Conformational-selective interference with subcellular pools of Aβ oligomers in living cells: a new strategy to decipher Alzheimer's Disease

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Keywords: recombinant antibody, intrabody, amyloid, oligomers

Studying and targeting different conformations and multimeric states of a given protein in the complex context of subcellular compartments of living cells is a big challenge. For instance, in living cells, the Amyloid-β (Aβ) peptides generate from the Amyloid Precursor Protein (APP) by a complex process of Regulated Intramembrane Proteolysis (RIP), before undergoing the processes of misfolding and aggregation. Along the pathways of aggregation, Aβ oligomers (AβOs) are recognized as the most neurotoxic proteinaceous forms in Alzheimer's Disease (AD) but they are still considered mysterious entities in terms of molecular and structural composition and activity.

Among new tools for analyzing amyloid assembly states and dynamics, conformational-sensitive antibodies deserve primary interest. In particular, recombinant antibody fragments can be exploited as intracellular antibodies (intrabodies) for a subcellular-localized interference to block or modulate the function of target molecules.

We firstly generated, by an *in vivo* intracellular selection in yeast cells[1], a panel of conformation-sensitive antibody fragments selectively recognizing AD-relevant AβO conformers[1]. The *in vivo* selected antibody fragments are ideal for the expression (as genes) in mammalian cells, as intrabodies targeted to different subcellular compartments. Recently, we expressed the anti-AβO single chain antibody fragment (scFv) A13[1] as an intrabody, with the aim of intercepting AβOs at subcellular sites of their putative formation, and of attempting their functional silencing. In this way, we established a new experimental paradigm of subcellular-localized and conformational-selective interference (CSI)[2].

As proof of concept, in this study the intrabody intrinsically equipped with conformational-sensitive binding properties is exploited for interference studies not currently feasible with nucleic acid targeting methods (i.e. RNA-interference or gene knock-out), that can silence entire gene products (i.e. APP or RIP machinery protein components) but not peculiar post-translational modification products (such as AβOs).

The intrabody-based CSI besides providing a novel approach to selectively control biologically-active AβO conformers in living cells, allows a new dissection of cellular mechanisms of AβO generation, trafficking and actions. Indeed, by exploiting CSI, we demonstrate that intracellular Aβ can oligomerize into pathological forms, through critical conformations formed inside the endoplasmic reticulum (ER).

As future perspective, CSI can be exploitable for *in vivo* therapeutic applications as well as to improve our understanding of the molecular and cellular processes of AD pathogenesis, thereby uncovering new targets for drugs development.

[1] G. Meli et al., J Mol Biol 387 (2009) 584-606
[2] G. Meli et al, Nature Communications 5 (2014) 3867