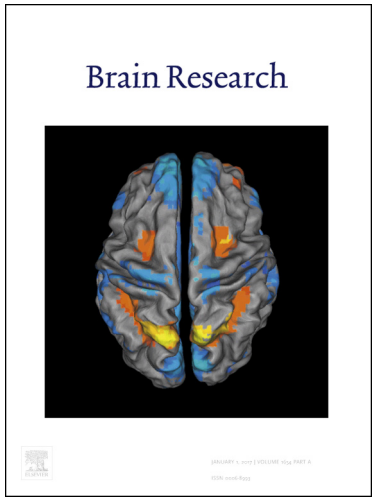

Research report

Aging dependent effect of nuclear tau

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PII: S0006-8993(17)30427-4
DOI: https://doi.org/10.1016/j.brainres.2017.09.030
Reference: BRES 45507

To appear in: *Brain Research*

Received Date: 5 May 2017
Revised Date: 11 August 2017
Accepted Date: 25 September 2017

Please cite this article as: L. Gil, C. Federico, F. Pinedo, F. Bruno, A.B. Rebolledo, J.J. Montoya, I.M. Olazabal, I. Ferrer, S. Saccone, Aging dependent effect of nuclear tau, *Brain Research* (2017), doi: https://doi.org/10.1016/j.brainres.2017.09.030

11 august 2017

Aging dependent effect of nuclear tau

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Running title: nuclear tau and cell aging

Key words: Aging, nuclear tau, AT100, Alzheimer disease, neurons, epithelial cells, colorectal mucosa, breast acinar cells, cancer, confocal microscopy, aging marker

ABSTRACT

Tau protein is characterized by a complex pattern of phosphorylation and is localized in the cytoplasm and nucleus in both neuronal and non-neuronal cells. Human AT100 nuclear tau, endowed by phosphorylation in Thr212/Ser214, was recently shown to decline in *cornus ammonis 1* (CA1) and *dentate gyrus* (DG) in Alzheimer's disease (AD), but a defined function for this nuclear tau remains unclear. Here we show that AT100 progressively increases in the nuclei of neuronal and non-neuronal cells during aging, and decreases in the more severe AD stages, as recently shown, and in cancer cells (colorectal adenocarcinoma and breast cancer). AT100, in addition to a co-localization with the DAPI-positive heterochromatin, was detected in the nucleolus of pyramidal cells from the CA1 region, shown to be at its highest level in the more senescent cells and in the first stage of AD (ADI), and disappearing in the more severe AD cases (ADIV). Taking into account the nuclear distribution of AT100 during cell aging and its relation to the chromatin changes observed in degenerated neurons, as well as in cancerous cells, which are both cellular pathologies associated with age, we can consider the Thr212/Ser214 phosphorylated nuclear tau as a molecular marker of cell aging.

1. Introduction

Cell degeneration and uncontrolled cell proliferation are two different paths of cell fate specifically related to diseases associated with aging, such as Alzheimer's Disease (AD) and cancer, respectively (**Ferrer 2012**; **Navarrete-Reyes *et al*., 2016**). A number of studies have shown an inverse association between neurodegeneration and cancer; namely, patients with AD very rarely showed cancer pathologies, and conversely patients with a cancer history showed a very low risk for AD (**Driver, 2014**). Moreover, other evidence indicates that AD and cancer seem to be related to similar molecular mechanisms, and modification of the nuclear chromatin organization possibly affects both pathologies (**Cruickshanks and Adams, 2011**).

For decades, tau has been considered a cytoplasmic tubulin binding neuro-protein, which plays an important role in the regulation of the microtubular network and in axonal formation (**Povlishock *et al*., 1999**). It has been found to be directly involved in the etiology of neurodegenerative disorders related to age, such as AD and other taupathies (**Panegyres and Zafiris-Toufexis, 2002**). However, more recently, the presence of tau has been demonstrated in non-neuronal cells, such as fibroblasts and lymphocytes, as well as in normal and transformed cell lines from different tissues (**Cross *et al*., 2000; Souter and Lee 2009**). Moreover, its expression level in several human cancers has been related to resistance to anti-mitotic treatments **(Rouzier *et al*., 2005; Smoter *et al*., 2013)**.

