行政院國家科學委員會補助專題研究計畫■ 成 果 報 告 □ 期中進度報告

樣澱粉β引發腦血管內皮細胞基質金屬蛋白酵素表現之探討

Amyloid β-induced matrix metalloproteinases expression in cerebral endothelial cells

計畫類別：□ 個別型計畫　　■ 整合型計畫
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執行單位：台北醫學大學醫學研究所

中　華　民　國　　95　年　　11　月　　26　日

英文摘要。

關鍵字：Alzheimer's disease (AD), amyloid β peptide (Aβ), cerebral amyloid angiopathy (CAA), cerebral endothelial cells (CECs), matrix metalloproteinase-9 (MMP-9)

The amyloid β peptide (Aβ) has been linked to both neuronal and vascular degeneration in Alzheimer's disease (AD). Amyloid deposition in cerebral vessels (cerebral amyloid angiopathy, CAA) is also a major cause of hemorrhagic and ischemic stroke in the elderly with or without AD. Matrix metalloproteinases (MMPs), a group of enzymes that regulate cell-matrix composition, has been implicated in various diseases including arthritis, atherosclerosis and tumor progression and metastasis. MMP-9 (gelatinase B) has received considerable attention recently because of its role in the pathogenesis of hemorrhagic transformation after cerebral ischemia. Recently, we examined the potential role of the MMP-9 in the pathogenesis of cerebral amyloid angiopathy (CAA), and the results suggested that the Aβ-induced incretion of vascular MMP-9 expression may play a role in the pathogenesis of spontaneous intracerabral hemorrhage in patients with CAA. We also demonstrated that MMP-9 can degrade Aβ fibrils (fAβ) and may contribute to extracellular brain Aβ clearance by promoting Aβ catabolism.

中文摘要。

關鍵字：阿茲海默式症、樣澱粉β胜肽、腦血管樣澱粉病變、腦內皮細胞、基質金屬蛋白酵素-9。

樣澱粉β胜肽的沈積是造成阿茲海默症與腦血管樣澱粉病變的主要成因。目前樣澱粉β對腦神經傷害之研究已累積許多，但其對腦血管內皮細胞之作用機制仍有待研究。基質金屬蛋白酵素-一群調控細胞外基質組成的酵素-與關節炎、粥狀冠狀動脈硬化、腫瘤之轉移與擴散之病理機制有關，其中基質金屬蛋白酵素-9 又被證實與中風性出血有關，故本計畫旨在探討樣澱粉β對小鼠腦內皮細胞之基質金屬蛋白酵素-9 表現與活性影響為何。本計畫結果證實經樣澱粉β處理後，腦內皮細胞之基質金屬蛋白酵素-9 表現量與活性皆顯著增加、 此增加為樣澱粉β刺激轉錄因子 AP-1 與 SP-1 的活化結果、基質金屬蛋白酵素-9 可分解樣澱粉β沉積所形成的 fibrils 與 plaques 以加速細胞外樣澱粉β的清除率。

報告內容[前言及文獻探討、研究目的、研究方法、結果與討論(含結論與建議)]

前言及文獻探討

The PI of this PPG, CY Hsu, has devoted his recent research effort to delineate the molecular mechanism of Aβ induced death of CECs and other cellular components in CNS. Recent publications on this topic and related fields are listed below:

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基質金屬蛋白酵素 (matrix metalloproteinase；MMP)

基質金屬蛋白酵素主要是一群調控細胞外基質組成的酵素，其受鋅離子活化後可切割一種或多種細胞外基質成分。這些蛋白酵素擁有一些共同的結構：一個 propeptide domain 和一個 catalytic domain。其中的 catalytic domain 包含一個 zinc binding site 和一個 conserved methionine (Massova *et al.*, 1998)。依據其所分解的細胞外基質種類可分為下列四大類：collagenase、gelatinase、stomelysins 與 matrilysins。其正常生理功能在於胚胎發育、器官成形、囊胚植入子宮壁、排卵、毛囊生長以及骨骼重組等 (Parks & Mecham, 1998)。然而，過量的基質金屬蛋白酵素則與多種疾病之生成有關，包括關節炎 (arthritis)、粥狀冠狀動脈硬化 (atherosclerosis)、腫瘤之轉移與擴散 (tumor progression and metastasis) (Nagase & Mecham, 1999)。基質金屬蛋白酵素-9 (MMP-9)又名 gelatinase B，其被分泌時為不活化之酵素前質 (proenzyme)。已知 MMP-9 與許多疾病隻病理生成機制有關，如：粥狀冠狀動脈硬化 (atherosclerosis) (Galis & Khatri, 2002)、腹部主動脈瘤 (abdominal aortic aneurysm) (Pyo *et al.*, 2000)以及中風後出血 (hemorrhagic transformation after ischemic stroke) (Lapchak *et al.*, 2000)。MMP-9 promotors 的活化是受到生長因子、細胞素 (cytokines)和腫瘤促進物質透過細胞內訊息傳遞過程而調控。目前已知 NF-κB 刺激 MMP-9 的表現 (Crowe *et al.*, 2001)、干擾素透過 STAT 與 IRF1 擷抗 NF-κB 以抑制 MMP-9 的表現 (Sanceau *et al.*, 2002)、活化 c-fos 會抑制 AP-1 (-79 bp)進而抑制 MMP-9 的表現 (Crowe & Brown, 1999)、JNK 則透過 c-jun 活化 AP-1 (-79 bp)而增加 MMP-9 的表現 (Crowe *et al.*, 2001)。但樣澱粉β如何刺激腦內皮細胞表現 MMP-9 仍不清楚，故此部分實驗將探討轉錄因子的活化的重要性。

研究方法與結果

Figure 1

1. Amyloid β peptide (Aβ) induces the synthesis and activation of matrix metalloproteinase (MMP)-9 in cerebral endothelial cells (CECs) and C6 glioma cells by Western blotting and gelatin-substrate zymography.
2. Aβ induces extracellular matrix (ECM) degradation in CECs.
3. Vascular matrix metalloproteinase-9 (MMP-9) colocalizes with amyloid β peptide (Aβ) and microhemorrhage in aged APPsw mice.

Figure 2

1. Transcription inhibitor (actinomycin D, Act-D) and translation inhibitor (cyclohexamide, CHX) attenuate Aβ-induced MMP-9 expression.
2. Inhibitors of AP-1 (curcumin, Cur) and SP-1 (mithramycin A, MMA) decreases Aβ-induced MMP-9 expression.
3. Levels of MMP-9 expression upon Aβ treatment are not attenuated by inhibitors of NF-κB.

