Roles of an HDL-Associated Anti-inflammatory Protein, Progranulin, in Atherosclerosis and Acute Coronary Syndrome

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COI Disclosure
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Reverse Cholesterol Transport

**Liver**
- **ApoA-Ⅰ**
- **ABCA1**
- **Discoidal HDL**
- **LDL receptor**
- **SR-BI**

**Intestine**
- **ABCA1**
- **ApoA-Ⅰ**

**Atheroma (Foam cell)**
- **ApoB-containing lipoprotein (VLDL, IDL, LDL)**
- **LDL receptor**
- **SR-BI**
- **CE**
- **TG**
- **CE**
- **CETP**

**Small HDL**
- **LCAT**

**Large HDL**
- **LCAT**

**Discoidal HDL**
- **ABCA1**

**LCAT**

**ABCG1**
Effects of Torcetrapib in Patients at High Risk for Coronary Events

◆ Death from Any Cause

Days after Randomization

Patients without Event (%)

100 99 98 97 96 95

0

HR=1.58 (95% CI: 1.14-2.19)
p=0.006

◆ Primary composite outcome (Major Cardiovascular Events)

Days after Randomization

Patients without Event (%)

100 99 98 97 96 95

0

HR=1.25 (95% CI: 1.09-1.44)
p=0.001

Anti-atherogenic Actions of HDL

- Cellular cholesterol efflux & reverse cholesterol transport
- Anti-inflammatory activity
- Anti-diabetic
- Anti-oxidative activity
- Anti-apoptotic activity
- Anti-infectious activity
- Anti-thrombotic activity
- Endothelial repair
- Vasodilatory activity

Functions of HDL-Associated Proteins

Lipid Metabolism

- CETP
- LCAT
- ApoC-I
- ApoC-II
- ApoC-III
- ApoC-IV
- PON1
- PON3
- SAA1
- SAA2
- SAA4

Proteinase Inhibitor

- AGT
- SERF2
- SERF1

Complement Regulation

- C3
- C4A
- C4B
- C9
- VTN

Acute Phase Response

- ORM2
- TTR
- ITIH4
- RBP4
- TF
- FGA
- HPX
- AMP
- KNG1
Functional HDL and Dysfunctional HDL

“Functional HDL”  “Dysfunctional HDL”

Quality is more important than Quantity?
Composition of HDL is important for playing its proper role?

(Ansell et al, JACC 2005; 10: 1792-1798)
Effects of HDL Obtained from Healthy Subjects, Patients with CAD or Acute Coronary Syndrome on NO Release from Human Aortic Endothelial Cells

Etiology of Dysfunctional HDL

Systemic inflammation / Oxidative stress
- Infection
- Coronary disease
- Diabetes mellitus
- Metabolic syndrome
- Smoking
- Rheumatic conditions
- Chronic kidney disease
- Surgery
- Obstructive sleep apnea
- Saturated fat diet

Chronic acute phase response

Proinflammatory HDL

LDL oxidation

Vascular inflammation

Reverse cholesterol transport

Anti-inflammatory HDL

HDL Cholesterol Efflux Capacity and Incident Cardiovascular Events

<table>
<thead>
<tr>
<th>Models</th>
<th>No. of Participants with Event/Total No. of Participants</th>
<th>Hazard Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HDL cholesterol</strong></td>
<td>132/2416</td>
<td></td>
</tr>
<tr>
<td>Unadjusted analysis</td>
<td></td>
<td>0.64 (0.40–1.03)</td>
</tr>
<tr>
<td>Analysis adjusted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>For traditional risk factors</td>
<td></td>
<td>0.80 (0.47–1.37)</td>
</tr>
<tr>
<td>For traditional risk factors and HDL particle concentration</td>
<td></td>
<td>1.08 (0.59–1.99)</td>
</tr>
<tr>
<td><strong>Cholesterol efflux capacity</strong></td>
<td>132/2416</td>
<td></td>
</tr>
<tr>
<td>Unadjusted analysis</td>
<td></td>
<td>0.44 (0.27–0.73)</td>
</tr>
<tr>
<td>Analysis adjusted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>For traditional risk factors</td>
<td></td>
<td>0.30 (0.18–0.50)</td>
</tr>
<tr>
<td>For traditional risk factors and HDL cholesterol</td>
<td></td>
<td>0.31 (0.18–0.52)</td>
</tr>
<tr>
<td>For traditional risk factors and HDL particle concentration</td>
<td></td>
<td>0.34 (0.20–0.56)</td>
</tr>
<tr>
<td>For traditional risk factors, HDL cholesterol, and HDL particle concentration</td>
<td></td>
<td>0.33 (0.19–0.55)</td>
</tr>
</tbody>
</table>
HDL/Apolipoprotein A-I Binds to Macrophage-Derived Progranulin and Suppresses its Conversion into Proinflammatory Granulins