In vitro experiments have demonstrated tau´s interaction with DNA and a number of studies have shown that tau protein binds to the minor groove of dsDNA in an unspecific manner via the proline-rich domain (PRD) and the microtubule-binding domain (MTBD) (**Wei *et al*., 2008; Qi *et al*., 2015**) and that presents, by nuclear magnetic resonance

spectroscopy, multiple points of contact both in AT-rich and GC-rich 22 bp oligonucleotides (**Qi *et al.*, 2015**). A specific binding of tau to AT-rich satellite DNA sequences in the nucleolus of human fibroblasts and HeLa cells was previously shown (**Sjöberg *et al.*, 2006**), in addition to binding of α-synuclein and tau to a GC-rich DNA oligonucleotide in the B- and Z-forms (**Vasudevaraju *et al.*, 2012**). This indicates a possible role for tau in nucleolar organization and gene regulation. Moreover, along this line of evidence, *in vitro* and *in vivo* experiments have revealed that the binding of tau to DNA plays a protective role in oxidative stress (**Wei *et al.*, 2008**) and heat-stress conditions (**Violet *et al.*, 2014**), and enhances chromosome stability (**Rossi *et al.*, 2008**), with the level of protection dependent on tau concentration (**Hua and He, 2003**).

The phosphoepitope AT100, which recognizes the phosphorylated Thr212/Ser214 residues, sequentially generated by glycogen synthase kinase and protein kinase A and induced *in vitro* by polyanions such as heparin and RNA (**Zheng-Fischhöfer *et al.*, 1998**), has been reported to be located in the nucleus of human (**Hernandez-Ortega *et al.*, 2015**) and mouse (**Gartner *et al.*, 1998**) neurons, and of cultured fibroblasts (**Rossi *et al.*, 2008**). However, in AD neurons, AT100 progressively decreases from the nuclei disappearing in neurons of advanced stages of AD (**Hernandez-Ortega *et al.*, 2015**), suggesting a strict relationship between Thr212/Ser214 phosphorylated tau with heterochromatin loss, global chromatin relaxation and gene expression deregulation in AD (**Mansuroglu *et al.*, 2016**).

The tau protein has not only been related to AD but also to cancer, both age-related diseases, and it has been suggested that aging may activate similar pathways in both (**Souter and Lee, 2009**). It is well known that nuclear aging is accompanied by chromatin

damage and genomic instability associated with changes in chromatin structure, such as heterochromatin loss (**Pegoraro and Misteli, 2009**).

The aim of the present study was to gain insight into the function of nuclear tau in relation to the age-related chromatin modification in neurons and epithelial cells, two cell type with different physiological and morphological features, and to their aging related diseases, namely Alzheimer's Disease (AD) and cancer respectively. We analyzed the cell distribution of the tau protein identified by the AT100 antibody, which recognizes phosphorylated Thr212/Ser214 residues. AT100 was used on human cells from healthy and degenerative tissues (brain, intestinal, and breast samples) at different aging stages, showing a progressive increase in the nucleus, during aging, especially in human senile hippocampal neurons. Moreover, in cancer cells AT100 signal almost disappears from the nucleus as well as in the AD cell nuclei, as recently shown in the case of AD neurons (**Hernandez-Ortega *et al*., 2015**). We also observed a nucleolar location of AT100 in the pyramidal cells from the CA1 region, not previously detected. The potential participation of the tau protein in the age-related change of chromatin organization is discussed.

2. Results

The nuclear tau protein was investigated here using the antibody that recognizes the AT100 epitope, corresponding to tau with phosphorylation at the Thr212/Ser214 residues. This antibody was previously shown to detect tau protein in the nucleus from mouse and human brain cells (**Gartner et al., 1998; Hernandez-Ortega et al., 2015**). The cell distribution of AT100 was performed in four different human cell types at different aging stages: pyramidal neurons from the CA1 region, granular neurons from the *dentate gyrus* (GD), epithelial cells from colorectal mucosa and from acinar cells. We also analyzed the distribution of AT100 in pyramidal neurons from CA1 region of subjects with AD, and in tissue sections of colorectal adenocarcinoma, or breast cancer (see **suppl. Tab. 1** for description of the cases used).