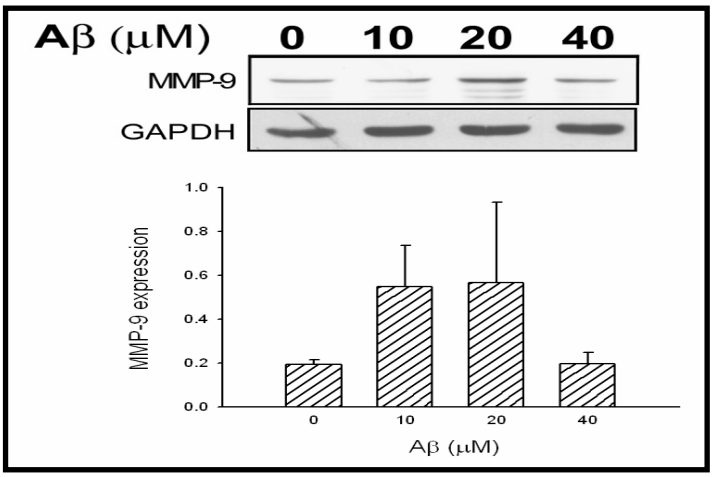
Figure 3

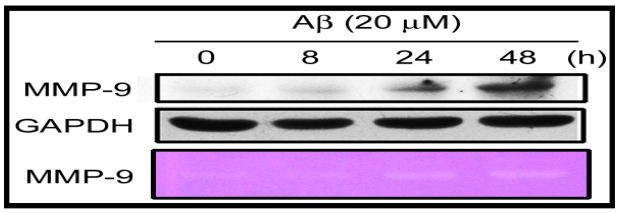
1. Mass spectra of soluble $A\beta_{1-42}$ digested with MMP-9.
2. To visualize ultrastructural changes in Aβ fibrils after incubation with Pro-MMP-9 and MMP-9, we performed transmembrane electoral microscopy (TEM) on preformed fAβ incubated in buffer with or without the MMP-9. Incubation of fAβ with activated MMP-9, fibrils were less abundant, and some amorphous globular structures were observed.
3. To explore the potential mechanism of fibril disruption by MMP-9, we determined whether Aβ fragments were released by MMP-9 digestion of fAβ. Aβ fragments generated by incubating purified fAβ with MMP-9 were isolated and analyzed by MALDI-TOF MS. MMP-9 produced Aβ fragment with molecular masses of 2461.68 and 3390.59 daltons corresponding to $A\beta_{1-20}$ and $A\beta_{1-30}$.
4. We examined MMP-9 expression in the brains of aged APPsw, APP/PS1, and wild-type littermate mice. Aged wild-type mice demonstrated a few isolated cells with MMP-9 immunoreactivity localized primarily in the corpus callosum, while APPsw mice had many more cells with prominent MMP-9 immunostaining throughout the brain. Double staining with ThS revealed that many of the MMP-9 immunoreactive cells appeared to surround ThS-positive compact plaques. Moreover, the cells had the appearance of activated astrocytes, based on their hypertrophic cell bodies and immunoreactivity with anti-GFAP antibodies.

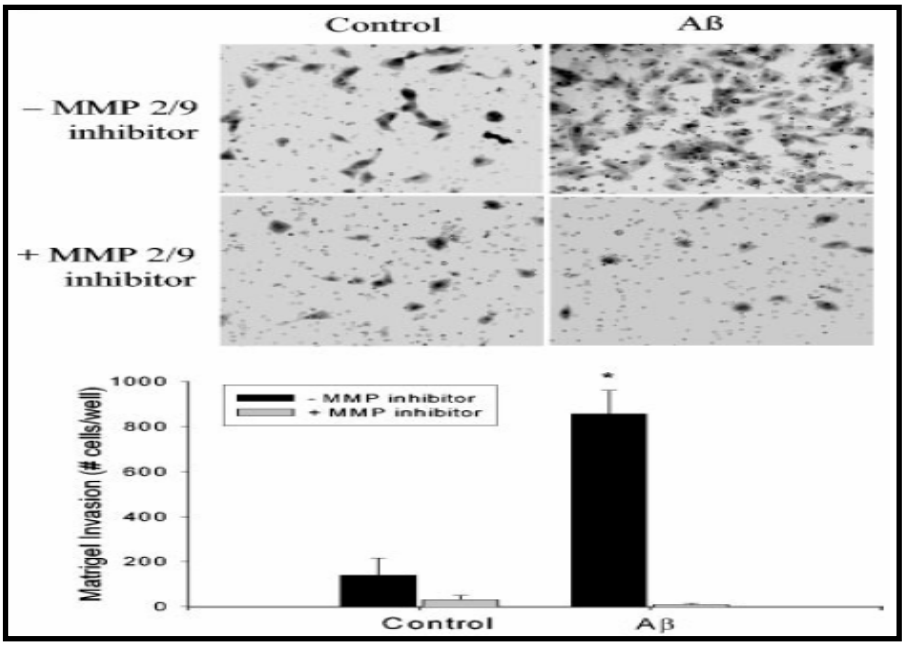
Figure 4

1. MMP-2 and -9 immunoreactivity is selectively increased in activated astrocytes surrounding amyloid plaques in aged APP/PS1 mice.
2. MMP-2 an- 9 mRNA levels are elevated in astrocytes surrounding amyloid plaques in aged APP/PS1 mice.
3. Astrocytes secrete Aβ-degrading activity in vitro producing characteristic Aβ fragments.
4. Aβ-degrading activity in ACM is mediated in part by MMP-2 and -9.
5. *mmp-2* and -9 gene deletion alters steady-state Aβ levels in mouse brain.
6. Basal expression of MMP-2 and -9 in mouse brain.

