[14:00 – 14:40]

P25: A hidden target for AD therapeutic.

Ilho Ha, Ph.D., Institute for Brain Science and Technology (IBST) and Graduate Program in Neuroscience, Inje University

Alzheimer's disease (AD) is an irreversible, progressive brain disorder that is characterized by dementia. Amounts of p25 and cdk5 kinase activity are specifically upregulated in AD patient's brain samples. Considerable evidence now points importance of p25/cdk5 in generation of Aβ peptides and hyperphosphorylation of tau linking amyloid plaques and neurofibrillary tangles, two major pathological hallmarks of AD. We demonstrated that P25/CDK5 phosphorylates BACE1, the first step protease to produce Aβ. P25/cdk5 inhibitors to reduce BACE1 phosphorylation and the secretion of Aβ are screened through *in silico, in vitro,* and cell-based assays. Out of 4.3 million chemicals we finally selected two compounds to have IC50 of 10 microM in cell-based assays. The inhibitors block Tau phosphrorylation as well as BACE1 phosphorylation. In conclusion P25 should be one of the best targets for AD therapeutics.

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P25:
A Hidden Target for AD Therapeutic

2006. 4. 26

Institute for Brain Science and Technology (IBST)
Inje University

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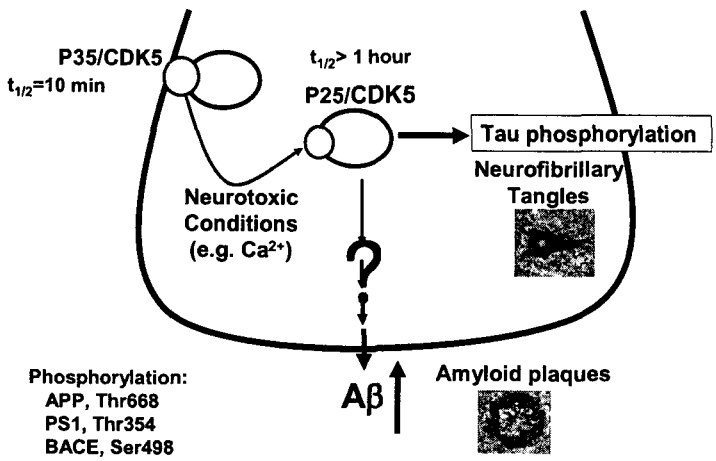
Alzheimer disease pathology
(Focused on P25/CDK5)



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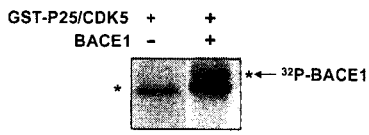
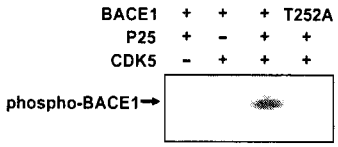
P25/CDK5 phosphrylates Thr_{252} of BACE1

Motif: T/S-P-X- K/R

Human	^{241}DHSLYTGSLWY**TPIR**REWYY^{260}
Rat	^{241}DHSLYTGSLWY**TPIR**REWYY^{260}
Mouse	^{241}DHSLYTGSLWY**TPIR**REWYY^{260}
Phosphopeptide	^{248}SLWY**T**PO_4**PIR**R^{256}





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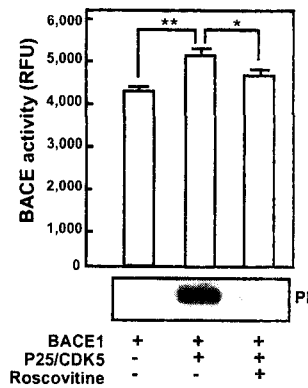
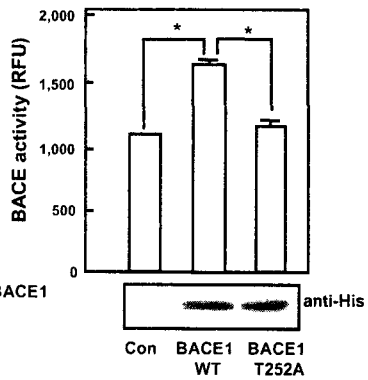
P25/CDK5-mediated phosphorylation of BACE1 increases its enzymatic activity





