

*S-6 [14:00-14:40]*

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P25: A hidden target for AD  
therapeutics

Prof. Il Ho Ha  
(*Inje University*)



[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/ckd5 in generation of A $\beta$  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 A $\beta$ . P25/ckd5 inhibitors to reduce BACE1 phosphorylation and the secretion of A $\beta$  are screened through *in silico*, *in vitro*, and cell-based assays. Out of 4.3 million chemicals we finally selected two compounds to have IC<sub>50</sub> of 10 microM in cell-based assays. The inhibitors block Tau phosphorylation as well as BACE1 phosphorylation. In conclusion P25 should be one of the best targets for AD therapeutics.

# P25: A Hidden Target for AD Therapeutic

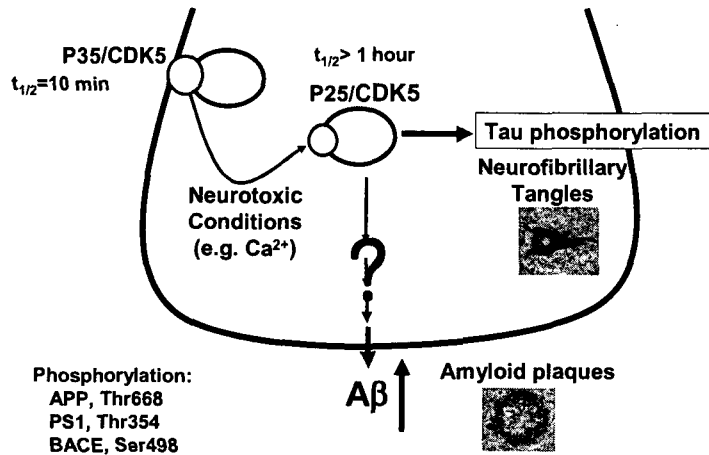


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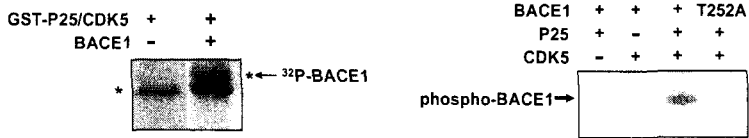
## Alzheimer disease pathology (Focused on P25/CDK5)



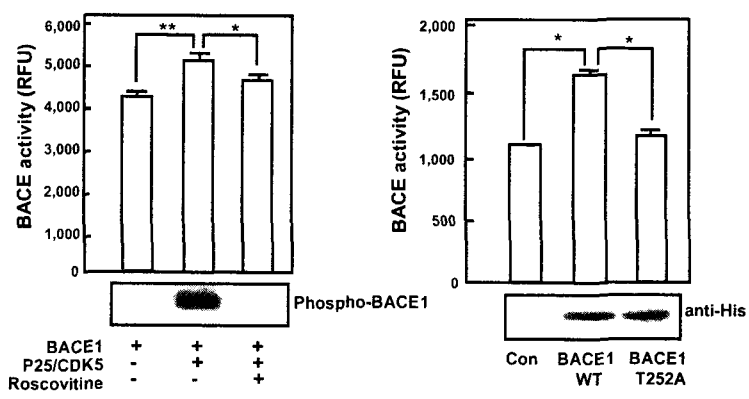
### P25/CDK5 phosphorylates Thr<sub>252</sub> of BACE1

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

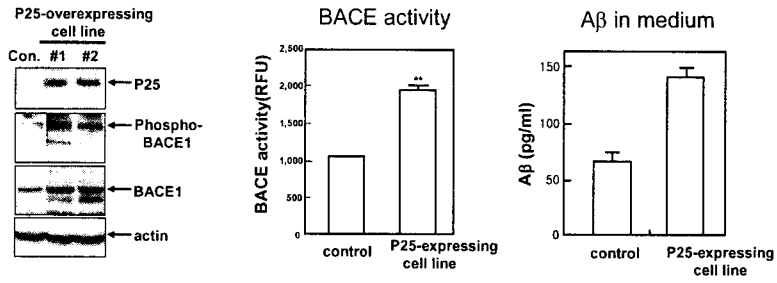
Human	241DHSLYTGSLWYTPIRREWYY <sup>260</sup>
Rat	241DHSLYTGSLWYTPIRREWYY <sup>260</sup>
Mouse	241DHSLYTGSLWYTPIRREWYY <sup>260</sup>
Phosphopeptide	248SLWYTPIRR <sup>256</sup> PO <sub>4</sub>