Fig. 1


Aβ (μM) 0 10 20 40
MMP-9
GAPDH
MMP-9 expression
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
40
Aβ (μM)


Aβ (20 μM)
0 8 24 48 (h)
MMP-9
GAPDH
MMP-9


Control
Aβ
– MMP 2/9 inhibitor
+ MMP 2/9 inhibitor
Matrigel Invasion (# cells/well)
1000
800
600
400
200
0
- MMP inhibitor
+ MMP inhibitor
*
Control
Aβ

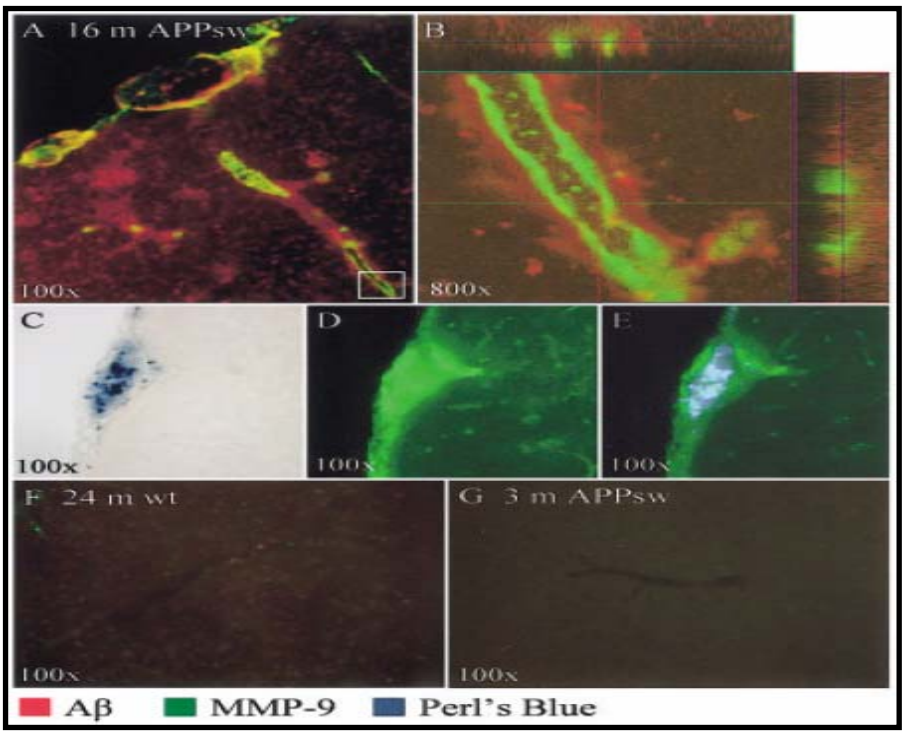
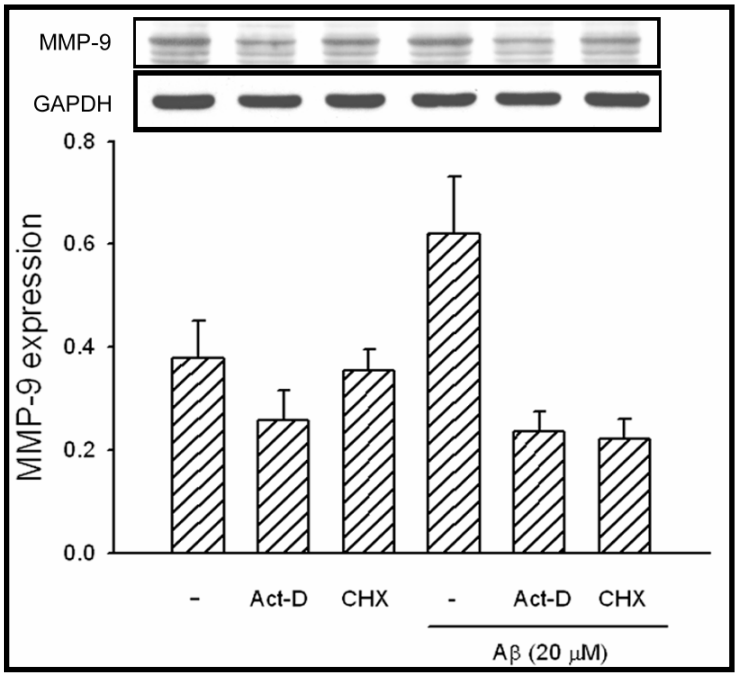
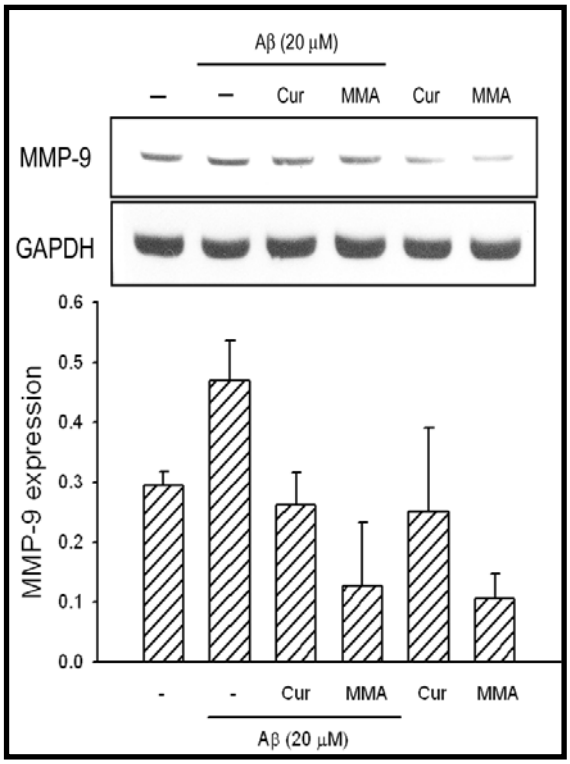

A 16 m APPsw
B
100x
800x
C
D
E
100x
100x
100x
F 24 m wt
G 3 m APPsw
100x
100x
Aβ
MMP-9
Perl's Blue

Fig. 2


MMP-9
GAPDH
MMP-9 expression
0.8
0.6
0.4
0.2
0.0
-
Act-D
CHX
-
Act-D
CHX
Aβ (20 μM)


Aβ (20 μM)
-
-
Cur
MMA
Cur
MMA
MMP-9
GAPDH
MMP-9 expression
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-
-
Cur
MMA
Cur
MMA
Aβ (20 μM)