2.1. AT100 in the human neurons from the CA1 region

We analyzed, by classical immunohistochemistry and by immunofluorescence analysis with confocal laser scanning microscopy (CLSM) of brain sections, the distribution of AT100 tau epitope in the CA1 region of the hippocampus. We observed AT100 in the nucleus of the pyramidal neurons with a progressively, statistically significant, increasing amount from healthy young to senile (**Fig. 1**). More precisely, AT100 is very low and in few cells in younger samples and progressively increases in the adult ages, reaching the highest levels at the senile stage (**Fig. 1H** and **Fig. 1I**). In this latter stage, AT100 is very evident in relation to the more compact chromatin, especially around the nucleolar region of the pyramidal cells (**Fig. 1F** and **Fig. 1G**). In the adult and

senile stages, a weak AT100 signal was seen in the cytoplasmic compartment in the initial part of the axonal dendrites (**Fig. 1**).

Immunofluorescence and CLSM analyses refined the nuclear distribution indicated above. AT100 co-localized with the more compact chromatin (DAPI-positive) and presented a detectable amount in the nucleolus of pyramidal cells from the CA1 region, as demonstrated by the co-localization with the nucleolar marker *upstream binding factor* (UBF) (**Fig. 2**). AT100 progressively increases in the nucleus, and more specifically in the nucleolus of the neurons from young to senile subjects and in the first stages of AD (ADI, classification according to Braak and Braak, 1991), disappearing from the nucleus of degenerated pyramidal cells at the AD-IV stage, corresponding to those cells with a high amount of neurofibrillar tangles (NFT) (**Fig. 2**). The difference of AT100 presence in the nuclei of ADI neurons respect to senile is not statistically significant (**Fig. 2H**).

In summary, the presence of AT100 in the nucleus increases through aging reaching the highest levels in senile neurons. The increase of AT100 is statistically significant considering the amount of protein in the nucleus (**Fig. 1I**) as well as considering the number of cells containing AT100 (**Fig. 1H** and **Fig. 3**). However, as has been previously described (**Hernandez-Ortega *et al*., 2015**), in AD neurons dramatic changes occur at nuclear AT100 that progressively disappears from the early stage. In more advanced stages, such as in ADIV, the AT100 signal disappears from the nuclear and nucleolar chromatin, concomitantly with its appearance in the cytoplasm forming the pathologic NFTs, even if other neurons lack AT100 in the nucleus and cytoplasm (**Fig. 2E**).

2.2. AT100 in the human neurons from the DG region

The presence of AT100 in the DG region in healthy young and senile samples was clearly visible, with a larger amount in the senile granular neurons compared to the younger ones (**Fig. 4**). However, AT100 did not reach the same level observed in the nuclei of the senile pyramidal neurons (see **Fig. 1I** and **Fig. 4H**). In senile granular neurons, AT100 forms large accumulation blocks, which are very evident at the periphery of the nucleus, a nuclear compartment largely corresponding to the GC-poor chromatin. No evident signals were detected in the cytoplasm of these cells. AT100 signals were not detectable, or detectable at very low amount, in fetal neurons (**Fig. 4**).

These results show a similar pattern of nuclear AT100 distribution in DG and CA1 neuronal cells, with an evident colocalization of AT100 with nuclear heterochromatin and its progressive increase in the nucleus related to age. However, the very high amounts observed in the senile pyramidal neurons are not seen in the granular neurons.

2.3. AT100 in the replicative human neoplastic and non-neoplastic cells

The AT100 epitope was detected in the nucleus of healthy young and senile replicative epithelial cells from intestinal mucosa (**Fig. 5**) and breast acinar cells (**Fig. 6**). In both types of cells, AT100 presents a distribution of scattered spots throughout the nuclear chromatin, prevalently at the nuclear periphery. This is more evident in the senile epithelial cells of colorectal mucosa where AT100 increases about four times respect to the normal young epithelial cells (**Fig. 4G**), forming larger blocks of signal. In the nucleus of the colorectal neoplastic cells, the signal amount of AT100 generally decreases at the level of the young epithelial cells but remains in some large, more compact spots that occupy nearly the entire periphery of the nucleus (**Fig. 4G**). However, in the neoplastic cells of

breast carcinoma the AT100 staining significantly increases in the degree I of the carcinoma (about five times respect to the healthy breast) and decreases in malignant cells of degree III (**Fig. 5J**). In cytoplasm from young and senile non-neoplastic epithelial cells of colorectal mucosa the presence of AT100 protein is low, and in the neoplastic cells it is always absent. A very similar situation in the cytoplasm was observed in breast cells, both neoplastic and non-neoplastic.

