Cathepsin K Deficiency Suppresses the Development of Experimental Intimal Hyperplasia in Response to Injury

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All other authors have reported that they have no relationships relevant to the contents of this paper to disclose
Atherosclerosis-based vascular disease and its complications

1) Plaque rupture
2) Thrombosis
3) Calcification
4) Restenosis
5) Aneurysm
6) Vasa vasorum

1) Atherosclerosis
2) Vascular injuries
3) Vascular responses
DES-related Restenosis in patients with DM or/and haemodialysis


DES: drug eluting stent; Non-HD, none-haemodialysis
Cats generally known as functioned in lysosomes, were discovered in the half of the 20\textsuperscript{th} century. There are 11 human Cats (B,C,F,H,K,L,O,S,V,W,and X) that belong to papain subfamily of cysteinyi proteases. Cystatin C (CystC) is one of the major endogenous Cat inhibitors.

Previously, It has been reported that Cats degrade apoptosis-related molecules such as Bcl-2, Bcl-xL, and Mcl-1, and contribute cell apoptosis.
CatK expression in the balloon-injured carotid artery and failing myocardium of rat

However, we found that the role of individual cathepsin for vascular remodeling and restenosis in response to injury is poorly understood.
The present study explored the possibility that CatK deficiency suppresses vascular remodeling and restenosis in response to injury, focusing on vascular smooth muscle cell (SMC) apoptosis and proliferation in mouse carotid artery injury model.
Murine carotid artery injury model

Exp: Protocol (1)

- Birth
- 9 weeks
- 13 weeks
- Day 0
- Day 7
- Day 28

Injuries
- Sampling

1. CatK^{+/+} ligation \((n = 15)\)
2. CatK^{-/-} ligation \((n = 16)\)
3. CatK^{+/+} ligation + cuff \((n = 14)\)
4. CatK^{-/-} ligation + cuff \((n = 13)\)

Methods
- Morphological analysis
- Immunohistochemistry
- Quantitative real-time PCR
- ELISA etc.
CatK\(^{-/-}\) reduces neointimal formation in response to injuries

CatK\(^{+/+}\)

CatK\(^{-/-}\)

Ligation

Ligation + Cuff

Neointima area (of 10\(^3\)/μm\(^2\))

P < 0.0001

P = 0.0003

P < 0.0001

P = 0.022

Ligation

Ligation+cuff

Ligation

Ligation+cuff
CatK^−/− reduces medial SMC proliferation and apoptosis response to injuries

CatK^−/− reduces medial SMC proliferation and apoptosis response to injuries.
CatK\(^{-/-}\) impairs aorta-derived SMC sprouting and invasion of SMC and macrophage.
CatK\(^{-/-}\) reduces superoxide production in response to both injuries

\[ L = \text{ligation} \]
\[ L+C = \text{ligation + cuff} \]
CatK\(^{-/-}\) reduces aortic SMC apoptosis induced hydrogen peroxide

CatK\(^{-/-}\) II = CatK specific inhibitor

* P < 0.001 vs control; † p < 0.05 vs corresponding control
Exp: Protocol (2)

- Morphological analysis
- Immunohistochemistry
- Quantitative real-time PCR
- ELISA etc.

<table>
<thead>
<tr>
<th>Event</th>
<th>Description</th>
<th>Sample Size</th>
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</thead>
<tbody>
<tr>
<td>① Injuries</td>
<td>E64d ligation + cuff</td>
<td>n = 12</td>
</tr>
<tr>
<td>② Injuries</td>
<td>E64d ligation + cuff</td>
<td>n = 11</td>
</tr>
</tbody>
</table>

Sampling points:
- Day 0
- Day 7
- Day 28
Efficacy of E64d on neointimal formation and SMC apoptosis and proliferation

E64d = non-specific CatK inhibitor

† p < 0.05 vs control
Exp: Protocol (3)

Methods
- Morphological analysis
- Immunohistochemistry
- Quantitative real-time PCR
- ELISA etc.

1. CystC\(^{+/+}\) ligation + cuff ($n = 14$)
2. CystC\(^{-/-}\) ligation + cuff ($n = 13$)
CystC\(^{-/-}\) reduces neointimal formation and SMC apoptosis
Observations

跚 On operative day 28, CatK deficiency significantly reduced neointimal formation in both single- and double-injured arteries as compared with corresponding control Cat K+/+ mice.

跚 At day 7, CatK deficiency reduced lesion macrophage content, medial cell proliferation and apoptosis, the mRNA levels of TLR-4, CCL12, and gelatinolytic activities of MMP-2 and -9, and increased the mRNA levels of TIMP-1 and -2.

跚 E64d decreased neointimal lesion formation and medial SMC apoptosis and proliferation.

跚 Cystatin C deficiency enhanced lesion macrophage content, neointimal lesion, medial cell proliferation and apoptosis.

跚 CatK deficiency reduced SMC or/and macrophage invasion proliferation, apoptosis.
Proposed mechanism

Injuries

$\text{O}_2^-$

Cats

SMC Apoptosis

SMC proliferation

Restenosis
Conclusion

This study demonstrates an essential role of Cat K in atherosclerotic neointimal formation in response to injury, possibly via the reduction of SMC apoptosis and proliferation associated inflammation and stress, suggesting a novel therapeutic strategy for the control of vascular intervention-related restenosis by regulating Cat K activity.
7 November 2011

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