From Biology to Therapy

The biology of PCSK9 in humans
Just LDL-cholesterol or more?

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PCSK9: The Enzyme
Proprotein Convertase Subtilisin Kexin type 9

Endoplasmic Reticulum

pro-domain
D H N S
C-terminal domain

Catalytic domain

Golgi

D H N S
C-terminal domain

Catalytic domain

Secretion

Lambert et al. (2009) Atherosclerosis
PCSK9: The Chaperone (Binds to the LDLR)

PCSK9

Catalytic domain

CHRD

Prodomain

LDLR

EGFA domain

Endocytosis

Seidah et al. (2014) Circ Res
PCSK9 Targets the LDLR to the Lysosome for Degradation

PCSK9-LDLR Binding
Kd= 750±80nM (at pH 7.5)
Kd= 10±1nM (at pH 5.5)

LDLR Conformation
Open (at pH 7.5)
Closed (at pH 5.5) alone
Open (at pH 5.5) bound to PCSK9

Surdo et al. (2011) *EMBO Reports*
PCSK9 Reduces LDLR Cell Surface Expression Dose Dependently

MFI: median fluorescence intensity
* p<0.05, ** p<0.01 vs. condition no PCSK9 (0)

Lambert et al. (2014) J Am Coll Cardiol
PCSK9: A Natural Inhibitor of the LDL Receptor

Statins Increase Circulating PCSK9 Levels in cohorts

Atorvastatin Dose

* p<0.01 vs. 10mg, ** p<0.01 vs. 10, 20mg

Lambert G. Unpublished data.
Emerging Therapy: PCSK9 Inhibitors

PCSK9 in Brief:

- PCSK9 is a natural circulating inhibitor of the LDLR that targets the receptor for degradation following endocytosis.

- PCSK9 and LDLR genes are co-regulated by intracellular cholesterol content and statin treatment.

- Targeting plasma PCSK9 is conceptually a promising approach to lower LDL-C levels in monotherapy as well as on top of statins.
FH: Four monogenic disorders

Class 3 & 4
- 90% ADH-1
- 5% ADH-2
- <1% ARH
- <1% ADH-3

Class 2

Class 1

Cholesterol release

Increased receptor degradation

Increased receptor recycling

Key to symbols:
- LDL
- LDL-receptor
- Clathrin
- LDLRAP1
- PCSK9
(1a) - PCSK9 modulates the FH phenotype

Normolipemic Individuals

Heterozygous FH patient

(Prevalence 1/250)
## Clinical characteristics

<table>
<thead>
<tr>
<th></th>
<th>CONTROLS (n=152)</th>
<th>FH-D206E (n=237)</th>
<th>FH-V408M (n=117)</th>
<th>FH-D154N (n=38)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>32 ± 18</td>
<td>39 ± 15</td>
<td>36 ± 16</td>
<td>39 ± 16</td>
</tr>
<tr>
<td><strong>Sex (M/F %)</strong></td>
<td>43/57</td>
<td>52/48</td>
<td>42/58</td>
<td>50/50</td>
</tr>
<tr>
<td><strong>CVD (%)</strong></td>
<td>0</td>
<td>24</td>
<td>33</td>
<td>23</td>
</tr>
<tr>
<td><strong>DM (%)</strong></td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td><strong>DBP (mmHg)</strong></td>
<td>-</td>
<td>130 ± 22</td>
<td>133 ± 21</td>
<td>129 ± 19</td>
</tr>
<tr>
<td><strong>SBP (mmHg)</strong></td>
<td>-</td>
<td>81 ± 12</td>
<td>80 ± 12</td>
<td>79 ± 11</td>
</tr>
<tr>
<td><strong>TC</strong></td>
<td>4.66 ± 0.96</td>
<td>8.64 ± 1.85*</td>
<td>9.30 ± 1.99†</td>
<td>8.75 ± 2.29*</td>
</tr>
<tr>
<td><strong>TG</strong></td>
<td>1.23 (0.38/2.1)</td>
<td>1.50 (0.48/2.52)</td>
<td>1.37 (0.58/2.15)</td>
<td>1.48 (0.33/2.63)</td>
</tr>
<tr>
<td><strong>HDL-C (mmol/L)</strong></td>
<td>1.28 ± 0.27</td>
<td>1.24 ± 0.40</td>
<td>1.20 ± 0.32</td>
<td>1.24 ± 0.33</td>
</tr>
<tr>
<td><strong>non-HDL-C</strong></td>
<td>3.39 ± 0.95</td>
<td>7.40 ± 1.86*</td>
<td>8.11 ± 2.01†</td>
<td>7.50 ± 2.35*</td>
</tr>
<tr>
<td><strong>LDL-C</strong></td>
<td>2.87 ± 0.86</td>
<td>6.74 ± 1.78*</td>
<td>7.50 ± 1.87†</td>
<td>6.83 ± 2.07*</td>
</tr>
<tr>
<td><strong>apoB (g/L)</strong></td>
<td>0.92 ± 0.26</td>
<td>1.59 ± 0.41*</td>
<td>1.72 ± 0.48*</td>
<td>1.57 ± 0.48*</td>
</tr>
<tr>
<td><strong>apoA-I (g/L)</strong></td>
<td>1.41 ± 0.19</td>
<td>1.18 ± 0.30</td>
<td>1.15 ± 0.32</td>
<td>1.14 ± 0.27</td>
</tr>
<tr>
<td><strong>PCSK9 (ng/mL)</strong></td>
<td>180 (65/357)</td>
<td>368 (111/497)*</td>
<td>411 (120/532)*</td>
<td>389 (95/511)*</td>
</tr>
</tbody>
</table>