3. Discussion

During cellular aging, alterations in the organelle function are progressively shown to be associated with biological wearing away and environmental stress. These alterations are related to changes in chromatin organization, which directly affects proper gene expression, suggesting that nuclear alteration could contribute to aging (**Feser and Tyler, 2011; Cruickshanks and Adams, 2011**). Tau is a relevant protein involved in the axonal formation and cytoplasmic aggregates NFT specifically observed in the age-related Alzheimer's disease (**Ayyadevara *et al.*, 2016**), but has also been biochemically demonstrated in the cell nuclei and by immunofluorescence in the nucleolus (**Sjöberg *et al.*, 2006; Bukar Maina *et al.*, 2016**). Here, we studied by immunohistochemistry and immunofluorescence the AT100 epitope of nuclear tau and its relation to cell aging and to aging pathologies.

3.1. Nuclear tau during cell aging

We detected AT100, the nuclear tau endowed by phosphorylation in Thr212/Ser214, in the nucleus of neuronal and epithelial cells, in agreement with some previous results showing AT100 in the nucleus of mouse and human neurons (**Gartner et al, 1998; Hernandez-Ortega *et al.*, 2015**), and of cultured fibroblasts (**Rossi *et al.*, 2008**). In addition, our results showed that the amount of AT100 increases with age, reaching its maximum level at the senile stage. Moreover, it co-localizes with the most intense DAPI staining that corresponds to the most compact chromatin prevalently present at the periphery of the nucleus, adjacent to the nuclear membrane, and containing DNA largely composed of the very GC-poor, and very gene-poor, L1 isochores (**Bernardi 2015**). The distribution of the chromatin in the nucleus according to the GC-level is evolutionarily conserved and related to the nuclear chromosomal organization (**Federico *et al.*, 2017**), determining an ordered gene positioning relevant for a number of cell activities, both normal (e.g., cell differentiation) and abnormal (e.g., the onset of genetic diseases) (**Ballabio *et al.*, 2009; Leotta et al., 2014**). We also detected a nucleolar location of AT100 in the pyramidal cells of the CA1 region, according to previous demonstration that tau interacts with the nucleolar repetitive alpha satellite DNA, in the AT rich sequences

(**Sjöberg *et al.*, 2006**), further indicating a correlation between tau and heterochromatin. Statistical evaluation of results indicated that tau increases during aging not only in the amount of protein present in the nuclei, but also in the number of cells with detectable amount of AT100, reaching its maximum levels at the senile stage.

Our data indicate a possibly different type of interaction between AT100 and nuclear DNA depending on whether the cell is young or senile. Moreover, the increase in the AT100 interaction with heterochromatin seems to be strikingly different in aging epithelial cells and granular neurons compared to pyramidal neurons of the CA1 region, possibly related to the differences in the proliferative *vs* post-mitotic status of the cells characterizing the epithelial and the DG tissues respect to CA1 (**von Bohlen und Halbach, 2011**). In the former case, AT100 forms large blocks of protein accumulation whereas in the latter it is more diffuse in the nuclei with few large blocks of accumulation, particularly in relation to the nucleolus. Thus, the reprogramming of chromatin organization during aging seems to be different in proliferative versus post-mitotic or differentiated cells.

Considering the above observations, we can speculate on a possible involvement of tau, and specifically of the AT100 epitope, in the heterochromatin organization and or protection during cell life. We propose that the AT100 phosphoepitope could interact with repetitive DNA from interphasic chromatin to maintain the more compacted status, thus increasing in amount with the increasing level of mutation/alteration affecting the more compact chromatin positioned at the nuclear periphery during aging (**Feser and Tyler, 2011**), interacting and protecting heterochromatin in the case of aged cells. Moreover, a high degree of heterochromatic pattern in rDNA is a normal condition in differentiated cells (**Sanij and Hannan, 2009**; **Takada and Kurisaki, 2015**), and the presence of tau in this context could enhance and protect this condition.

3.2. Nuclear tau and aging pathologies

AT100, further to the increase in the nucleus during cell aging, drastically decreases in derived pathological conditions such as AD and cancer. Recently, the work of **Mansuroglu *et al.* (2016)** and **Hernandez-Ortega *et al.* (2015)** have revealed a relationship between nuclear tau and conformational changes in global chromatin in

pyramidal neurons of early AD, which is maintained throughout the development of the disease. It is also known the dramatic nuclear change takes place between aging neurons and AD neurons, possibly related to an aberrant attempt to re-enter into the cell cycle associated with aging stress (**Zhu *et al*., 1999**) and these events may begin the neurodegenerative process associated with age (**McShea *et al*., 2007**). In this sense, the changes observed in the nuclear tau distribution in aging cells with respect to younger cells, and even more so with AD neurons, could be associated to modifications of the chromatin organization determining alterations in gene expression (**Tan *et al*., 2010; Arhondakis and Kossida, 2011**).

