**Presenter Disclosure Information**
Yang Zhang, PhD
Novel Role of Nlrp3 Inflammasome Activation beyond Canonical Inflammation: Coronary Endothelial Injury and Repair

**FINANCIAL DISCLOSURE:**
No relevant financial relationship exists
Novel Role of Nlrp3 Inflammasome Activation beyond Canonical Inflammation: Coronary Endothelial Injury and Repair

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Virginia Commonwealth University
Richmond, VA
Endothelial dysfunction

Risk factors:
- High blood pressure
- High cholesterol
- Obesity
- Diabetes mellitus
- Smoking

Vascular injury

CVD (Atherogenesis)
Question

What is the early, initiating mechanism mediating the response of endothelial cells to endogenous danger signals such as increased plasma cholesterol, cytokine or adipokines leading to endothelial dysfunction and vascular injury?
Inflammasome

Domains:
- LRR
- NACHT
- NAD
- Pyrin
- CARD
- Caspase
- FIIND
- HIN200
- ASC
- Caspase-1
- Caspase-5

Muramyl dipeptide
*B. anthracis* lethal toxin

Flagellin
Rod
Needle

dsDNA

DAMPs
PAMPs
TLR-mediated "priming" signal

- Extracellular Ca^{2+}

- NADPH oxidase

- MAVS

- Frustrated phagocytosis

- Cathepsins

- IL-1β

- LCWE

- Crystals

- Particulates

- TXNIP

- Crystals

- Particulates

- NLRP3 inflammasome activation

- Bioactive IL-1β

- Active Caspase-1

- Pro-IL-1β

- Ub

- BRCC3

- Deubiquitinase

- Inactive NLRP3

- NF-κB

- NLRP3 & pro-IL-1β

- K+ efflux

- ATP

- K+ extrusion

- K+ influx

- Extracellular

- Ca^{2+}

- Mitochondrial rupture

- ER Stress

- ROS

- NADPH oxidase

- TXNIP

- Ub
Specific questions

- Whether endothelial inflammasome is activated in the early stage of obesity?

- Whether activation of endothelial inflammasomes by endogenous danger signals directly induces alterations in coronary endothelial function?
Experimental design

- Nlrp3 genotyping
  - WT PCR
  - KO PCR
  - bp
  - WT
  - Nlrp3 KO

- ND or HFD 6 weeks
- Nlrp3+/+ or Nlrp3-/-

- Endothelial inflammasome activation
- Ultrasound imaging: coronary artery diameter and blood flow
- Endothelium-dependent vasodilation in isolated coronary arteries
- Endothelial junction integrity
- EC repair by EPCs: EPC mobilization/recruitment
Activation of Nlrp3 inflammasome in coronary arterial endothelium
Experimental design

Nlrp3 genotyping

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<tr>
<th>bp</th>
<th>WT PCR</th>
<th>KO PCR</th>
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<td>+/+</td>
<td>+/-</td>
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<td>330-</td>
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ND or HFD 6 weeks

Nlrp3+/+ or Nlrp3-/-

- Endothelial inflammasome activation
- Ultrasound imaging: coronary artery diameter and blood flow
- Endothelium-dependent vasodilation in isolated coronary arteries
- Endothelial junction integrity
- EC repair by EPCs: EPC mobilization/recruitment
Coronary blood flow in mice
Experimental design

Nlrp3 genotyping

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- Endothelial inflammasome activation
- Ultrasound imaging: coronary artery diameter and blood flow
- Endothelium-dependent vasodilation in isolated coronary arteries
- Endothelial junction integrity
- EC repair by EPCs: EPC mobilization/recruitment

