Design of two single-domain intrabodies to reduce intracellular Aβ production

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Introduction

Alzheimer's disease is the major cause of dementia in the elderly population, and its figures will increase as aging is the major risk and the population's lifespan is increasing. There is a promising gene-based therapy which turns the humoral immune system outside in for intracellular immunization. Intracellular antibodies (intrabodies) are antibody fragments fusioned to signal sequences which allow them to stay within the cell and, if desired, direct them to specific compartments and organelles. In the present work we present a therapeutic alternative for combating intracellular amyloid-β production.

APP processing and Alzheimer

The amyloid hypothesis affirms that initiation of Alzheimer's Disease (AD) is elicited by excessive amyloidogenic processing of the Amyloid Precursor Protein (APP), producing toxic amyloid-β. Significant *in vitro* and human pathological data suggest that intraneuronal accumulation of Aβ peptides plays an early role in the neurodegenerative cascade.

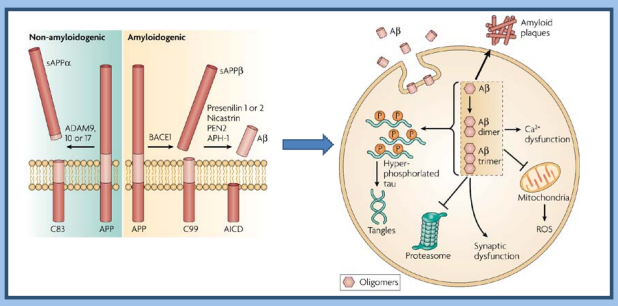

Fig 1. Amyloidogenic cleavage of APP is mediated by β-secretase (BACE) followed by γ-secretase activity (presenilins 1/2, nicastrin, PEN2 and APH-1). An early event in AD is the intracellular oligomerization of Aβ, leading to toxic oligomers that can aggregate extracellularly and impair synaptic functionality, mitochondria homeostasis and affect the protein folding quality control of neurons.

Intrabody approach

Intrabodies are intracellularly retained antibodies fusioned to signal sequences specific for intracellular compartments. Originally intrabodies derive from single-chain variable fragments (scFv), but recently smaller versions are being developed (i. e. single-domain intrabodies (sdAb)).

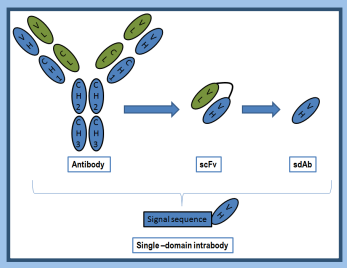

Fig 2. Rationale of the intrabody approach. Intrabodies are generated by fusing the desired format of an antibody with a signal sequence specific for the desired intracellular compartment or organelle where the intrabody will be retained. The most used versions of intrabodies are scFv (a light chain variable fragment linked to a heavy chain variable fragment) and sdAb (a single fragment retaining both affinity and specificity for a certain antigen).

Our hypothesis is that β-cleavage site-specific intrabodies can reduce amyloidogenic processing of APP, leading to less intracellular Aβ accumulation, thus preventing or slowing-down the onset of the disease in transgenic mice harboring mutant presenilin 1, mutant APP and mutant tau protein (3xTg AD mice).

Methodology

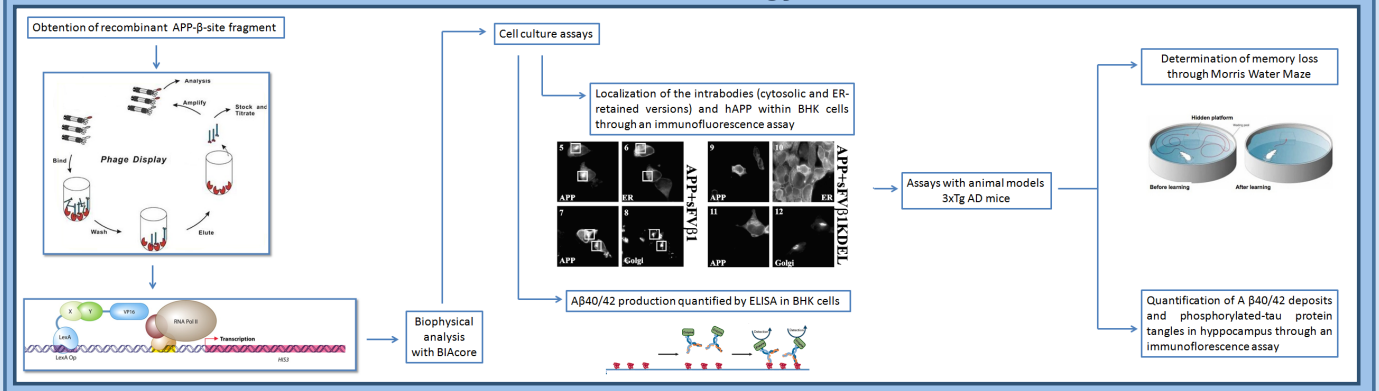

Fig 3. β-cleavage site-specific intrabodies will be produced by panning *in vitro* a sdAb phage library. Binders will be used for an *in vivo* panning round using an auxotrophy-based yeast-two-hybrid assay. Best binders will be used for assays in cell cultures and triple-transgenic AD mice. Their ability to bind APP and reduce Aβ deposition will be tested as well as their ability to reduce memory loss.

Expected results

	Cytosolic sdAb	sdAb-KDEL	
Time to complete Morris Water Maze	↓ vs control	↓↓ vs control	Slower progression of AD
Amount of Aβ42 deposits	↓ vs control	↓↓ vs control	
Amount of phospho-Tau tangles	↓ vs control	↓↓ vs control	

More promising results of the ER-retained version (sdAb-KDEL) are expected, since the processing of APP occurs mainly in the secretory pathway. However, results from this sdAb format are expected to overcome those obtained with conventional intrabodies formats.

References

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Images in Methodology come from: [2] and [3]
Image of APP processing comes from: [5] Frank M. LaFerla, Kim N. Green & Salvatore Oddo, (2007). Intracellular amyloid-β in Alzheimer's disease, Nature Reviews Neuroscience 8, 499-509.