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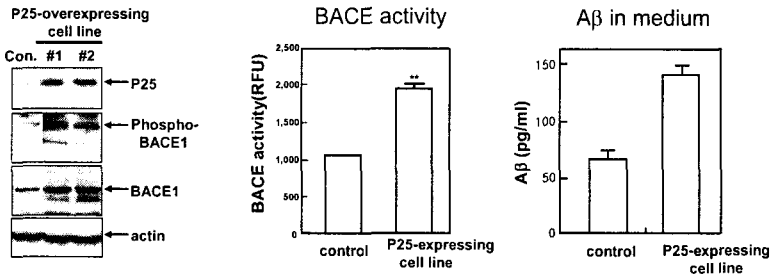
Over-expression of P25 increases BACE activity and Aβ secretion



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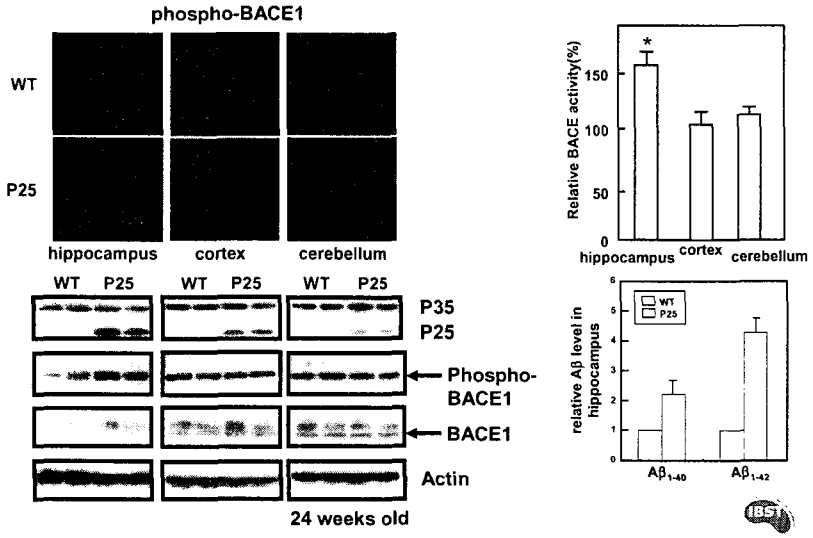
Increase of phospho-BACE1 in p25 Tg mouse

Ahlijanian *et al.* PNAS 92:2910(2000)



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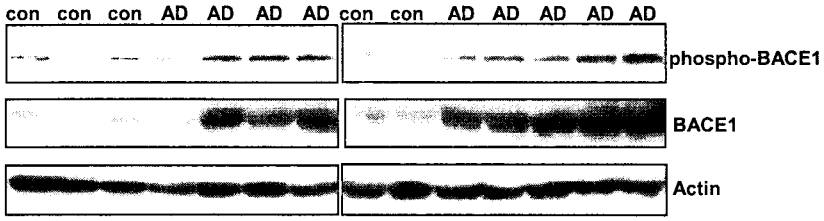
Increase of phospho-BACE1 in AD patient brain*



* Samples from McLean Hospital, Harvard Medical School

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Strategy for screening P25/CDK5 inhibitors

Step1. *In silico*

1. **Molecular modeling of P25/CDK5 complex**
2. ***In silico* screening for a library of 3.4 Million chemicals**

Step2. *In vitro*

1. Phophorylation of histone H1 with ^{32}P-ATP
2. α-phospho-histone H1 antibody (ELISA)
3. Phosphorylation of BACE1 (western analysis)

Step3. *In vivo*

1. Two tet-off stable cell lines (APPsw38- and P25- expressing)
2. Phosphorylation of BACE1 (western analysis)
3. Aβ secretion (sandwich ELISA)

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In silico screening based on a new P25/CDK5 3D model

(Eur. J. Biochem., 269: 4427, **2002**; *J. of Neurosci.*, 23:1189, **2003**)

Target : P25 not CDK5

- CDK5 expresses in the whole body
- P35: predominantly in brain
- P25: produced in neurotoxic condition