A number of studies have demonstrated the presence of non-phosphorylated tau (Tau-1) in the nucleolus (**Loomis et al., 1990; Thurston et al., 1996, 1997; Sjoberg et al., 2006; Rossi et al., 2008**), and now we observed for the first time a nucleolar location of AT100 in the pyramidal cells of the CA1 region, that could indicate a further involvement in chromatin organization, in this case related to the rDNA sequences, as the loss of nucleolar heterochromatin has been shown to trigger accelerated aging and that heterochromatin prevents recombination between rDNA repeats, thereby preserving the nucleolar structure (**Sinclair and Guarente, 1997; Peng and Karpen, 2007**). Besides, during the development of AD we observed that, at the start of disease (stage I), the nucleolar AT100 increases in consonance with the nucleolar hypertrophy described in hippocampal neurons, while the evident reduction in nucleolar volume observed in stages IV-V matches the absence of AT100 in the nucleolus (**Iacono *et al*., 2008**). Globally, the data presented may suggest that the absence of AT100 at the more severe AD stage could be related to the loss of cell control on the genomic DNA and more specifically, in the maintenance of the correct heterochromatic patterns in the cell nuclei, including the nucleolus (**Falk *et al*, 2014**).

In epithelial cells the chromatin dynamics during aging are manifested differently, but it is widely accepted that aging stress can lead to tumoral development (**Serrano and Blasco, 2007**). Our results show for the first time a regression of the amount of nuclear tau in cancer cells, especially in the more severe stages, compared to senile cells or to the less severe stage of the disease. Until now, research performed on tau and cancer have focused on the expression levels in relation to the cytoplasmic microtubular function in cell

division and to the anti-mitotic activity of taxol (**Rouzier *et al.*, 2005; Smoter *et al.*, 2013**). Nevertheless, it has been shown that a large proportion of tau does not bind MTs in prostate cancer cells, suggesting a different role for tau in cancer cells (**Souter and Lee, 2009**). The presence of AT100 in the nucleus of the colorectal and breast carcinoma neoplastic cells should encourage new investigations to understand the function of nuclear tau and whether this is related in the change of chromatin organization in tumoral cells (**Carone and Lawrence, 2013**).

3.3 Conclusions

In this work we showed for the first time a gradual accumulation of nuclear tau in human cells during aging and its general co-localization with the DAPI-positive heterochromatin. This could indicate a possible role of nuclear tau in the organization and/or protection of chromatin in the cells during aging (**Pegoraro and Misteli, 2009**). Moreover, this seems to be related to aging pathologies (neurodegenerative or cancerous diseases), where nuclear AT100 decreases drastically, a condition very evident in the more severe stages of the diseases. Anyway, further investigation is needed to better understand the role of phosphorylated AT100 nuclear tau in chromatin aging.

4. Experimental procedures

4.1. Tissue samples

Brain samples were obtained from the Institute of Neuropathology HUB-ICO-IDIBELL Biobank. Colon mucosa, appendiceal, breast cancer and colorectal adenocarcinoma samples were obtained from Department of Pathology of Hospital Universitario Fundación Alcorcón (HUFA) (see **suppl. Tab. 1**). These patients had not received previous radiation or chemotherapy. All procedures performed in the present study, involving human participants, 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.

Tissues and sections were prepared as previously described (**Hernandez-Ortega *et al*, 2015**). The brain regions examined in the present study were the CA1 region of the hippocampus and the Dentate Gyrus. Non-neoplastic colon mucosa and adenocarcinoma tissues from the appendix and colon were selected to compare. Age groups were: young (between 20 to 40 years), adult (between 40 to 60 years) and senile (more than 60 years).