* p<0.01 vs. controls
Correlation Studies

NORMOLIPEMIC CONTROLS

$R=0.29$

$p=0.01$

FH (LDLR-D206E)

$R=0.27$

$p=0.02$

FH (LDLR-V408M)

$R=0.34$

$p=0.01$

FH (LDLR-D154N)

$R=0.35$

$p=0.04$
Mechanistic Studies

Control
WT/WT

FH patient
WT/D206E

FH patient
WT/V408M

FH patient
WT/D154N

SKIN FIBROBLASTS

20% FCS
0.5% FCS
0.5% FCS + mevastatin 10ug/mL
0.5% FCS + mevastatin 20ug/mL
0.5% FCS + mevastatin 40ug/mL

rPCSK9
(0, 150, 300 or 600 ng/mL)

LDLr Expression
by FC

FH patient
WT/D154N
PCSK9 Similarly Reduces LDLR Cell Surface Expression in non-FH and HeFH fibroblasts.
PCSK9 levels are increased in asymptomatic FH patients with coronary artery calcifications

<table>
<thead>
<tr>
<th></th>
<th>Agatston Score = 0</th>
<th>Agatston Score &gt; 0</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=63)</td>
<td>(n=98)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>39 ± 1</td>
<td>50 ± 1</td>
<td>3.10^{-9}</td>
</tr>
<tr>
<td>High intensity statin Rx</td>
<td>46%</td>
<td>74%</td>
<td>0.0003</td>
</tr>
<tr>
<td>TC bas. (mg/dL)</td>
<td>343 ± 54</td>
<td>384 ± 74</td>
<td>0.0004</td>
</tr>
<tr>
<td>LDL-C bas. (mg/dL)</td>
<td>267 ± 53</td>
<td>308 ± 70</td>
<td>0.0005</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>44%</td>
<td>56%</td>
<td>0.0009</td>
</tr>
<tr>
<td>PCSK9 incl. (ng/mL)</td>
<td>421 (340-494)</td>
<td>500 (403-578)</td>
<td>0.0028</td>
</tr>
<tr>
<td>TC incl. (mg/dL)</td>
<td>198 ± 35</td>
<td>218 ± 51</td>
<td>0.0086</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.9 ± 3.9</td>
<td>26.6 ± 4</td>
<td>0.0164</td>
</tr>
<tr>
<td>TG incl. (mg/dL)</td>
<td>73 (59-86)</td>
<td>86 (57-128)</td>
<td>0.0195</td>
</tr>
<tr>
<td>Blood Glucose (g/L)</td>
<td>0.87 ± 0.91</td>
<td>0.92 ± 0.12</td>
<td>0.0220</td>
</tr>
<tr>
<td>ApoB incl. (g/L)</td>
<td>1.08 ± 0.26</td>
<td>1.18 ± 0.32</td>
<td>0.0482</td>
</tr>
<tr>
<td>LDL incl. (mg/dL)</td>
<td>127 ± 37</td>
<td>142 ± 49</td>
<td>0.0496</td>
</tr>
<tr>
<td>Lp(a) incl. (mg/dL)</td>
<td>25 (8-67)</td>
<td>34 (13-69)</td>
<td>0.0698</td>
</tr>
</tbody>
</table>
Elevated circulating PCSK9 levels aggravate the hypercholesterolemic phenotype of heterozygous FH patients.