Chemicals

1. M.W. < 600 Dalton
2. Commercially available

3.4 M. chemicals → *in silico* → 109 chemicals
(64 chemicals for *in vitro* assay)

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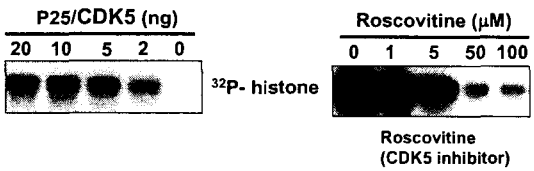
In vitro assay: Phosphorylation of histone H1

P25/CDK5 + Histone H1 + ^{32}P-ATP(50 μM)

+ / - Inhibitors (50 μM)

Phospho(^{32}P)- Histone H1



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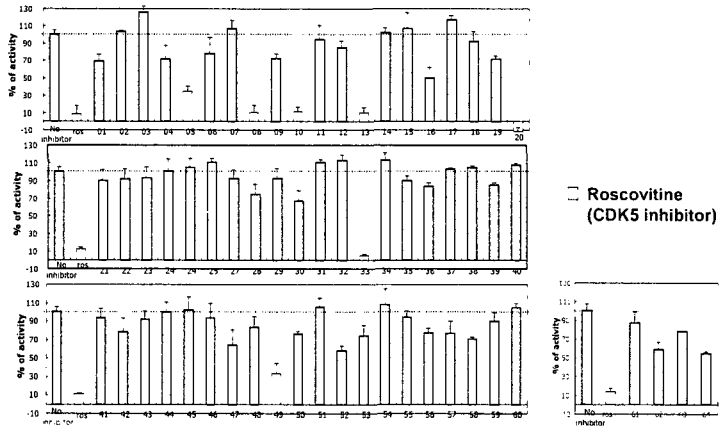
In vitro screening: radioactive ^{32}P phosphorylation



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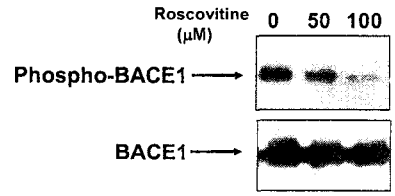
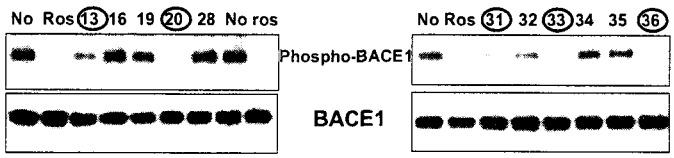
In vitro screening: Phosphorylation of BACE1

P25/CDK5 + BACE1 ± inhibitor → Phosphorylation of BACE1





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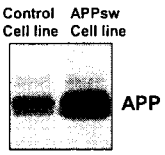
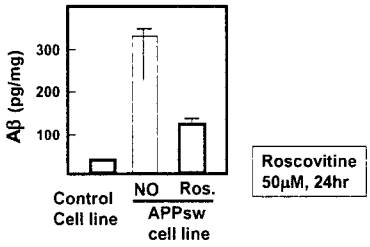
In vivo assay(1): APPsw38$_{tet\text{-}off}$ stable cell lines (PC12)





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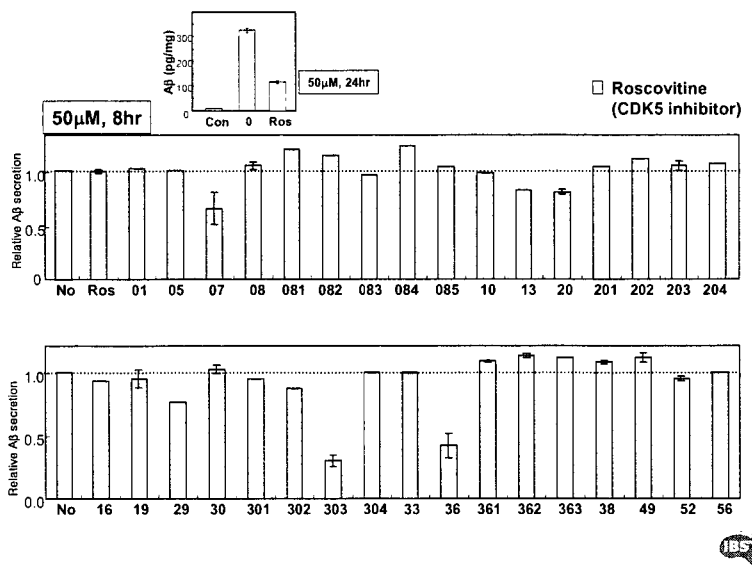
Cell-based screening: Aβ secretion from APPsw38 cells



