INCREASED EXPRESSION OF THE OLIGOPEPTIDASE THOP1 IS A NEUROPROTECTIVE RESPONSE TO AMYLOID BETA TOXICITY

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Identification of THOP1 as a protein differentially modulated by Aβ25-35 in cortical neurons

Primary rat cortical neurons

50 μM Aβ 25-35

control, 5 min treatment, 4 hr treatment

Purification of phosphoproteins

2D gel analysis

Mass spectrometry
Identification of THOP1 as a protein differentially modulated by Aβ25-35 in cortical neurons (G. Pollio et al., Neurobiology of Disease 31, 2008)

2D gel analysis:

Profile of THOP1 containing spot after 2D gel analysis (repeated experiments):

* 1.21 fold change, P<0.05
two-way-ANOVA, FDR adjusted

mRNA expression levels of THOP1 after Aβ treatment:
THOP1: Thimet oligopeptidase 1 (EC 3.4.24.15)

- Alternative names: Endopeptidase 24.15, MP78
- Lenght: 689 aa, Mass: 78 kDa
- Belongs to the family of zinc metalloendopeptidases
- THOP1 is a Zn$^{2+}$-dependent enzyme that preferentially cleaves peptides 6–17 amino acids in length
- Relative high expression levels in brain, pituitary and testes
- Localization in cytosol, but also less predominantly in nuclear fractions and extracellular space
THOP1 context map generated using CellDesigner

G. Pollio et al., Neurobiology of Disease 31 (2008)
THOP1 is involved in proteolysis of pre-antigens after proteasomal degradation

THOP1 limits antigen presentation by MHCI by breakdown of antigenic peptides (Kim et al., 2003; York et al., 2003)

→ Suggested role for THOP1 in regulation of MHCI expression in immune-privileged sites, like the brain
THOP1 is involved in the metabolism of neuropeptides: bradykinin, neurotensin and gonadotropin-releasing hormone.

G. Pollio et al., Neurobiology of Disease 31 (2008)
THOP1 is present in neurofibrillary tangles and senile plaques

**THOP1 immunohistochemistry**
Conn et al., 1996

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G. Pollio et al., Neurobiology of Disease 31 (2008)
THOP1 indirectly degrades Aβ by activating serine proteases

G. Pollio et al., Neurobiology of Disease 31 (2008)

THOP1 mediated Aβ degradation is inhibited by α1-antichymotrypsin
Yamin et al., 1999

α1-antichymotrypsin immunohistochemistry
Rozemuller et al., 1991
THOP1 immunohistochemistry in nondemented-control and Alzheimer’s disease brain (G. Pollio et al., Neurobiology of Disease 31, 2008)
THOP1 immunohistochemistry in nondemented-control and Alzheimer’s disease brain (G. Pollio et al., Neurobiology of Disease 31, 2008)
THOP1 colocalizes with phospho-Tau (AT8)

G. Pollio et al., Neurobiology of Disease 31 (2008)
THOP1 colocalizes with ubiquitin
THOP1 and Aβ colocalize in the temporal cortex of AD patients
THOP1 protein levels are increased in Alzheimer disease brain compared with nondemented control brain (G. Pollio et al., Neurobiology of Disease 31, 2008)

Western blot analysis on protein lysates of temporal cortex

**AD vs CON**

**Pathology**

* P<0.05, ANOVA
THOP1 and Alzheimer disease

Provisional conclusions

- This study confirms the association of THOP1 with neurofibrillary tangles, neuropil threads, and senile plaques.

- THOP1 is closely associated with the neuritic changes and accumulation of ubiquitin in AD neurodegeneration.

- Protein levels of THOP1 are increased in the early pathological stages of AD (Braak stage III-IV).
Role of THOP1 in AD pathogenesis

*Ability of THOP1 to degrade the beta-amyloid precursor protein and generate amyloidogenic fragments?*

- THOP1 has been suggested to have β-secretase activity demonstrated by peptide cleavage, but was unable to cleave membrane bound APP (Brown et al., 1996; Koike *et al*., 1999).
- THOP1 is indirectly involved in the pathway leading to the degradation of Aβ (Yamin *et al*., 1999).

*How is THOP1 involved in amyloid driven pathology in AD?*
THOP1 protects against Aβ neurotoxicity in primary rat cortical neurons (G. Pollio et al., Neurobiology of Disease 31, 2008)

Effects on neuronal viability after treatment with 25 μM Aβ25-35 (48 hrs)

THOP1 overexpression protects against Aβ toxicity

THOP1 silencing results in increased Aβ toxicity

* P<0.05, ANOVA

Expression construct

Concentration siRNA
THOP1 protects against Aβ neurotoxicity in primary rat cortical neurons (G. Pollio et al., Neurobiology of Disease 31, 2008)

Effects on neurite length after treatment with 25 μM Aβ25-35 (48 hrs)

Expression after co-transfection

THOP1 overexpression

Expression construct
THOP1 increases with age in TgCRND8 APP transgenic mice
(G. Pollio et al., Neurobiology of Disease 31, 2008)

TgCRND8: APP Swedish (K670M/N671L) and Indiana (V717F) mutations

THOP1 protein levels in the hippocampus

N= 4-5 in each group, * P <0.05, ANOVA
THOP1 immunoreactivity in CA1 neurons and plaques in TgCRND8 mice
(G. Pollio et al., Neurobiology of Disease 31, 2008)
THOP1 and Aβ_{1-42} colocalize in the neocortex (Ctx) and hippocampus (CA1) in 12-month-old TgCRND8 mice
(G. Pollio et al., Neurobiology of Disease 31, 2008)
**Conclusions**

- THOP1 protects neurons from Aβ toxicity in vitro
- A prominent increase in THOP1 immunoreactivity is observed in amyloid plaques in APP transgenic mice
- Protein levels of THOP1 are increased in the early pathological stages of AD and associated with amyloid deposits.
THOP1 and Alzheimer disease

Relative occurrence

No cognitive impairment  Transitional phase  Alzheimer's disease

AD pathology

0  I-II  III-IV  V-VI

Amyloid beta deposition
THOP1
Glial response
Tangles

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Prague, March 10, 2009
THOP1 and Alzheimer disease: hypothesis based on current data

THOP1

? Neuroinflammation

MHC class I

THOP1

inactive multimer (accumulates with tau and ubiquitin)

Aβ degradation

α1-antichymotrypsin (increased expressed in AD)

Serine proteinases

α1-antichymotrypsin

Aβ degradation

MHC class I

THOP1

α1-antichymotrypsin

inactive multimer (accumulates with tau and ubiquitin)