Elevated circulating PCSK9 is an independent predictor of coronary arteries calcifications in heterozygote FH patients.
(1b)- PCSK9 in Homozygote FH

- Normolipemic Individuals
- Heterozygous FH patient (Prevalence 1/250)
- Homozygous FH patient (Prevalence 1/300,000)
- LDLR defective
- LDLR negative
Statins modulate LDLR expression in HoFH Defective but not in HoFH Negative Fibroblasts
PCSK9 modulate LDLR expression in HoFH Defective but not in HoFH Negative Fibroblasts

Lambert et al. (2014) J Am Coll Cardiol
Statins increase whereas PCSK9 reduces LDLR cell surface expression in controls, heterozygote FH and homozygote FH cells carrying at least one receptor defective LDLR allele but not in those isolated from carriers of two receptor negative LDLR alleles.

PCSK9 inhibition will reduce LDL-C levels in all HeFH irrespective of their mutation status and in the majority of HoFH patients.
(2)- PCSK9 in Autosomal Recessive Hypercholesterolemia (ARH)

<table>
<thead>
<tr>
<th></th>
<th>ADH 90%</th>
<th>LBD 5%</th>
<th>ARH &lt; 1%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Homozygous FH</strong></td>
<td>LDLR (n=42)</td>
<td>ARH (n=42)</td>
<td></td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>18.4 ± 3.6</td>
<td>16.3 ± 2.5</td>
<td></td>
</tr>
<tr>
<td>LDLC (mmol/L)</td>
<td>16.9 ± 3.5</td>
<td>14.6 ± 2.4</td>
<td></td>
</tr>
</tbody>
</table>

Key to symbols:
- LDL
- LDL-receptor
- Clathrin
- LDLRAP1
# LDLR expression in ARH lymphocytes

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th></th>
<th></th>
<th>ARH</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nucleus</td>
<td>LDLR (Z stack)</td>
<td>LDLR (central Z)</td>
<td>Nucleus</td>
<td>LDLR (Z stack)</td>
<td>LDLR (central Z)</td>
</tr>
<tr>
<td>w/o Meva</td>
<td><img src="image1" alt="Image" /></td>
<td><img src="image2" alt="Image" /></td>
<td><img src="image3" alt="Image" /></td>
<td><img src="image4" alt="Image" /></td>
<td><img src="image5" alt="Image" /></td>
<td><img src="image6" alt="Image" /></td>
</tr>
<tr>
<td>+ Meva</td>
<td><img src="image7" alt="Image" /></td>
<td><img src="image8" alt="Image" /></td>
<td><img src="image9" alt="Image" /></td>
<td><img src="image10" alt="Image" /></td>
<td><img src="image11" alt="Image" /></td>
<td><img src="image12" alt="Image" /></td>
</tr>
<tr>
<td>+ Meva + PCSK9</td>
<td><img src="image13" alt="Image" /></td>
<td><img src="image14" alt="Image" /></td>
<td><img src="image15" alt="Image" /></td>
<td><img src="image16" alt="Image" /></td>
<td><img src="image17" alt="Image" /></td>
<td><img src="image18" alt="Image" /></td>
</tr>
<tr>
<td>+ Meva + PCSK9 + Alicorumb</td>
<td><img src="image19" alt="Image" /></td>
<td><img src="image20" alt="Image" /></td>
<td><img src="image21" alt="Image" /></td>
<td><img src="image22" alt="Image" /></td>
<td><img src="image23" alt="Image" /></td>
<td><img src="image24" alt="Image" /></td>
</tr>
</tbody>
</table>
LDL uptake in ARH lymphocytes

![Graph showing the internalized LDL (Δ MFI with cell autofluorescence) for different conditions.]

