Introduction

- Alzheimer’s disease (AD) is the most common form of dementia and one of the most common cause of death worldwide.
- The pro-amyloidogenic cleavage of Amyloid Precursor Protein (APP) leading to amyloid beta peptide (Aβ) formation, and the subsequent misfolding and aggregation into neurotoxic oligomers, remains one of the most supported theories for AD pathogenesis.
- Aging, the strongest risk factor for AD, and subsequent decreased efficiency of chaperones might be an important contributor for the spreading of the disease. Chaperones are a class of proteins that protect cells against protein misfolding.

In the present study, we investigated the effect of Aβ-Hsp60 protein-protein interaction using biological, in vitro and ex vivo approaches. We further tested whether Hsp60 interacted with pre-formed Aβ oligomers (Aβo) and if this interaction resulted in a change of the Aβo-derived synaptic toxicity.

Materials and Methods

Cell culture: Human neuronal cells (H9 and SH-SY5Y) were generously donated by Dr. Dennis Selkoe (Harvard Medical School, Boston, MA). Conditions: DMEM, 1% FBS, 1% p/s without CHO cells or with G418 (7PA2 cells). Transfection: Empty pcMV6 or pcMV6-Hsp60, Lipofectamine 2000 according to the manufacturer.

Results

1. Hsp60 overexpression in vitro modulates the release of Aβ in the extracellular compartment without affecting APP levels.
2. Hsp60 overexpression significantly reduces secreted levels of Aβ, thus resulting in a downstream reduced cytotoxicity of secreted media (CM).
3. Hsp60 directly binds to pre-formed Aβo and modulates their conformation.
4. Hsp60 protects against Aβo-derived cytotoxicity.
5. Aβo treated with Hsp60 no longer impair synaptic plasticity.
6. Pre-treatment of Hsp60 reduces Aβo binding to synaptosomes ex vivo.

Conclusions

- Our results suggest that Hsp60 overexpression influences the turn-over of APP protein and Aβ peptide toxicity in vitro.
- The reduction of secreted Aβ peptide in the media of 7PA2/1H60 cell line suggests that Hsp60 might directly or indirectly activate a mechanism of APP degradation or post-translational modification leading to a reduction of Aβ release.
- Hsp60 protects against the cytotoxicity of pre-formed Aβo in vitro and prevents the binding to synapses and the impairment of synaptic plasticity observed by Aβo treatment ex vivo, thus suggesting a direct effect of Hsp60 as an “oligomer scavenger” of toxic oligomeric conformations.
- Investigations of the mechanism of Hsp60-Aβo protein-protein interaction suggest a direct binding of the two proteins thus resulting in a modification of toxic conformations toward less toxic ones as suggested by the different patterns of PK digestion and different binding to Bio-ANS probe upon Hsp60 treatment of Aβo.
- Targeting Hsp60 could be a viable molecular element in devising future innovative therapies for AD centered on targeting Aβo oligomer toxicity.

References


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