THE ROLE OF HISTONE H1 IN ALZHEIMER'S DISEASE

The design of a research project

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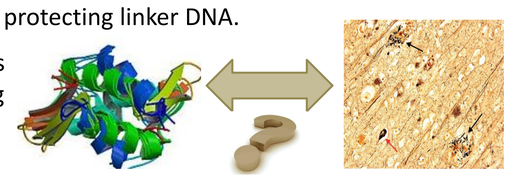
INTRODUCTION

Histone H1

- Unphosphorylated, binds to the DNA entering and exiting the nucleosoma, stabilizing higher order chromatin conformations and protecting linker DNA.

- Its phosphorylation reduces its affinity for DNA, promoting its release to the cytoplasm.



Alzheimer's disease (AD)

-A chronic disorder that causes serious cognitive disability.
-Characterized by:

✓senile plaques: deposition of abnormal protein β (Aβ)
✓neurofibrillary tangles (NFTs): aggregates of hyperphosphorylated tau protein
✓ loss of synapses and neurons.

Main goal: define a hypothesis about **the implication of histone H1 in the pathological profile of AD** and attempt to prove it by the use of analytical techniques.

Figures obtained from [4], [5].

Main background

- Non-nuclear histone H1 is upregulated within the cytoplasm of neurons and astrocytes from areas affected by disease as well as amyloid plaques in both prion disease and AD but not acute neurodegeneration [1].

- H1 was identified as a major extracellular candidate released by damaged adult brain that causes neurotoxicity and activation of the innate system [2].

- H1 was the strongest detected interacting protein to the amyloid-like motifs of Aβ [3].

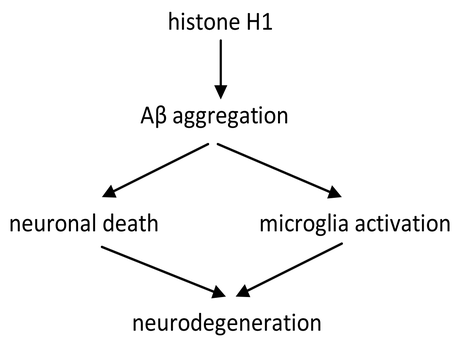
- An upregulation of Cdk5 (kinasa responsible for H1's phosphorylation) in neurons with early stage AD has been reported. Therefore, it seems feasible that increases in Cdk5 may not only abnormally phosphorylate tau but also increase phosphorylated histone H1 [3].

HYPOTHESIS

Our hypothesis postulates that **the phosphorylated form of histone H1 recognizes and binds preferentially to the amyloid motif of the Aβ peptide stabilizing its amyloid-like fibrils aggregation and protecting them against proteolysis**, at intracellular and extracellular level.

This aggregation causes the formation of NFTs and senile plaques, the two features defining Alzheimer's disease.

the direct binding of Aβ fibrils to C1q enhances the classical complement activation *via* C1q, which exerts a detrimental effect on neuronal integrity by the recruitment of activated glia.



AIMS

1- To demonstrate **the real interaction between histone H1 and aggregated Aβ** (immunohistochemistry assay).

2- To prove that it **is just the phosphorylated form of H1 the one in contact with the Aβ aggregates** and to determine the **H1 subtype involved** in the process (MALDI TOF mass spectrometry analysis).

3- To elaborate a **transgenic mice strain** by knocking the gene codifying for the H1 subtype it and analyze the effect of the absence of the histone on both the Aβ aggregation and the immune system activation.

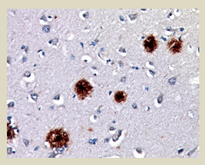
4- By inducing Aβ aggregation of synthetic protein β:

4.1- To study **the effect of H1 phosphorylated and unphosphorylated on the Aβ aggregation process** (Thioflavin T spectroscopic assay)

4.2- To test the **capacity of H1 of protecting Aβ fibrils from proteolysis** (by exposition to proteolitic enzymes and analyzed by capillary electrophoresis with laser-induced fluorescence detection, CE/LIF).

METHODOLOGY

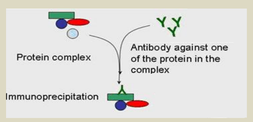
***In vivo* tests:** comparing *wt* with C57BL/6-Tg (an AD model) mice



Immunohistochemistry

-anti-H1 ANA108 for H1
-WO2 for Aβ aggregates

Co-Immunoprecipitation

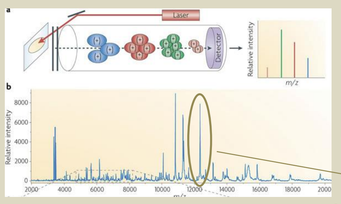


Complex Aβ aggregates – H1

H1 purification

Acid perchloric extraction

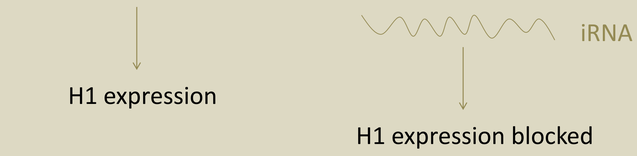
SDS-PAGE electrophoresis



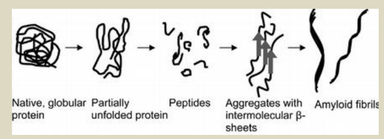
MALDI TOF mass spectrometry

H1 subtype (un- or phosphorylated)

Transgenic elaboration:

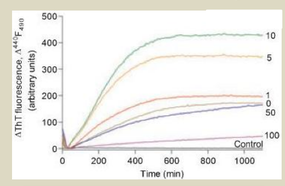


***In vitro* tests:** Fibril formation

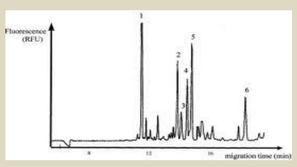


H1 addition (un- and phosphorylated)

proteolitic enzyme



Thioflavin T spectroscopic assay



CE/LIF

Figures obtained from [6], [7], [8], [9], [10], [11]

TIMELINE

Aim	1st year		2nd year		3rd year	
	1st semester	2nd semester	1st semester	2nd semester	1st semester	2nd semester
Animals obtaining	x					
In vivo tests	x	x				
Transgenic elaboration			x			
In vivo tests with the transgenic				x	x	
In vitro tests		x	x			
Results analysis						x

EXPECTED RESULTS AND CONCLUSIONS

1- Co-localization of both markers in the immunohistochemistry (confirmation of real interaction).

2- The sequenced peptides analyzed by MALDI TOF to correlate with the phosphorylated form of H1 and to one of the H1 subtypes defined in the literature.

3- Absence or decrement of Aβ aggregation and immune system activation in the transgenic strain.

4- Aβ aggregation and proteolysis resistance only or significantly increased when tests carried on with the phosphorylated form of the H1 subtype defined in 2.

Then, we could dare to say that inhibition of the interaction between histone H1 and amyloid-like fibrils may impede the common characteristics in AD and may therefore represent a potential therapeutic target for its treatment.

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