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

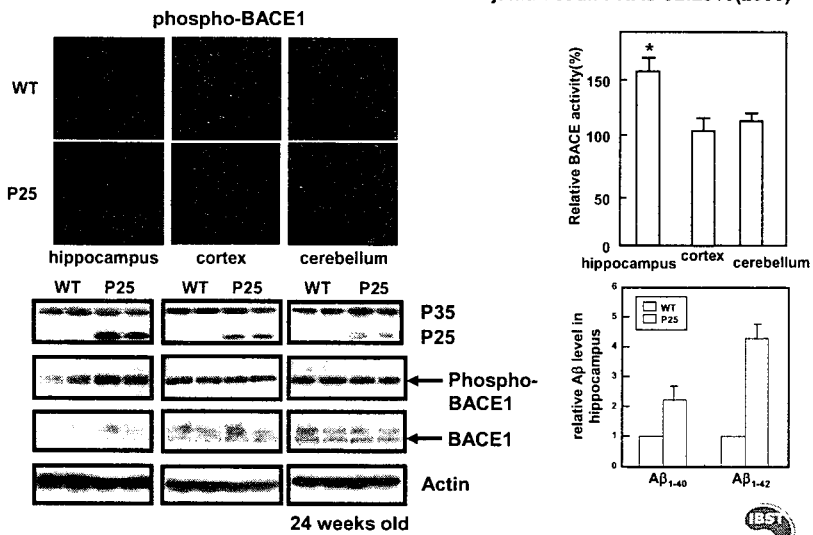


### Over-expression of P25 increases BACE activity and A $\beta$ secretion

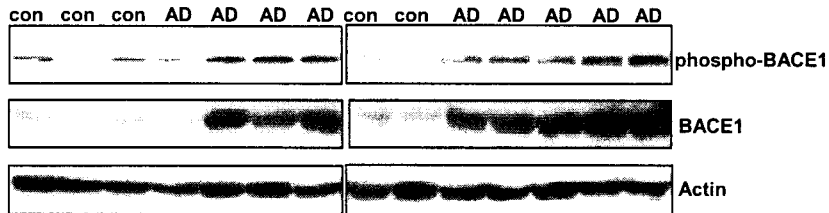


### Increase of phospho-BACE1 in p25 Tg mouse

Ahlijanian et al. PNAS 92:2910(2000)



### Increase of phospho-BACE1 in AD patient brain\*



\* Samples from McLean Hospital, Harvard Medical School



### 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. Phosphorylation of histone H1 with  $^{32}\text{P}$ -ATP
2.  $\alpha$ -phospho-histone H1 antibody (ELISA)
3. Phosphorylation of BACE1 (western analysis)

#### Step3. *In vivo*

1. Two tet-off stable cell lines (APP<sup>sw38</sup>- and P25- expressing)
2. Phosphorylation of BACE1 (western analysis)
3. A $\beta$  secretion (sandwich ELISA)



