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The Nuclear Tau as an Early Molecular Marker of Alzheimer's Disease †

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Abstract: The age-related neurodegenerative diseases drew the interest of the scientific community, due to the gradual increase of the average age in the World's population. Recent studies indicated the altered cell cycle in the triggering of neurodegenerative diseases such as Alzheimer's disease (AD). This process seems to involve the nuclear tau, a protein which we previously shown to have a central role in the neuronal in vitro differentiation. In this work, we studied the role of the nuclear tau protein, specifically of the AT8 epitope, in the onset of AD, to evaluate its possible use as an early molecular marker. The immunolocalization in neurons of CA1 region of the human hippocampus from normal, senile and AD subjects, shown that AT8 epitope decreases in senile neurons respect to youngers, indicating its possible role in the ectopic activation of the cell cycle in differentiated cells. Here we show data that improve the knowledge on the role of nuclear tau in neuronal differentiation and cell degeneration in AD, involving the presence/absence of AT8 in the nucleolus of neurons related to a re-entering in the cell cycle. The molecular mechanisms related to the start of AD are not yet clear, so their understanding is relevant if we consider the social impact of this disease in the human populations.

Keywords: keyword 1; keyword 2; keyword 3 (List three to ten pertinent keywords specific to the article yet reasonably common within the subject discipline.)

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1. Introduction

Alzheimer's disease (AD) is an age-related neurodegenerative disease characterized by progressive loss of memory and deterioration of cognitive functions. The molecular mechanisms that beginning neurodegeneration, in AD patients, are still unclear and indeed, even today, there aren't early diagnostic methods for AD. The literature data on the degeneration of neurons showed that most of neurodegenerative diseases are caused by misfolding of proteins and consequently, aggregation of proteins in the brain [1,2]. Moreover, recent studies suggested a predominant role of the altered cell cycle in the triggering of neurodegenerative diseases such as AD [3].

Tau protein has a central role in AD, it is characterized by a high number of phosphorylations that bring to the development of "Paired Helical Filaments" (PHFs) representing the "core" of the "NeuroFibrillary Tangles" (NFTs). The presence of NFTs, highly insoluble fibrillar aggregates, present in the soma of the pyramidal neurons of the hippocampus, are a result of abnormal hyperphosphorylation of tau protein and represent an

important neuropathological hallmark of AD [4,5]. The tangles lead to progressive neuronal loss in the central nervous system (CNS) correlated with the clinical progression of AD. The NFTs are evident in specific, vulnerable brain areas and the hippocampus is one of the earliest brain structures to be affected [6].

Although tau protein has been described mainly as a cytoplasmic protein, nuclear localization isoforms in neuronal and non-neuronal cells have been observed whose function is not yet elucidated [7–9]. Literature data indicate that the tau protein could interact with the genome and especially the nucleolar localization isoforms could be involved in the early events of AD [10]. Nuclear and nucleolar tau isoforms seem to play a protective role against DNA and RNA from damage events such as oxidative and thermal stress, also suggesting its potential role in ribosome biosynthesis and/or in the organization of genes for rRNA [9,11]. Further studies demonstrated that nuclear Tau is detected in the Nucleolar Organizing Regions (NORs) of the acrocentric chromosomes, and in the fibrillary region of the nucleolus of neuronal and non-neuronal cells [8,10]. In this compartment, Tau may bind rDNA, and it was shown that Tau dysfunction following phosphorylations, such as in AD, can induce disaggregation from DNA [12].

As demonstrate recently, the nucleolar phosphoepitopes of tau protein, with a mechanism that would involve a probably direct interaction with the DNA and/or the RNA in the nucleolus in vitro models, could play a role in the early events of AD. In particular the shorter isoform of tau 0N3R was detected in neuroblastoma cells during neuronal differentiation, and specifically the epitopes Tau-1 and AT8, detecting the unphosphorylated and the phosphorylated Ser202/Thr205 region, respectively. The unphosphorylated Tau-1 epitope was observed in both replicative and differentiated cells, in a spot-like distribution colocalizing with the Upstream Binding Transcription Factor (UBTF); instead AT8 is undetected in replicative neuroblastoma cells and appears during neuronal cell differentiation. Moreover, AT8 epitope appears in the nucleolus when the transcriptional activity in blocked or largely reduced, such as in cells exposed to Actinomycin-D, or in neuronal differentiated cells [10].

Some tau hyperphosphorylated epitopes was observed in the neurons of the hippocampus region in the brains of patients with AD and, moreover, it has been verified the close relationship between nuclear tau in the structure and stabilization of the global heterochromatin blocks [13–15], pericentromeric heterochromatin [16] and perinucleolar heterochromatin [9]and its direct involvement in the development of AD. The phosphoepitope AT100, a specific nuclear tau epitope, detected close to the nuclear global chromatin of human neurons, was also observed to increase during aging in the nucleus of neurons from DG and CA1 regions of the human hippocampus, and the interaction between AT100 and global chromatin progressively decreases until it disappears in more advanced stages of AD [15].

Over the years, many studies that have led to the most recent biological hypothesis of Alzheimer's disease, namely the "Cell cycle hypothesis", are also interesting, according to which AD, could be considered a disease caused by the neuron's cell cycle deregulation and that it is possible aberrant drivers of cell cycle re-entry reported in the literature deregulate the neuronal cell cycle machinery, trigger cell cycle checkpoints, and prevent cell cycle completion [3,17]. Indeed, several lines of evidence indicate a predominant role of cell cycle malfunction in the pathogenesis of AD. Has been proposed that tau-induced heterochromatin loss may be related to the cell cycle re-entry and to the degeneration of postmitotic neurons [13].