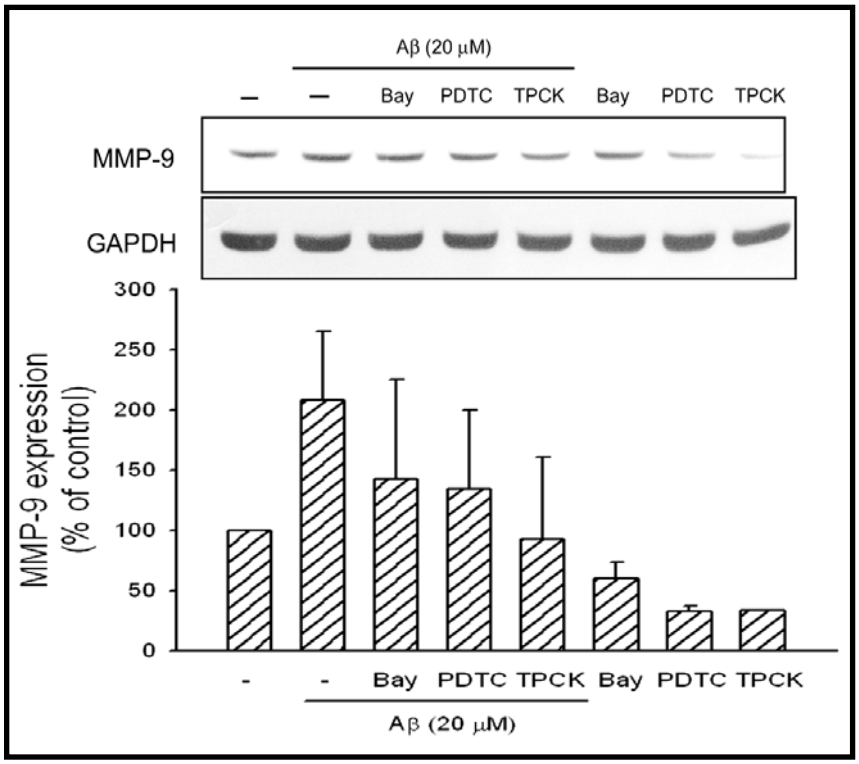
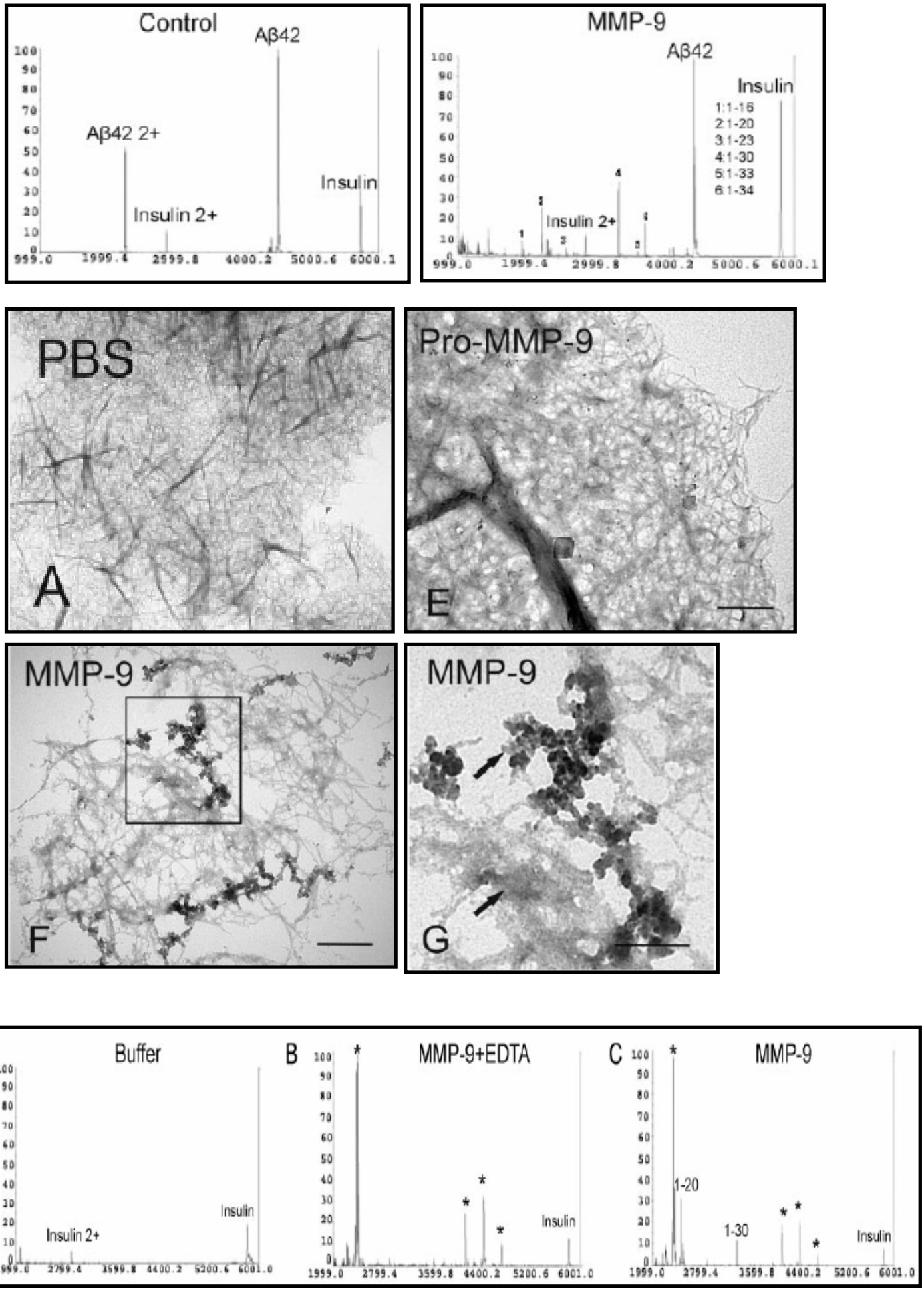

Aβ (20 μM)
-
-
Bay
PDTC
TPCK
Bay
PDTC
TPCK
MMP-9
GAPDH
MMP-9 expression
(% of control)
300
250
200
150
100
50
0
-
-
Bay
PDTC
TPCK
Bay
PDTC
TPCK
Aβ (20 μM)

Fig. 3


Control
Aβ42
Aβ42 2+
Insulin
Insulin 2+
MMP-9
Aβ42
Insulin
1:1-16
2:1-20
3:1-23
4:1-30
5:1-33
6:1-34
Insulin 2+
PBS
A
Pro-MMP-9
E
MMP-9
F
MMP-9
G
A
Buffer
Insulin 2+
Insulin
B
MMP-9+EDTA
Insulin
C
MMP-9
1-20
1-30
Insulin