Internal amino acid sequencing after V8 endopeptidase treatment

Progranulin
HDL/Apolipoprotein A-I Binds to Macrophage-Derived Progranulin and Suppresses Its Conversion into Proinflammatory Granulins

- Progranulin derived from macrophages is bound to Apolipoprotein A1

- Granulin, which is the cleaved product of progranulin, released proinflammatory cytokines from macrophages

- Progranulin associated with apolipoprotein A1 might suppress the conversion into proinflammatory granulin, leading to inhibition of inflammation

Okura H et al: Journal of Atherosclerosis and Thrombosis 2010
Progranulin is a multifunctional protein.

**Structures**

- P
- G
- F
- B
- A
- C
- D
- E

**Tissue Distribution**

Progranulin ubiquitously expresses in almost all tissues. Expression levels of PGRN are increased in some stress conditions such as hypoxia, acidosis and age.

**Functions**

- Growth factor (neuronal growth factor) | BBRC 1990
- Cancer progression | J Mol Med 2003
- Wound healing | Nat Med 2003
- Systemic inflammation | Cell 2002
Phenotypes of Reduced Progranulin Levels in Humans and Mice

Consequenses of Reduced Progranulin Levels

★ Cause Neurological Diseases (Affected Neurons and Microglia)

- Heterozygous deficiency causes frontotemporal dementia in humans (Nature 2006)
- Homozygous deficiency causes neuronal ceroid lipofuscinosis in mice (Am J Hum Genet 2012)
- Reduced progranulin levels might be a risk factor for Alzheimer disease (JAMA Neurol 2013)

★ Modulate Metabolic Diseases (Affected Adipocytes and Macrophages)

- Homozygous deficiency protects against diet-induced obesity and insulin resistance in mice (Cell Metab 2012)

Trends in Endocrinology & Metabolism 2013
1. Roles of an HDL-associated Anti-inflammatory Protein, Progranulin, in Atherosclerosis
PGRN Was Expressed in Foam Cells of Human Aortic Atherosclerotic Plaques

PGRN Is Co-localized with CD68 to a Greater Extent Than with Smooth Muscle Actin
Deletion of Progranulin Exacerbates Atherosclerosis (1)

ApoE KO

DKO

* $p<0.0005$

Deletion of Progranulin Exacerbates Atherosclerosis (2)