### ***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

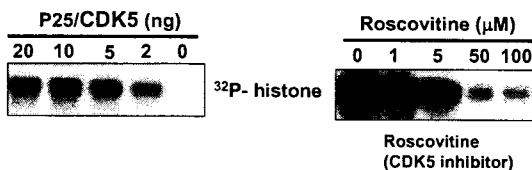
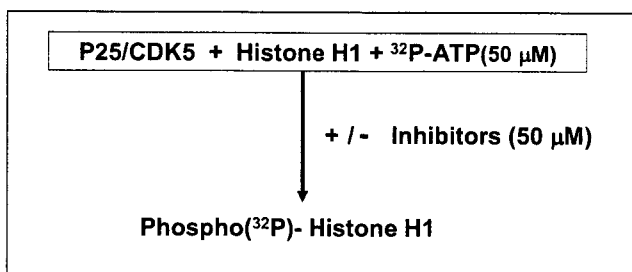
#### **Chemicals**

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

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

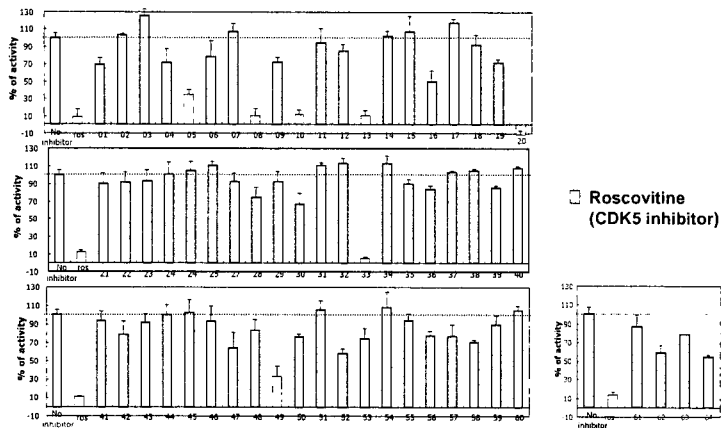


### ***In vitro* assay: Phosphorylation of histone H1**



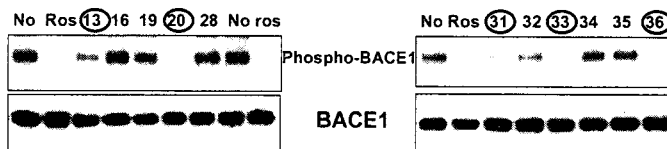
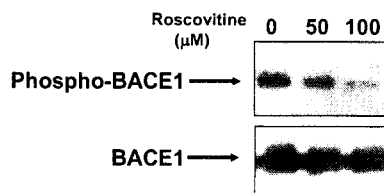


### In vitro screening: radioactive <sup>32</sup>P phosphorylation

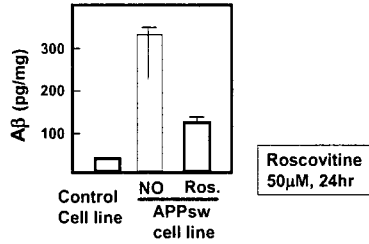
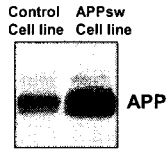


### In vitro screening: Phosphorylation of BACE1

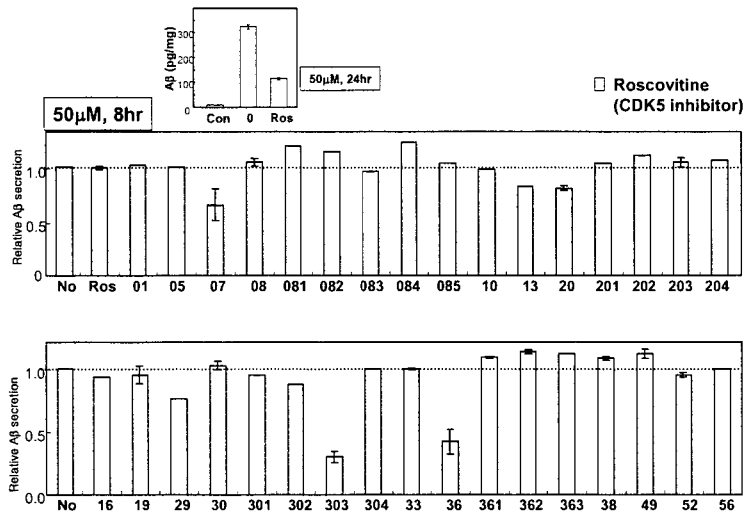
P25/CDK5 + BACE1 ± inhibitor → Phosphorylation of BACE1