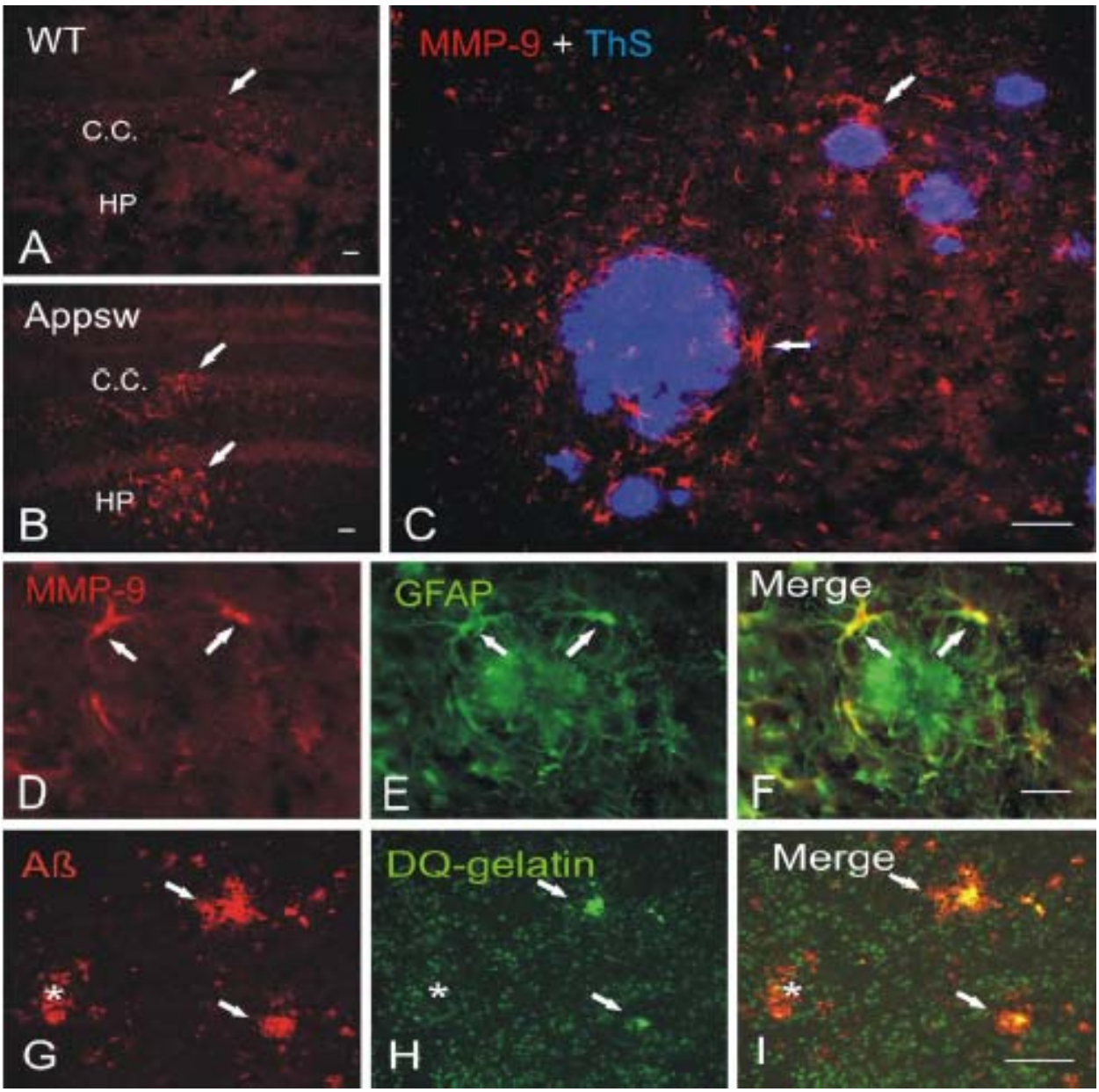
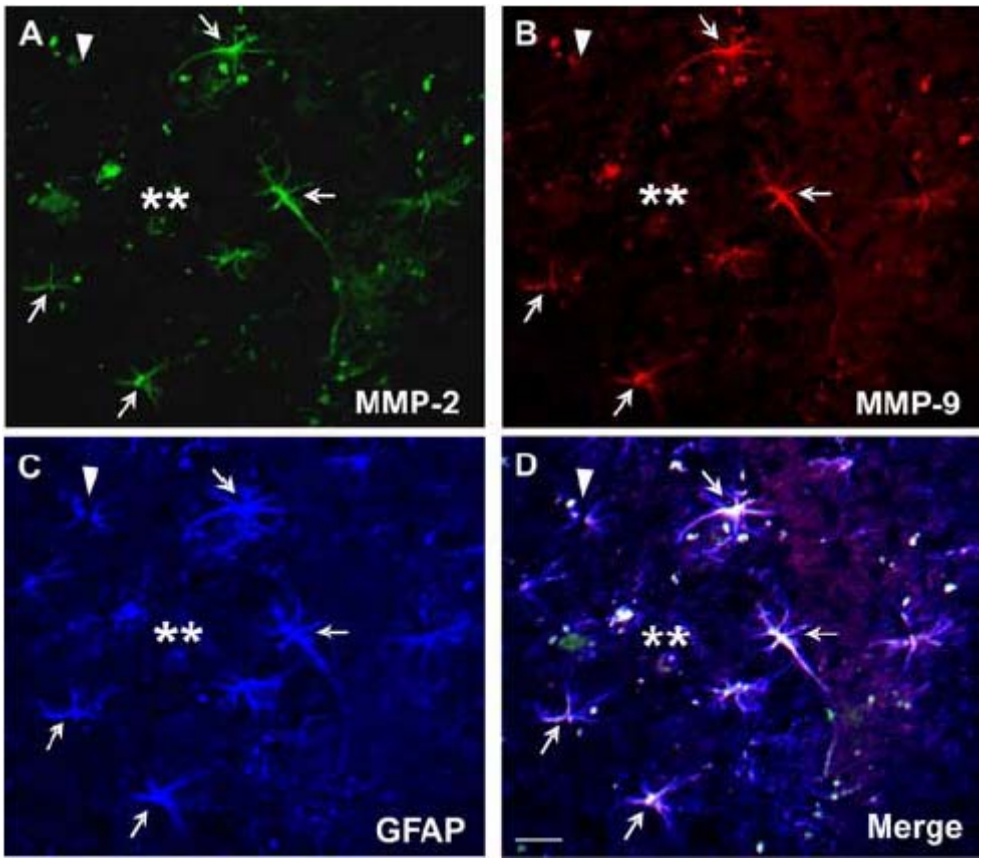
