Mechanism of Macrophage Trapping in Atherosclerosis

Young Mi Park

Department of Molecular Medicine
Ewha Womans University School of Medicine
Atherosclerosis

Monocyte migrating in
Monocyte
endothelium
macrophage
CD36
OxLDL
myeloperoxidase
lymphocytes
cytokines
arterial intima
Mobilization of macrophages as a new therapeutic strategy for the treatment of atherosclerosis that reverses the disease.
Interaction between oxidized LDL and CD36 modulates macrophage cytoskeletal function and inhibits migration;

A Mechanism of Macrophage Trapping
Oxidized lipoproteins

CD36

NH₂-GCDRNC

CACRSKTIK-COOH

Atherosclerosis
CD36 in Atherosclerosis

- Macrophages from CD36 null mice are profoundly defective in uptake of oxLDL and foam cell formation
- CD36 null mice demonstrate a dramatic decrease in atherosclerotic lesion development
**In vivo macrophage migration assay**

1. **Thioglycolate I.P. injection**
   - 4 days
   - Monocytes come into peritoneal cavity and differentiate to macrophages

2. **LPS I.P. injection**
   - 4 hours
   - Macrophages migrate out to regional lymph nodes

3. **Peritoneal lavage and count macrophages**

4. **CD36**

5. **oxLDL**
OxLDL inhibits macrophage migration *in vivo*
OxLDL inhibition of macrophage migration *in vivo* is CD36-dependent

![Bar chart showing migration index for different treatments](chart.png)

- No treatment
- LPS
- OxLDL / LPS

Legend:
- **WT**
- **CD36 null**

Statistical significance: P < 0.001
Macrophage migration is inhibited by ox-LDL

No MCP-1

+ MCP-1

Without oxLDL

+ oxLDL 50µg/ml
Ox-LDL induces rapid macrophage spreading; CD36 null cells show less spreading in response to oxLDL
OxLDL induces sustained activation of FAK

![Graph showing the level of change in p-FAK (Tyr 576/577) with time after oxLDL and TSP-1 treatment. The graph indicates a peak at 10 minutes for oxLDL and 30 minutes for TSP-1, followed by a decrease over time.](image-url)
Inactivation of PTP is due to oxidation of the essential cysteine in the active site of PTP

Wu RF, Terada LS, Sci STKE, 2006(332):pl2
OxLDL induces oxidative modification of SHP-2

oxLDL (50ug/ml) No treatment

15’ 5’ 0

IP: SHP-2

Fluorescein-bound SHP-2
Anti-oxidant and NADPH oxidase inhibitor restore dynamic phosphorylation of pFAK by oxLDL

**Pre-treatment**

<table>
<thead>
<tr>
<th>oxLDL</th>
<th>None</th>
<th>NAC</th>
<th>Apocynin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>30'</td>
<td>60'</td>
<td>90'</td>
</tr>
<tr>
<td>0</td>
<td>30'</td>
<td>60'</td>
<td>90'</td>
</tr>
<tr>
<td>0</td>
<td>30'</td>
<td>60'</td>
<td>90'</td>
</tr>
</tbody>
</table>

**Fold Change**

pFAK (Y576/577)

- **untreated**
- **NAC**
- **Apocynin**

**Graphs**

- **pFAK**
- **tFAK**

**Graphs**

- **Fold Change pFAK (Y576/577)**
- **oxLDL**

- **untreated**
- **NAC**
- **Apocynin**
Blockade of ROS generation restores macrophage migration

- DPI 4µM
- Apocynin 10µM
- NAC 20mM
- Resveratrol 100µM
- Apocynin 2µM
- Apocynin 100µM

Migrated cells (per 10x field)
OxLDL inhibits macrophage migration by CD36-dependent modulation of cytoskeletal function.