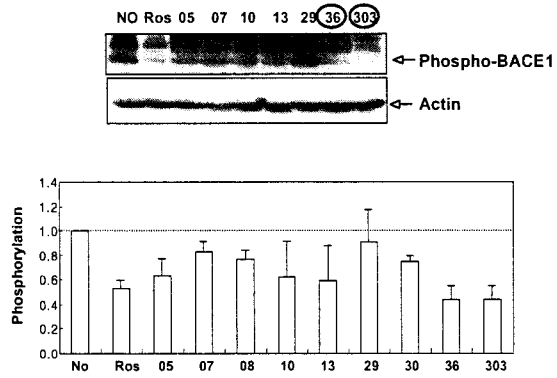
**In vivo assay(1): APPsw38<sub>tet-off</sub> stable cell lines (PC12)**



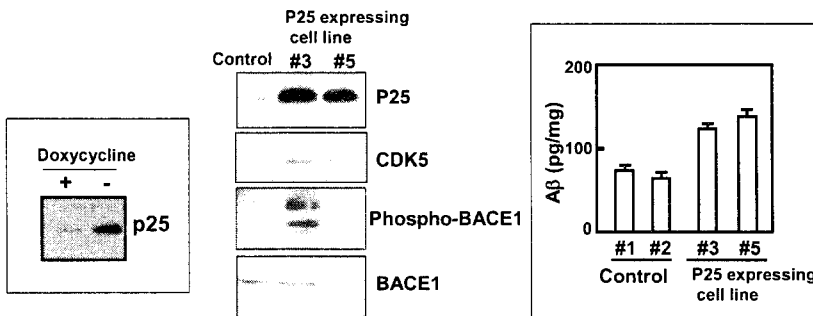
**Cell-based screening: Aβ secretion from APPsw38 cells**



### Cell-based screening: Phospho-BACE1 in APPsw38 cells

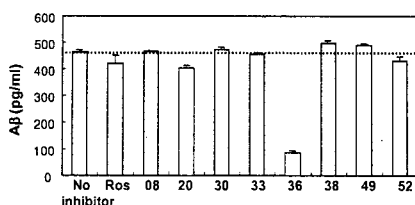


### In vivo assay(2): p25<sub>tet-off</sub> stable cell lines (PC12)

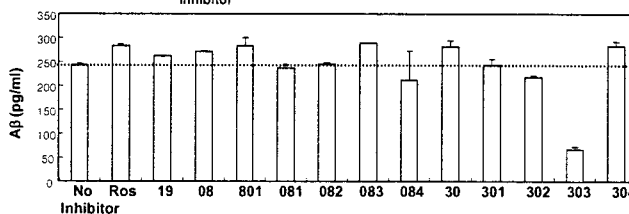


**In vivo screening: A $\beta$  and phopho-BACE1 from p25<sub>tet-off</sub> cells**

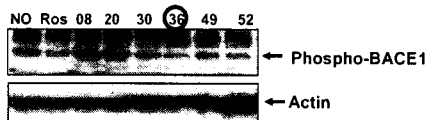
**1. A $\beta$**



50 $\mu$ M, 8 hrs



**2. Phopho-BACE1**



**Summary of *in vitro* and *in vivo* screening**

<i>in vitro</i> screening	active chemicals	
Phophorylation of histone H1 with P <sup>32</sup> -ATP	08 10 13 20 30	33
$\alpha$ -phospho-histone H1 (ELISA)	08 13 20 30	33 36 52
Phosphorylation of BACE1 (western analysis)	10 13 20 30	31 32 33 36
<i>in vivo</i> 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 A $\beta$  pathway and Tau pathway.**



## Acknowledgement



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