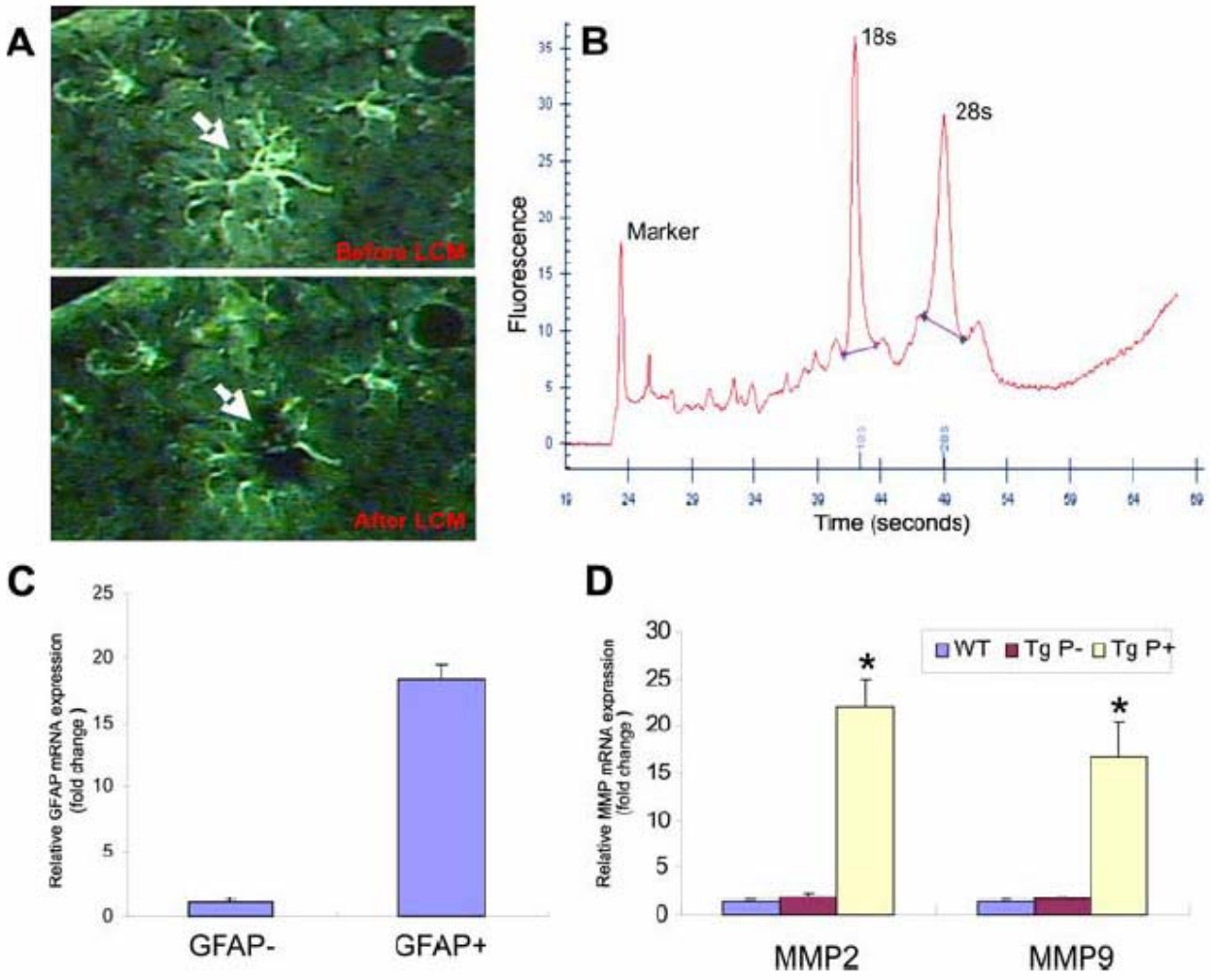