Nlrp3+/+ or Nlrp3-/-

ND or HFD 6 weeks

Mouse
Endothelium-dependent vasodilation

A  Nlrp3+/+

B  Nlrp3-/-
Experimental design

- Nlrp3 genotyping
  - WT PCR: +/+  +/-  -/-
  - KO PCR: +/+  +/-  -/-

- ND or HFD 6 weeks
  - Nlrp3+/+ or Nlrp3-/-

- \[\text{Endothelial inflammasome activation}\]
- \[\text{Ultrasound imaging: coronary artery diameter and blood flow}\]
- \[\text{Endothelium-dependent vasodilation in isolated coronary arteries}\]
- \[\text{Endothelial junction integrity}\]
- \[\text{EC repair by EPCs: EPC mobilization/recruitment}\]
Transmembrane adhesive proteins at endothelial junctions

ZO-1

VE-Cadherin

Nature Reviews Molecular Cell Biology 5, 261-270
Tight and adherens junction protein

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<th>Nlrp3+/+</th>
<th>Nlrp3-/−</th>
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<td>HFD</td>
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ZO-1

VE-Cadherin

50 μm
Experimental design

- **Nlrp3 genotyping**
  - WT PCR
    - +/+ 330 bp
    - +/- 254 bp
    - -/- 254 bp
  - KO PCR
    - +/+ 254 bp
    - +/- 254 bp
    - -/- 254 bp

- **ND or HFD 6 weeks**
  - Nlrp3+/+ or Nlrp3-/-

- **Endothelial inflammasome activation**
- **Ultrasound imaging**: coronary artery diameter and blood flow
- **Endothelium-dependent vasodilation in isolated coronary arteries**
- **Endothelial junction integrity**
- **EC repair by EPCs**: EPC mobilization/recruitment
EPC in EC repair

- Pro-angiogenic factors
- Oestrogen
- NO bioavailability

- Oxidative Stress
- Inflammation
- Ang II
- ox-LDL
- Telomere shortening

- Impaired EPC mobilisation/generation
- Circulating EPCs
- Impaired function
  - EPC Senescence
  - Antioxidant capacity

- Impaired EPC migration and homing to sites of vascular injury
  - Pro-angiogenic factors
  - Altered HSPG structure

- Injured endothelium
**Nlrp3 inflammasome in EPC mobilization**

EPC markers: CD133+, KDR+ (Flk1+; VEGFR2+)

![EPC image](image)

**Circulating CD133+/KDR+ EPCs (%)**

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<th>HFD</th>
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- *: Significant difference
- #: Significant difference compared to ND

**Plot2: Gated by: Gate 1**
Nlrp3 in adhesion protein expression in arterial endothelium

Colocalization coefficient (PCC)

NDHFD

VWF / VCAM-1

Nlrp3+/+ Nlrp3+/-

ND

HFD

Nlrp3+/+ Nlrp3+/-

* *
Summary

- Nlrp3 is involved in HFD-induced impairment on endothelium-dependent vasodilation in coronary arteries.

- Nlrp3 is needed for in HFD-induced junction disruption in coronary arteries.

- Nlrp3 activation impairs EPC-mediated repair by inhibiting EPC mobilization.

- These actions are distinct from canonical inflammatory responses such as inflammatory cell recruitment and infiltration in the vasculature.

- It is proposed that these uncanonical actions initiates or exacerbates endothelial dysfunction in coronary arteries contributing to vascular inflammation and sclerosis.
Coronary risk factors (FFA, adipokines, free cholesterol, etc.)

Nlrp3 inflammasome activation

- Impaired vasomotor response
- EC junction disruption
- Impaired EPC repair

Vascular injury

Vascular Inflammation

IL1β

CVD (Atherogenesis)
Clinical Significance

- The current clinical practice shows that the only use of anti-inflammatory therapy alone (IL1R antagonism) has low efficiency in treatment and prevention of cardiovascular diseases, particularly, coronary atherosclerosis.

- Clarification of the roles of Nlrp3 inflammasomes beyond canonical inflammation in endothelial dysfunction and vascular injury and related molecular mechanisms will help identify novel targets for development of more efficient therapies for such early stage injury preventing atherosclerosis.
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