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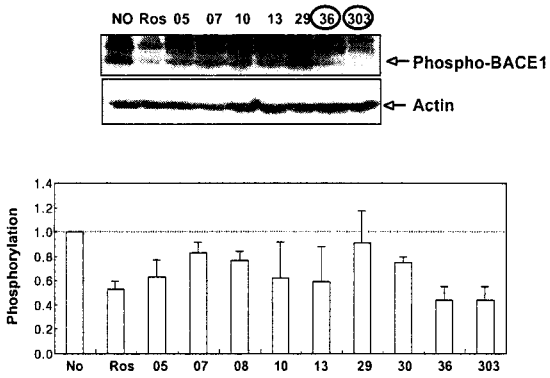
Cell-based screening: Phospho-BACE1 in APPsw38 cells



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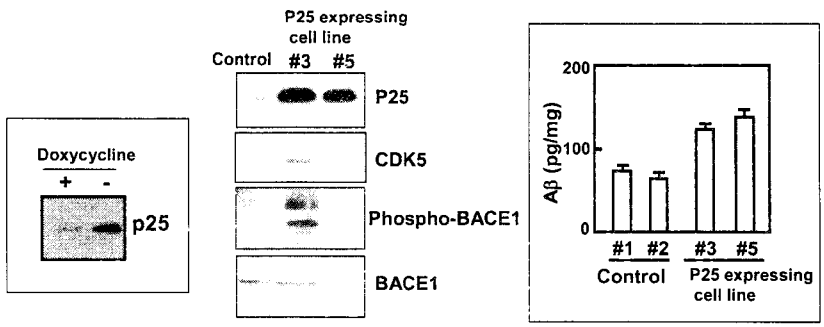
In vivo assay(2): $p25_{tet\text{-}off}$ stable cell lines (PC12)



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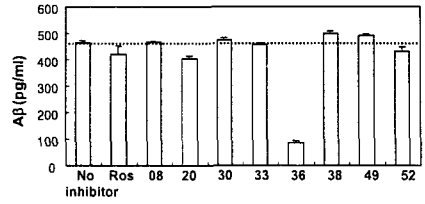
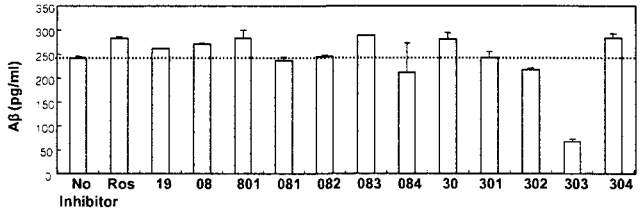
In vivo screening: Aβ and phopho-BACE1 from $p25_{tet\text{-}off}$ cells

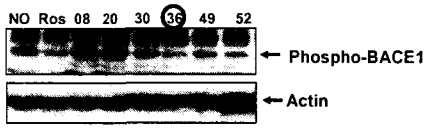
1. Aβ



50μM, 8 hrs



2. Phopho-BACE1



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Summary of *in vitro and in vivo* screening

in vitro screening	active chemicals
Phophorylation of histone H1 with P^{32}-ATP	08 10 13 20 30 33
α-phospho-histone H1 (ELISA)	08 13 20 30 33 36 52
Phosphorylation of BACE1 (western analysis)	10 13 20 30 31 32 33 36
in vivo screening	13 20 30 303 36

Summary

- P25/CDK5 phosphorylates BACE1.

2. P25 is a new target for AD therapeutics.

3. P25 inhibitors should block
both Aβ pathway and Tau pathway.

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