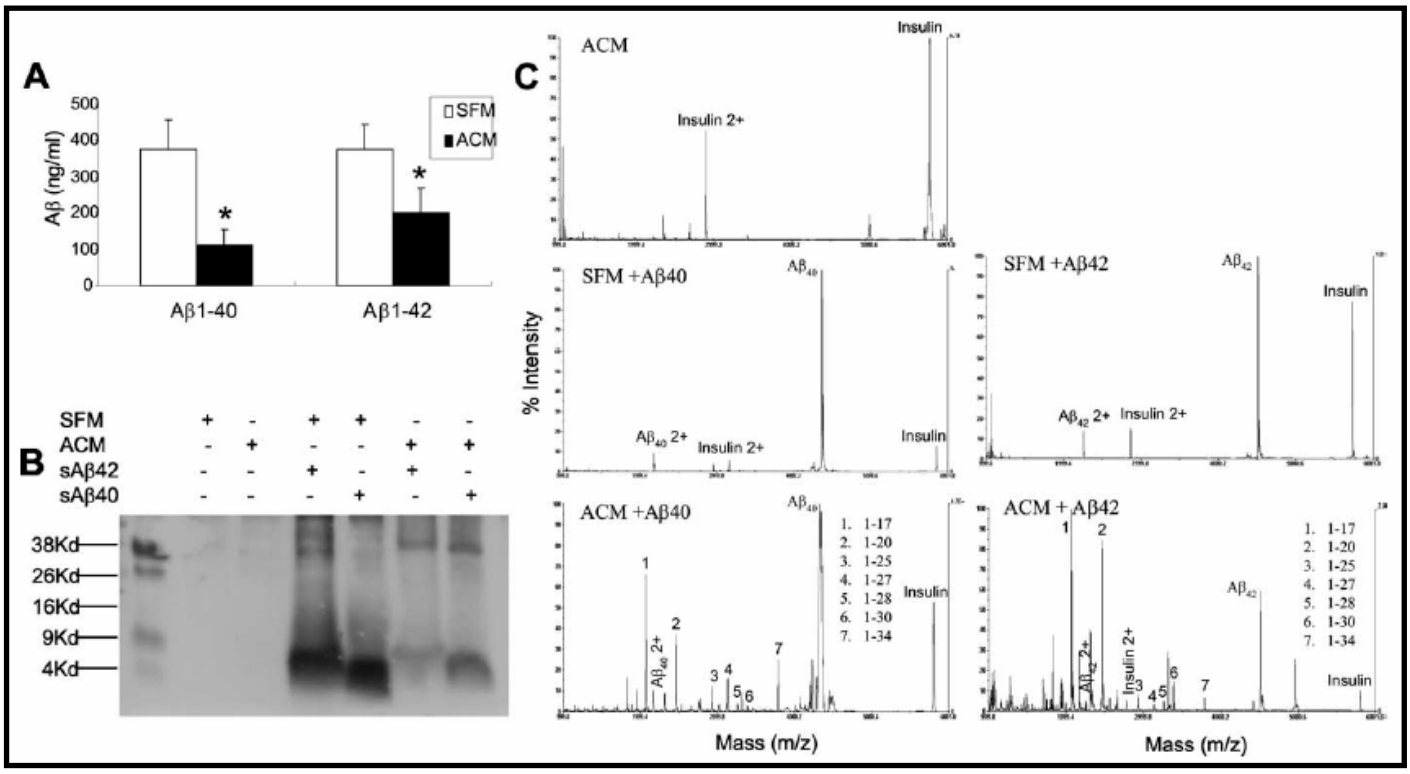
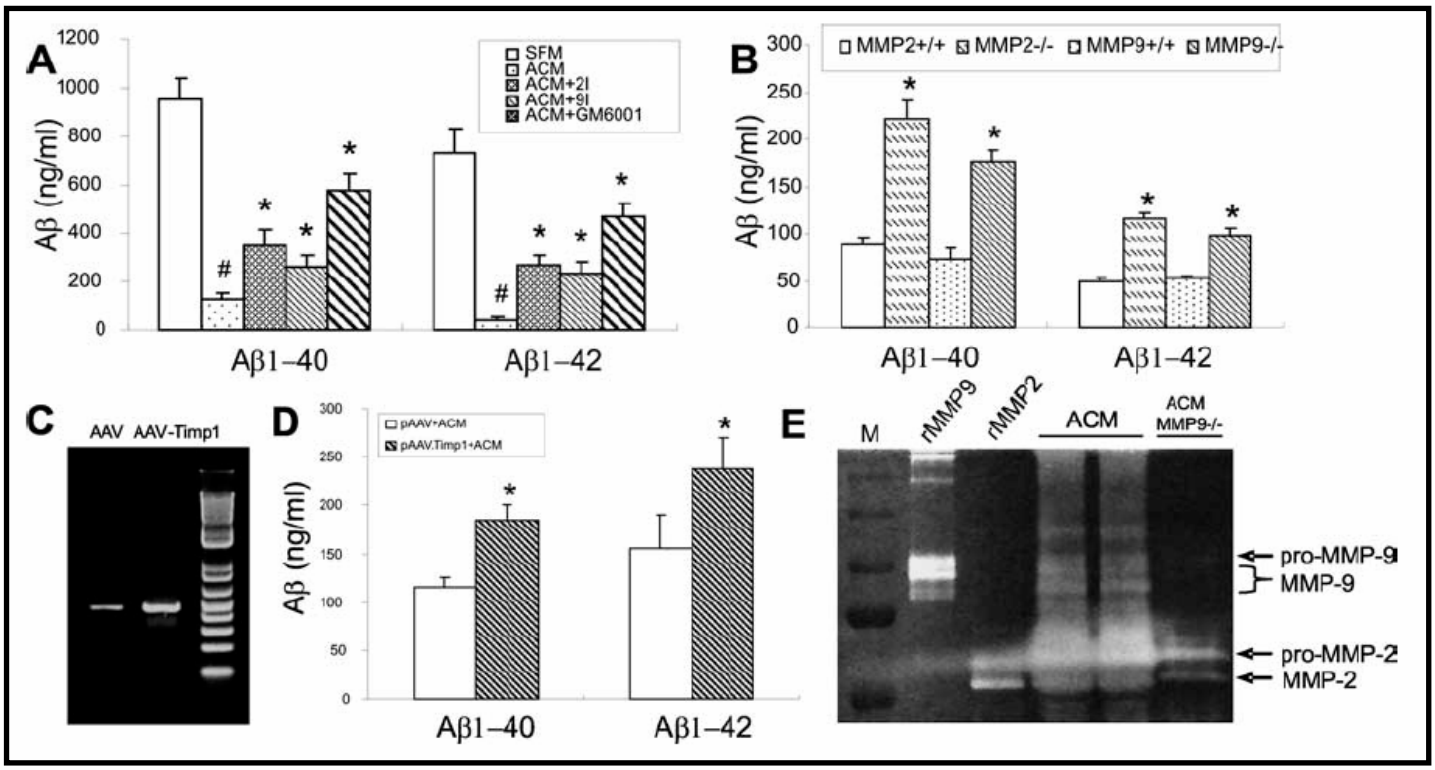

