Evaluating the roles of PCSK9 and specific receptors in lipoprotein(a) catabolism

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Lp(a) catabolism

- Liver is main site of Lp(a) clearance
  - Cain WJ et al., J Lipid Res 2005;46:2681
- Identity of receptors unclear
  - Plasminogen receptors, LDL-R, VLDL-R, LRP8, SR-B1 have been suggested
    - Hoover-Plow J, Metabolism 2013;62:479
- PCSK9 inhibitors decrease plasma Lp(a) levels ~30%
  - Desai NR et al., Circulation 2013;128:962, Gaudet D et al., Am J Cardiol 2014;114:711
    - Suggests role for LDL-R
      - Notable that statins do not have same effect on Lp(a), possibly because of upregulation of PCSK9
    - Controversy over correlation between extent of Lp(a) and LDL lowering
      - Desai NR et al., Circulation 2013;128:962, Gaudet D et al., Am J Cardiol 2014;114:711
- Our own studies in HepG2 cells indicate PCSK9 decreases Lp(a) internalization via LDL-R
  - Romagnuolo R et al., J Biol Chem 2015;290:11649
PCSK9 regulates Lp(a) internalization by HuH7 hepatocyte cell line

* p< 0.05 compared to control
PCSK9 decreases apo(a) internalization in primary human hepatocytes

<table>
<thead>
<tr>
<th>εACA (mM)</th>
<th>0</th>
<th>0</th>
<th>0</th>
<th>0</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCSK9 (µg/mL)</td>
<td>0</td>
<td>1</td>
<td>10</td>
<td>20</td>
<td>0</td>
</tr>
</tbody>
</table>

17K apo(a)

β-actin
Lp(a) clearance is enhanced in PCSK9\(^{-/-}\) mice
Overexpression of LDL-R-related receptors does not affect Lp(a) internalization

![Graph showing the relative Lp(a) internalized without and with PCSK9 for pCMV6, LRP8, LRP1, and VLDL-R.](image)

<table>
<thead>
<tr>
<th>PCSK9 (µg/mL)</th>
<th>0</th>
<th>20</th>
<th>0</th>
<th>20</th>
<th>0</th>
<th>20</th>
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<tbody>
<tr>
<td>Lp(a)</td>
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<td>β-actin</td>
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</tr>
</tbody>
</table>

* p< 0.05 compared to no PCSK9
Sortilin overexpression enhances Lp(a) internalization in HepG2 cells

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>pcDNA Vector</th>
<th>Sortilin wt</th>
<th>ΔCT</th>
<th>Y792A</th>
<th>L829A / 830A</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCSK9 (μg/mL)</td>
<td>0</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>[Image]</td>
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<td>β-actin</td>
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</tbody>
</table>
Effect of sortilin on Lp(a) internalization is independent of LDLR-R

Experiment conducted in FH fibroblasts lacking functional LDL-R

* p< 0.05 compared to control
Lp(a) does not bind to sortilin, unlike LDL
Sortilin overexpression enhances apo(a) secretion without affecting kinetics of intracellular maturation
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Sortilin overexpression enhances apo(a) secretion without affecting kinetics of intracellular maturation
Conclusions

- PCSK9 inhibits Lp(a) internalization by hepatocytes through the LDL-R
- LRP1, LRP8, VLDL-R do not appear to function as internalization receptors
- Sortilin promotes Lp(a) internalization
  - Effect independent of LDL-R and direct binding of Lp(a)
  - Dependent on carboxyl-terminal domain, in part through sorting motifs
- Sortilin promotes apo(a) secretion
  - Dependent on carboxyl-terminal domain, in part through sorting motifs
  - May prevent presecretory degradation of apo(a)
Funding

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