Here, we present results concerning the presence of AT8 epitope in hippocampal neurons during the life of normal humans, and in subjects at the first stage of the Alzheimer's disease. The rationale of the study was to evaluate the implication of AT8 in the early events leading to the AD.

2. Materials and Methods

2.1. Tissue Samples

Human brain sections, of the hippocampus CA1 region, were obtained from the Institute of Neuropathology HUB-ICO-DIBELL Biobank. All experiments performed in the present study were in accordance with the ethical standards of the institutional and/or national research committees and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Brain samples were prepared from normal human subjects at different age: fetus, young (between 20 to 40 years), and senile (more than 60 years). Moreover, samples from subjects with AD at the first stage (AD-I) were also used.

2.2. Indirect Immunofluorescence

Paraffin-embedded sections, for indirect immunofluorescence (IIF) experiments, were prepared as previously described [18,19]. Briefly, slides with the tissue sections were placed at 55 °C for 20 min, de-paraffined in xylene and rehydrated in graded alcohols. To reduce auto-fluorescence, sections were then boiled in citrate buffer (10 mM sodium citrate, pH 6) and treated with Sudan Black B for 30 min at room temperature. Before the incubation with the primary antibody, a pre-incubation step of 1 h at 37 °C with blocking solution (non-fat dry milk or bovine serum albumin) was carried out.

Immunodetection of AT8 epitope were obtained by overnight incubation at 4 °C with the specific primary antibody AT8 (Thermo Scientific, Cat. MN1020) to detect pSer202/Thr205. After PBS rinses, specimens were incubated at 37 °C for 1 h with FITC-conjugated sheep anti-mouse secondary antibody (Sigma-Aldrich, 1:100 dilution in blocking solution).

Experiments were repeated at least three times. Images were recorded with confocal laser scanning microscope (CLSM) Zeiss LSM700 equipped with 40x and 63x objectives. ZEN 2010 software was used for image acquisition. Cell counting and statistical analyses were performed as previously described [14]. In details, cells were counted on 0.5-μm scanned images for the immunofluorescence data obtained with CLSM. Three to six cases per group were analyzed.

3. Results

Indirect immunofluorescence (IIF) experiments were performed on sections of human brain tissue from the CA1 region of the hippocampus at different ages, from the fetal brain to the senile brain, further to brain tissue sections of subjects with AD at the first stage. IIF analysis, to detect cells with AT8 epitope, were performed by using confocal laser scanning microscopy (CLSM). The brain sections detected, and more precisely the CA1 region of the hippocampus, were analyzed by counting the number of neurons showing the presence of AT8 in the nucleolus respect to the total number of neurons. This should define the percentage of undifferentiated neurons, being these neurons that not showing AT8, as we previously demonstrated in the replicative neuroblastoma SK-N-BE cells or after differentiation by retinoic acid treatment. In the case the degenerative neuronal cells correspond to the cells with an ectopic restart of the cell cycle, the degenerated neurons in the CA1 region of the brain from AD subjects should be identified by the absence of AT8 epitope.

Our data indicated an increasing percentage of AT8 positive cells from fetus to young samples. On the contrary, the percentage of AT8 positive cells largely decreases from young to senile or AD-I brain tissue sections. The number of neurons with AT8 epitope showed a statistically significant decrease from young to senile brain. The same result was obtained in AD-I samples, with the percentage of positive AT8 cells in the nucleolus decreasing from young to AD-I and from senile to AD-I. These data indicate a significant decrease of differentiated cells from young to senile or AD-I brain (CA1 region of the hippocampus).

4. Discussion

We previously described, in the human neuroblastoma SK-N-BE cell line, a specific expression of the AT8 epitope in the replicative vs differentiated cells [10]. Indeed, AT8 is absent in the SK-N-BE replicative cells and present in the nucleolus of these cells after differentiation induced by retinoic acid treatment. The disappearance of AT8 from the nucleus of differentiated cells is related to the activation of the replicative cell cycle, a condition that seems one of the first events leading to the AD, as previously suggested by other authors [3,17], namely neurons degenerate because an ectopic cell cycle starts in the differentiated cells.

To demonstrate this hypothesis, we analyzed the presence of AT8 in the neurons of the region involved in the AD, namely the CA1 region of the hippocampus. Coherent with this hypothesis, AT8 disappears in a significant number of neurons from young to senile and to AD-I brain, indicating that a statistically significant number of neurons from the CA1 region tried to re-enter in replicative status, starting cell degeneration.

AT8 epitope corresponds to the pSer202/pThr205 region of tau protein, a region of tau protein that seems relevant in its interaction with DNA, and conformational changes related to this phosphorylation can induce chromatin alteration and cell degeneration [10,15]. Indeed, the presence of phosphorylation in the AT8 region is related to the nucleolus and cell differentiation functionality. Here we showed Tau-1 epitope located in the nucleolus of neuronal cells of the hippocampus CA1 region in all ages analyzed and in AD cases, and that AT8 gradually disappears in old age as well as in the replicative neuroblastoma cell line.

The present results improve the knowledge on the role of nuclear tau in the neuronal differentiation and cell degeneration that happens in the AD, a role that seems to involve the presence/absence of AT8 epitope in the nucleolus of neurons, and a re-entering in the cell cycle that seems to have a central role in the start of AD. This event seems to happen very early during neuronal degeneration, and understanding when and how this occurs in neuronal cells can be considered a relevant progress non only for the use of AT8 as an early AD biomarker, but also to search natural or synthetic compounds pharmacologically active against ectopic cell cycle restart related to the AT8 epitope.

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