WT
C.C.
HP
A
Appsw
C.C.
HP
B
MMP-9 + ThS
C
MMP-9
D
GFAP
E
Merge
F
Aß
*
G
DQ-gelatin
*
H
Merge
*
I

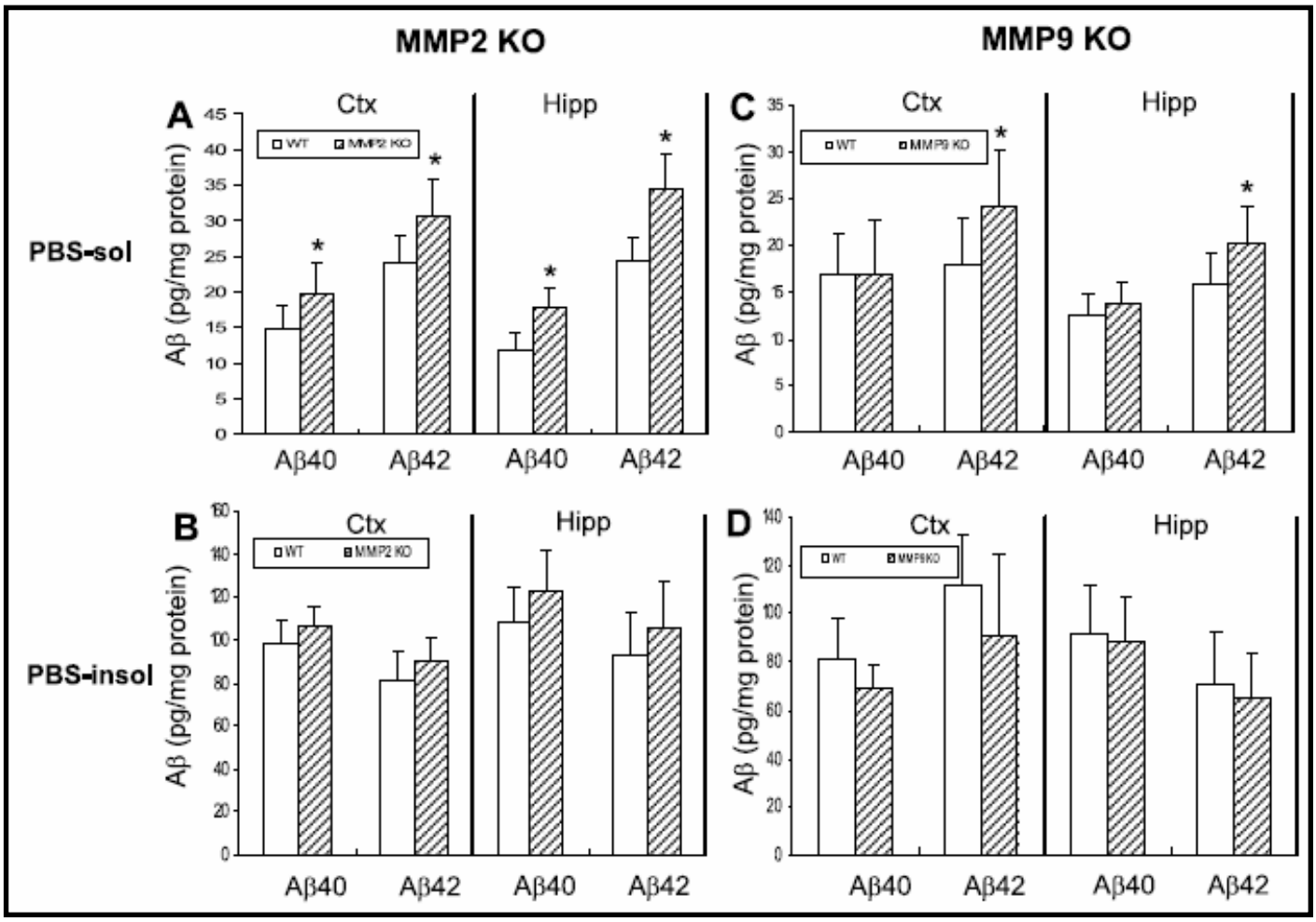
Fig. 4

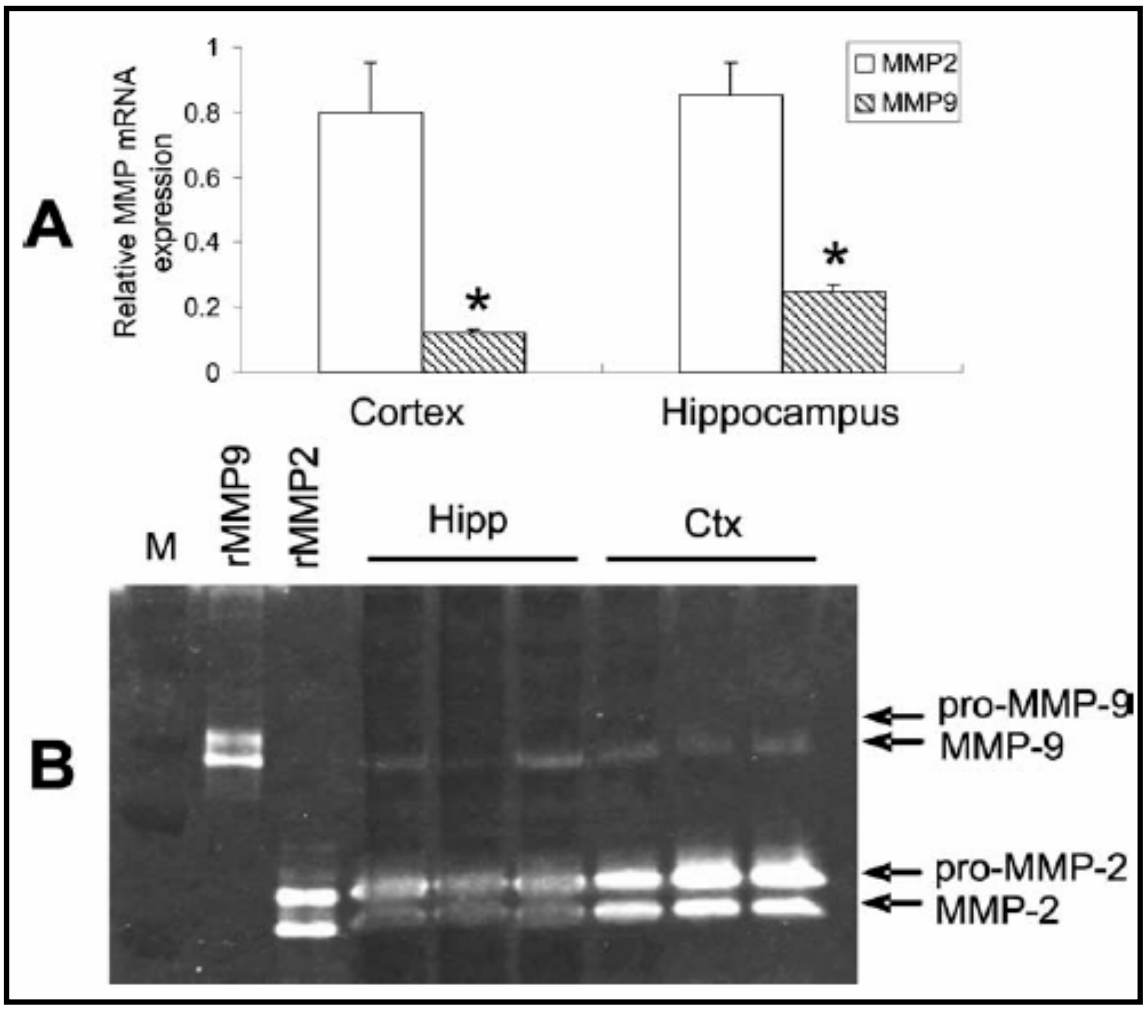

A
B
C
D
MMP-2
MMP-9
GFAP
Merge


A
Before LCM
After LCM
B
18s
28s
Marker
Fluorescence
Time (seconds)
C
Relative GFAP mRNA expression (fold change)
GFAP-
GFAP+
D
Relative MMP mRNA expression (fold change)
WT
Tg P-
Tg P+
MMP2
MMP9


A
Aβ (ng/ml)
SFM
ACM
Aβ1-40
Aβ1-42
B
SFM
ACM
sAβ42
sAβ40
38Kd
26Kd
16Kd
9Kd
4Kd
C
ACM
Insulin
Insulin 2+
SFM +Aβ40
SFM +Aβ42
ACM +Aβ40
ACM +Aβ42
% Intensity
1. 1-17
2. 1-20
3. 1-25
4. 1-27
5. 1-28
6. 1-30
7. 1-34
Mass (m/z)


A
SFM
ACM
ACM+2I
ACM+9I
ACM+GM6001
Aβ (ng/ml)
Aβ1–40
Aβ1–42
B
MMP2+/+
MMP2-/-
MMP9+/+
MMP9-/-
C
AAV
AAV-Timp1
D
pAAV+ACM
pAAV.Timp1+ACM
E
M
rMMP9
rMMP2
ACM
ACM MMP9-/-
pro-MMP-9
MMP-9
pro-MMP-2
MMP-2


MMP2 KO
MMP9 KO
PBS-sol
PBS-insol
A
B
C
D
Ctx
Hipp
WT
MMP2 KO
MMP9 KO
Aβ (pg/mg protein)
Aβ40
Aβ42


A
Relative MMP mRNA expression
MMP2
MMP9
Cortex
Hippocampus
B
M
rMMP9
rMMP2
Hipp
Ctx
pro-MMP-9
MMP-9
pro-MMP-2
MMP-2

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