ApoE KO  DKO

Bar: 500μm

# p<0.0001
Deletion of PGRN Leads To Severe Atherosclerosis Despite Lower Plasma Cholesterol Level

A  Total Cholesterol

B  Cholesterol

C  Triglyceride

D  Triglyceride

DKO Mice Exhibit Increased Expression of Proinflammatory Cytokines

A mRNA Expression Levels in the Liver

- **TNF-α**
- **IL-6**
- **IL-1β**
- **MCP-1**
- **IL-10**

Comparisons:
- *P < 0.05 and #P < 0.01 compared with ApoE KO mice

B mRNA Expression Levels in the Aorta

- **TNF-α**
- **IL-6**
- **MCP-1**
- **ICAM-1**
- **VCAM-1**

Comparisons:
- *P < 0.005, compared with ApoE KO mice

Progranulin Suppresses TNF-α-induced Expression of ICAM-1 and VCAM-1 in HUVEC

DKO Mice Exhibit Decreased Expression of Endothelial NOS (eNOS) in the Aorta

<table>
<thead>
<tr>
<th></th>
<th>ApoE KO</th>
<th>DKO</th>
</tr>
</thead>
<tbody>
<tr>
<td>eNOS</td>
<td><img src="eNOS_ApoE_KO.png" alt="Image" /></td>
<td><img src="eNOS_DKO.png" alt="Image" /></td>
</tr>
<tr>
<td>p-eNOS</td>
<td><img src="p-eNOS_ApoE_KO.png" alt="Image" /></td>
<td><img src="p-eNOS_DKO.png" alt="Image" /></td>
</tr>
<tr>
<td>β-actin</td>
<td><img src="%CE%B2-actin_ApoE_KO.png" alt="Image" /></td>
<td><img src="%CE%B2-actin_DKO.png" alt="Image" /></td>
</tr>
</tbody>
</table>

Lack of Progranulin Leads to Accumulation of Excessive Cholesterol in Macrophages

ApoE KO

DKO

A

B

SRA

CD36

ABCA1

ABCG1

Relative values

apoE KO

DKO

Relative values

apoE KO

DKO

Relative values

apoE KO

DKO

Relative values

apoE KO

DKO

Relative values

apoE KO

DKO


*P <0.005, #P <0.05 compared with ApoE KO mice
Lack of Progranulin Altered the Proportion of HDL-Associated Proteins

A

ApoE KO      DKO

PGRN
ApoA-I
PAF-AH
SAA1

B

Cholesterol Efflux Capacity

ApoE KO      DKO

0  0.5  1  1.5  2  2.5  3  3.5  4

C

Paraoxonase Activity

0  0.5  1  1.5

*P < 0.005, #P < 0.05 compared with ApoE KO mice

Systemic inflammation ↑
Inflammation of aorta ↑
TNF alpha ↑
Adhesion molecule ↑
eNOS ↓

Scavenger receptors ↑
ABCG1 ↓
Up take of ox-LDL ↑

Change of component proteins
PAF-AH ↓  SAA1 ↑
Dysfunctional HDL?
anti-oxidative capacity ↓
PGRN Directly Binds to TNFR and Antagonizes TNFα Actions

A

B

C

KO

WT KO

 PBS rhPGRN
Possibility of Receptors Other Than Tumor Necrosis Factor (TNF) Receptors

(Using peritoneal macrophages in TNF receptor 1/2 KO mouse)

Kawase R, Unpublished data

PGRN (ng/ml)

200

p-Akt

0 100

pan-Akt

p-Akt/pan Akt

(ng/ml)
Deletion of progranulin exacerbates atherosclerosis partly through suppressing inflammation.

Progranulin might be a promising therapeutic target for atherosclerotic cardiovascular diseases.
2. Roles of an HDL-associated Anti-inflammatory Protein, Progranulin, in Acute Coronary Syndrome
We hypothesized that progranulin may play some roles in the coronary plaque stability in patients with acute coronary syndrome (ACS)
We enrolled consecutive 51 patients with ACS who underwent emergent PCI at Saiseikai Senri Hospital and 158 controls without CAD

Clinical characteristics of ACS patients

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>51</td>
<td>40</td>
<td>11</td>
</tr>
<tr>
<td>Age (y)</td>
<td>65.6±10.3</td>
<td>62.7±9.1</td>
<td>76.0±7.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.5±3.6</td>
<td>24.7±3.6</td>
<td>23.9±3.5</td>
</tr>
<tr>
<td>Hypertension</td>
<td>57%(29)</td>
<td>53%(21)</td>
<td>73%(8)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>35%(18)</td>
<td>38%(15)</td>
<td>27%(3)</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>53%(27)</td>
<td>53%(21)</td>
<td>55%(6)</td>
</tr>
<tr>
<td>Smoker</td>
<td>67%(34)</td>
<td>80%(32)</td>
<td>18%(2)</td>
</tr>
<tr>
<td>Previous MI</td>
<td>4%(2)</td>
<td>5%(2)</td>
<td>0%(0)</td>
</tr>
</tbody>
</table>

PGRN concentration in peripheral vein in both groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ACS</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>158</td>
<td>51</td>
</tr>
<tr>
<td>male / female</td>
<td>79 / 79</td>
<td>40 / 11</td>
</tr>
<tr>
<td>Age (y)</td>
<td>61.8±13.5</td>
<td>65.6±10.3</td>
</tr>
<tr>
<td>PGRN (ng/ml)</td>
<td>2.9±0.6</td>
<td>3.0±0.5</td>
</tr>
</tbody>
</table>
We obtained peripheral venous blood samples, arterial blood samples as well as the aspirated coronary arterial blood samples obtained from the culprit lesion at the time of emergent PCI.