- **CONTROLS**
- **ARH**

<table>
<thead>
<tr>
<th>Meva</th>
<th>-</th>
<th>+</th>
<th>+</th>
<th>+</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCSK9 D374Y (ng/ml)</td>
<td>-</td>
<td>-</td>
<td>600</td>
<td>600</td>
</tr>
<tr>
<td>Alirocumab</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
(2) PCSK9 in Autosomal Recessive Hypercholesterolemia (ARH)

- Statins increase whereas PCSK9 reduces LDLR cell surface expression in lymphocytes isolated from control and ARH patients.

- Cellular LDL uptake is increased by statin treatment and reduced by PCSK9 and increased back to baseline levels by the PCSK9i Alirocumab in ARH lymphocytes but to much lower extents than in control cells.

- These in vitro findings suggest that PCSK9 inhibition may potentially reduce LDL-C in ARH patients currently treated with statins and LDL apheresis.
(3)- PCSK9 in Autosomal Dominant Hypercholesterolemia 3
PCSK9 inhibition for PCSK9 GOF FH patients

A

LDLR cell surface expression (MFI)

Control
Father
Mother
Daughter

B

LDL uptake (MFI)

Mevastatin
PCSK9 D374Y
Alirocumab
(3)- PCSK9 inhibition with mAbs for
FH patients to reduce LDL-C

- ADH1: All heterozygotes (irrespective of their LDLR mutation status)
- ADH1: Most homozygotes (carrying at least one LDLR defective allele)
- ADH2 (LDB): Not known but likely to work for HeFH and HoFH LDB.
- ARH: Cellular “resistance” to statins and PCSK9, but worth to test in patients.
- ADH3 (PCSK9 GOF): Likely to work for all PCSK9 GOF mutations carriers.
(4)- Not just LDL-C… also Lp(a)

PCSK9 inhibition by monoclonal antibody therapy was shown to be very effective at reducing circulating LDL-C and, by unexplained mechanisms, to decrease Lp(a) plasma levels.

- PCSK9 effects on apo(a)/Lp(a) levels synthesis 
  *in human primary hepatocytes*
- PCSK9 effects on Lp(a) cellular catabolism 
  *in primary hepatocytes and dermal fibroblasts*
Not just LDL... also Lp(a)

PCSK9 increased Lp(a) levels in cell media

PCSK9 increased apo(a) gene expression

Lp(a) and apo(a) level in hepatocytes media

n=3 independent experiments

** p<0.01 vs 0 PCSK9; two-way ANOVA on Rank-transformed values

n=7 independent experiments from 3 hepatocytes donors

* p=0.02 on log-transformed data analyzed by one-way ANOVA
PCSK9 did not modify Lp(a)-bodipy uptake in hepatocytes

$n=10$ independent experiments from 2 hepatocyte donors and 2 Lp(a) isoforms at 7μg/mL

*** $p<0.001$ vs basal on log-transformed data analyzed by two-way ANOVA
PCSK9 did not modify Lp(a)-bodipy uptake in fibroblasts

Plasma Lp(a) decrease associated with PCSK9 inhibition in clinical trials may result primarily from impaired Lp(a) production
CONCLUSIONS

- PCSK9 is a natural circulating inhibitor of the LDLR that targets the receptor for degradation following endocytosis.

- Targeting plasma PCSK9 is conceptually a promising approach to lower LDL-C levels particularly for FH patients (HeFH, receptor defective HoFH, PCSK9 GOF, and maybe some ARH patients)

- PCSK9 modulates LDL-C levels via a functional LDLR pathway (requiring ARH). Our in vitro data suggest that PCSK9 may not modulate Lp(a) levels through the same cellular mechanism but rather by impacting Lp(a) production.