4.2. Immunohistochemistry

Immunohistochemical localization of the phosphorylated tau at Thr212/Ser214 residues was carried out with the AT100 antibody (Thermo Fisher Scientific, Rockford, USA) on 4 µm paraffinated sections. Tissue sections were dewaxed using standard protocols. Antigen retrieval was performed using a low pH 6.0 citrate buffer (Dakocytomation, Glostrup, Denmark) at 96 °C for 20 minutes in a Dako PTLink

(Dakocytomation, Glostrup, Denmark) automated retrieval unit. The procedure was carried out with a Dako Autostainer Link48 (Dakocytomation, Glostrup, Denmark).

Briefly, after blocking of endogenous peroxidases with Dako Peroxidase Blocking Reagent (DakoCytomation, Glostrup, Denmark), and a pre-incubation step of 1 hour at 37°C with a blocking solution containing bovine serum albumin, specimens were incubated with primary antibody AT100 diluted 1:100 in Dako Antibody Diluent (Dakocytomation Glostrup, Denmark) for 30 minutes at room temperature. The staining was visualized using EnVision FLEX+mouse (linker) (DakoCytomation Glostrup, Denmark), and with 3'3-diaminobenzidine tetrahydrochloride (DAB) chromogenic reagent and hematoxylin counterstain. All incubation steps were performed at room temperature and between incubations sections were washed with Dako wash buffer. The IHC staining includes a positive control for the AT100 antibody.

4.3. Immunofluorescence and CLSM analyses

Paraffin-embedded sections for immunofluorescence experiments were treated as previously described (**Oliveira *et al.*, 2010; Scuderi *et al.*, 2014**). Briefly, they were placed at 55° C for 20 minutes, dewaxed 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 primary antibody, a pre-incubation step of 1 hour at 37°C with blocking solution (non-fat dry milk or bovine serum albumin) was carried out. Double immunodetections were obtained by incubation with specific primary mouse AT100 (Thermo Fisher Scientific, Rockford, USA, 1:100 dilution) and goat UBF-1 (Thermo

Fisher Scientific, Rockford, USA 1:100 dilution) antibodies. After PBS rinses, specimens were incubated with FITC-conjugated anti-mouse and TRITC-conjugated anti-goat secondary antibodies (Sigma-Aldrich, 1:700 dilution). Experiments were repeated at least three times to confirm results. Images were obtained with a confocal laser scanning microscope (CLSM) Zeiss LSM700 equipped with 40x and 63x objectives. ZEN2010 software was used for image acquisition and 3D reconstructions.

4.4. Cell counting and statistical analysis

Cell counting and statistical analyses were performed as previously described (**Hernandez-Hortega *et al.*, 2015**). In details, cells were counted on 4-µm-thick sections for the immunohistochemistry, and on 0.5-µm scanned images for the immunofluorescence data obtained with CLSM. Three to six cases per group were analyzed. The number of AT100 positive cells was obtained in three optical fields at 400X magnification; the total number and the percentage of AT100 positive cells per each section were taken in consideration. Intensity level of AT100 signal was evaluated using the ImageJ software (NIH, Bethesda, MD; available at http://rsb.info.nih.gov/ij/index.html). Mean data were analyzed with one-way ANOVA followed by Tukey's test post hoc analysis or by two tails T-test. Differences between groups were considered significant at *P<0.05, **P<0.01 and ***P<0.001.

Acknowledgements

Authors thank Giorgio Bernardi, Maite Serra, Salvatore Motta for the encouragement to research and critical reading of the manuscript. Biobanco of Hospital Universitario Fundación Alcorcón for providing biological samples. Foundation Universidad Alfonso X el Sabio (FUAX), and University of Catania for financing the research project.

Author contributions

LG, CF, SS designed and planned the experiments, analyzed the data and wrote the manuscript. LG, CF, FB, SS performed immunofluorescence and confocal microscopy analyses. LG, ABR performed immunohistochemistry. ABR prepared the cancer and brain slices. FP, IF enrolled the patients with AD and cancer. FP, IMO, JJM, IF contributed to the interaction analyses and manuscript writing.

References

Arhondakis S, Kossida S (2011) Compositional perspectives on human brain aging. *Biosystems* **104**, 94-98.

Ayyadevara S, Balasubramaniam M, Parcon PA, Barger SW, Griffin WST, Alla R, Tackett AJ, Mackintosh SG, Petricoin E, Zhou W, Reis RJ (2016) Proteins that mediate protein aggregation and cytotoxicity distinguish Alzheimer's hippocampus from normal controls. *Aging cell*15, 924-939.