Serial blood samples were obtained from arrival to at discharge from the hospital and at the later outpatient visit (0h, 2h, 6h, 9h, 12h, 24h, 48h, 72h after emergent PCI and later outpatient visit).

We also underwent immunostaining of aspirated samples.

Osaka Univ. IRB and Saiseikai Senri Hospital IRB approved this study: UMIN000004241
PGRN Was Mainly Expressed in Macrophages

Immunostaining of aspirated samples at the culprit region showed that PGRN was mainly expressed in macrophages.
PGRN Concentration in Coronary Arterial Samples Was Significantly Lower

《Samples at emergent PCI 》

![Graph showing PGRN concentration in different blood samples](image)

- Peripheral venous blood
- Peripheral arterial blood
- Coronary arterial blood obtained from the culprit

*: p<0.05
PGRN Concentration Gradually Increased After PCI

〈Serial blood samples〉

PGRN Concentration Gradually Increased After PCI

Later outpatient visit

* : p<0.05
Progranulin Can Be Cleaved by MMP-9

<table>
<thead>
<tr>
<th>rPGRN</th>
<th>rPGRN</th>
<th>rPGRN</th>
</tr>
</thead>
<tbody>
<tr>
<td>+0.1μM</td>
<td>+0.3μM</td>
<td></td>
</tr>
<tr>
<td>MMP9</td>
<td>MMP9</td>
<td></td>
</tr>
</tbody>
</table>

1st antibody: c-PGRN (Invitrogen)

- progranulin (PGRN)
- PGRN cleavage products
TNF-\(\alpha\)-induced Expression of MMP-9 Is Suppressed by PGRN

\[\text{in THP-1 cells}\]

MMP-9

\[
\begin{array}{cccc}
\text{PGRN (ng/ml)} & 0 & 100 & 200 \\
\text{Relative value} & 1 & 1 & 0.8 \\
\end{array}
\]

\(*: p<0.05\)
PGRN is mainly expressed in macrophages and monocytes in aspiration samples from the culprit lesion.

Compared to peripheral venous and arterial samples, PGRN concentration in the coronary arterial samples was significantly lower in patients with ACS.

PGRN concentration gradually increased after PCI and became highest after 48 hours.

PGRN can be cleaved by MMP-9.

PGRN suppresses expression of MMP-9 in macrophages.
Possible Roles of Progranulin in Acute Coronary Syndrome

《NORMAL STATE》

PGRN

MMP-9

MMP-9

Plaque stability

《ACS》

MMP-9

PGRN cleavage

PGRN

MMP-9

Plaque instability & plaque rupture

Possible Roles of Progranulin in Acute Coronary Syndrome

《NORMAL STATE》

PGRN

MMP-9

MMP-9

Plaque stability

《ACS》

MMP-9

PGRN cleavage

PGRN

MMP-9

Plaque instability & plaque rupture
Various Phenotypes of PGRN Knockout Mice

- MetS/Obesity
- Fatty liver/NASH
- Inflammatory bowel
- Dermatitis
- Arthritis/Osteoporosis
- Atherosclerosis
- Early death
- Tumor

PGRN Deficiency

- High Fat Loading
- ApoE Deficiency

High Fat Loading

ApoE Deficiency
Various Phenotypes of Apo E/Progranulin Double Knockout Mice

Dermatitis  Arthritis  Tumor (Cancer)

Kaplan-Meier

Early Death

Survival

Follow-up (weeks)

ApoE KO  DKO
Anti-inflammatory protein, progranulin, may be involved in the pathogenesis of atherosclerosis and stabilization of vulnerable coronary arterial plaques possibly through inhibition of MMP-9
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