Vav and CD36

- Guanine nucleotide exchange factor (GEF)
- Activates small MW g proteins, Rac1/RhoA/RhoG
- Fibrillar beta amyloid binding to CD36 induces phosphorylation (activation) of Vav in monocytes and microglia
- Ox-LDL / CD36 interaction induces phosphorylation of Vav in murine macrophages
Time lapse microscopy to analyze macrophage cytoskeleton

Peritoneal macrophages from wild type, CD36 null, and Vav null mice loaded onto glass coverslips

Cell Polarization

oxLDL

Time lapse microscopy

1 hour 1 hour

?
OxLDL induced loss of cell polarity with lamellipodial retraction, decreased locomotion and decreased dynamic movement.
OxLDL effects on macrophage polarity depend on CD36

No Treatment

oxLDL
Summary
(Time lapse microscopy)

$\text{NO}_2\text{LDL} \rightarrow \text{Lamellipodial retraction with retraction fiber formation}$

$\rightarrow \text{Loss of cell polarity}$

$\rightarrow \text{Inhibition of cellular locomotion/migration}$

These effects are CD36 and Vav dependent
Non-Muscle Myosin II

Cell polarity determinant
Myosin regulatory light chain phosphorylation is decreased by oxLDL (murine macrophage)

<table>
<thead>
<tr>
<th>WT</th>
<th>CD36 null</th>
<th>Vav null</th>
</tr>
</thead>
<tbody>
<tr>
<td>oxLDL(min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.34</td>
<td>1.0</td>
</tr>
<tr>
<td>10</td>
<td>1.0</td>
<td>1.19</td>
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<tr>
<td>30</td>
<td>0.40</td>
<td>0.98</td>
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<tr>
<td>60</td>
<td>1.0</td>
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</tr>
<tr>
<td>Peak</td>
<td>0.39</td>
<td>0.98</td>
</tr>
<tr>
<td>Fold change</td>
<td>0.40</td>
<td>1.14</td>
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</tbody>
</table>

P-MRLC (T18/S19)

α-tubulin
OxLDL induced activation of Rac1-GTPase
6-thio GTP inhibits MRLC dephosphorylation by oxLDL

<table>
<thead>
<tr>
<th>WT</th>
<th>no pretreatment</th>
<th>WT</th>
<th>6-thio GTP</th>
<th>5μM, 4 hr</th>
<th>WT</th>
<th>6-thio GTP</th>
<th>5μM, 16 hr</th>
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</thead>
<tbody>
<tr>
<td>oxLDL(min)</td>
<td>0</td>
<td>10</td>
<td>30</td>
<td>0</td>
<td>10</td>
<td>30</td>
<td>0</td>
</tr>
</tbody>
</table>

Phospho-MRLC (Ser19)

α-tubulin
Rac inhibitor, 6-thio GTP blocks the effect of oxLDL
oxLDL

Actin polymerization

SHP-2 (PTP)

NADPH oxidase

Macrophage trapping

Inflammation

Lesion Formation

CD36

src

FAK

Myosin II

Loss of Cell Polarity

Migration

Rac GTPase

Myosin II

Spreading

Migration


Park et al. Mol Biol Cell, 23(16), 2012

Actin polymerization

Inflammation

Lesion Formation

Park et al. Mol Biol Cell, 23(16), 2012
Understanding of the mechanisms of macrophage trapping as well as foam cell emigration may lead to development of novel strategies for the treatment of atherosclerosis.
Dr. Roy L. Silverstein  
(Univ. of Wisconsin at Milwaukee)

Dr. Maria Febbraio  
Dr. Thomas Egelhoff  
Dr. Martha Cathcart  
Dr. Paul Fox  
Dr. Judy Drazba  
Dr. Amit Vasanjii  
(Cleveland Clinic)

Dr. Clifford Harding  
Dr. Alan Tartakoff  
Dr. Laura Nagy  
(Case Western Reserve Univ.)

Dr. Josephine Adams  
(Univ. of Bristol)

Dr. Won Kyung Cho  
Young Eun Yoon  
Myung Sook Cheon  
(Ewha Womans Univ.)