fat diet for 27 wk</td>
<td>CD45&lt;sup&gt;+&lt;/sup&gt; leukocytes from Ldlr&lt;sup&gt;−/−&lt;/sup&gt; (8-wk male) fed a high cholesterol diet for 12 wk</td>
<td>CD45&lt;sup&gt;+&lt;/sup&gt; leukocytes from Apoe&lt;sup&gt;−/−&lt;/sup&gt; (6-wk male) fed Western diet for 11 wk</td>
</tr>
<tr>
<td><strong>Validation</strong></td>
<td>FACS, histology, and RNA-seq for foamy and nonfoamy macrophages, In situ hybridization</td>
<td>Mass Cytometry and FACS for 3 B-cell subsets</td>
<td>Immunohistochemistry for 3 macrophage subsets</td>
</tr>
<tr>
<td><strong>Human translation</strong></td>
<td>In situ hybridization of JUN mRNA and K3-67 staining in human lesional macrophages</td>
<td>Enumerate leukocyte frequencies in 126 human plaques by a genetic deconvolution strategy</td>
<td>Immunohistochemistry of human lesions</td>
</tr>
</tbody>
</table>

**Consistency of seq data on Mphs Clusters**

*Cochain et al., Circ Res 2018
Single cell RNA seq on aortic immune cells*

LDLR KO WD 11 weeks
CD45<sup>+</sup> scRNA-seq

Enriched genes in Inflammatory Macrophages
(cZ score >3.0, significantly higher expression vs Trem2<sup>+</sup> macrophages, CD45<sup>+</sup> and Resident-like macrophages)

**Enriched genes in Inflammatory Macrophages**

<table>
<thead>
<tr>
<th>Gene</th>
<th>cZ Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trem2</td>
<td>&gt;3.0</td>
</tr>
<tr>
<td>Cxcl10</td>
<td>&gt;3.0</td>
</tr>
<tr>
<td>Il6</td>
<td>&gt;3.0</td>
</tr>
<tr>
<td>Tnf</td>
<td>&gt;3.0</td>
</tr>
<tr>
<td>Bcl10</td>
<td>&gt;3.0</td>
</tr>
<tr>
<td>Ifi30</td>
<td>&gt;3.0</td>
</tr>
</tbody>
</table>

**Enriched genes in Resident-like Macrophages**

<table>
<thead>
<tr>
<th>Gene</th>
<th>cZ Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd68</td>
<td>&gt;3.0</td>
</tr>
<tr>
<td>Fizz1</td>
<td>&gt;3.0</td>
</tr>
<tr>
<td>P2ry1</td>
<td>&gt;3.0</td>
</tr>
<tr>
<td>Pdhk1</td>
<td>&gt;3.0</td>
</tr>
<tr>
<td>Atg5a</td>
<td>&gt;3.0</td>
</tr>
<tr>
<td>Col1a1</td>
<td>&gt;3.0</td>
</tr>
</tbody>
</table>

**Enriched genes in Resident-like Macrophages**

<table>
<thead>
<tr>
<th>Gene</th>
<th>cZ Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd68</td>
<td>&gt;3.0</td>
</tr>
<tr>
<td>Fizz1</td>
<td>&gt;3.0</td>
</tr>
<tr>
<td>P2ry1</td>
<td>&gt;3.0</td>
</tr>
<tr>
<td>Pdhk1</td>
<td>&gt;3.0</td>
</tr>
<tr>
<td>Atg5a</td>
<td>&gt;3.0</td>
</tr>
<tr>
<td>Col1a1</td>
<td>&gt;3.0</td>
</tr>
</tbody>
</table>

Kim & Choi, Circ Res 2018
Letter to editor
## Acknowledgement

<table>
<thead>
<tr>
<th>Hanyang University</th>
<th>Washington University School of Medicine</th>
<th>IRCM, Canada</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kyungdae Kim</td>
<td>Gwendalyn J Randolph</td>
<td>Cheolho Cheong</td>
</tr>
<tr>
<td>Dahee Shim</td>
<td>Maxim Artyomov</td>
<td>Jun Sung Lee</td>
</tr>
<tr>
<td>Hyung Seok Jang</td>
<td>Konstantin Zaitsev</td>
<td>Tae Jin Yun</td>
</tr>
<tr>
<td>Seung Hyun Lee</td>
<td>Jesse Williams</td>
<td>Nabil Seidah</td>
</tr>
<tr>
<td>Ki Byung Kim</td>
<td>Ki-Wook Kim</td>
<td>Annik Prat</td>
</tr>
<tr>
<td>Jin-Wu Nam</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sang-ho Yoon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Junssoon Choi</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Ewha Womans University                 | Seoul National University Hospital       |                               |
| Goo Taeg Oh                            | Seung-Pyo Lee                            |                               |

| Seoul National University              | KRIIBB                                   |                               |
| Je Kyung Seong                         | Won Kee Yoon                             |                               |