Ballabio E, Cantarella CD, Federico C, Di Mare P, Hall G, Harbott J, Hughes J, Saccone S, and Tosi S (2009) Ectopic expression of the HLXB9 gene is associated with an altered nuclear position in t(7;12) leukaemias. *Leukemia* **23**, 1179-1182.

Bernardi G (2015) Chromosome architecture and genome organization. *Plos One* **10**, e0143739.

Braak H, Braak E (1991) Neuropathological staining of Alzheimer-related changes. *Acta Neuropathol.* **82**, 239-259.

Bukar Maina M, Al-Hilaly YK, Serpell LC (2016) Nuclear tau and its potential role in Alzheimer's disease. *Biomolecules* **6**, 9.

Carone DM, Lawrence JB (2013) Heterochromatin instability in cancer: from the Barr body to satellites and the nuclear periphery. *Semin Cancer Biol.* **23**, 99-108.

Cross DC, Munoz JP, Hernandez P, Maccioni RB (2000) Nuclear and cytoplasmic tau proteins from human nonneuronal cells share common structural and functional features with brain tau. *J Cell Biochem.* **78**, 305–317.

Cruickshanks HA, Adams PD (2011) Chromatin: a molecular interface between cancer and aging. *Curr Opin Genet Dev.* **21**, 100–106.

Driver JA (2014) Inverse association between cancer and neurodegenerative disease: review of the epidemiologic and biological evidence. *Biogerontology* **15**, 547-557.

Falk M, Lukášová E, Štefančíková L, Baranová E, Falková I, Ježková L, Davídková M, Bačíková A, Vachelová J, Michaelidesová A, Kozubek S (2014) Heterochromatinization associated with cell differentiation as a model to study DNA double strand break induction and repair in the context of higher-order chromatin structure. *Appl Radiat Isot.* 83PtB, 177-185.

Federico C, Pappalardo AM, Ferrito V, Tosi S, Saccone S (2017) Genomic properties of chromosomal bands are linked to evolutionary rearrangements and new centromere formation in primates. *Chromosome Res* **in press**. DOI: https://doi.org/10.1007/s10577-017-9560-1.

Ferrer I (2012) Defining Alzheimer as a common age-related neurodegenerative process not inevitably leading to dementia. *Prog Neurobiol* **97**, 38-51.

Feser J, Tyler J (2011) Chromatin structure as mediator of aging. *FEBS Letters* **585**, 2041-2048.

Gartner U, Janke C, Holzer M, Vanmechelen E, Arendt T (1998) Postmortem changes in the phosphorylation state of tau-protein in the rat brain. *Neurobiol Aging* **19**, 535-543.

Hernández-Ortega K, Garcia-Esparcia P, Gil L, Lucas JJ, Isidre Ferrer (2015) Altered machinery of protein synthesis in Alzheimer's: from the nucleolus to the ribosome. *Brain Pathology* doi:10.1111/bpa.12335.

Hua Q, He RQ. (2003) Tau could protect DNA double helix structure. *Biochim Biophys Acta* **1645**, 205-211.

Iacono D, O´Brien R, Resnick SM, Zonderman AB, Pletnikova O, Rudow G, An Y, West MJ, Crain B, Troncoso JC (2008) Neuronal hypertrophy in asymptomatic Alzheimer disease. J. Neuropathol. *Exp. Neurol.* **67**, 578-589.

Leotta CG, Federico C, Brundo MV, Tosi S, Saccone S (2014) *HLXB9* gene expression, and nuclear location during *in vitro* neuronal differentiation in the SK-N-BE neuroblastoma cell line. *PLoS ONE* 9(8): e105481.

Loomis PA, Howardt TH, Castleberryt RP, and Binder LI. (1990) Identification of nuclear τ isoforms in human neuroblastoma cells. *Proc. Natl. Acad. Sci USA* **87**: 8422-8426.

Mansuroglu Z, Benhelli-Mokrani H, Marcato V, Sultan A, Violet M, Chauderlier A, Delattre L, Loyens A, Talahari S, Bégard S, Nesslany F, Colin M, Souès S, Lefebvre B, Buée L, Galas MC, Bonnefoy E. (2016) Loss of Tau protein affects the structure, transcription and repair of neuronal pericentromeric heterochromatin. *Sci Rep* **6**, 33047. Doi: 10.1038/srep33047.

McShea A, Lee HG, Petersen RB, Casadesus G, Vincent I, Linford NJ, Funk JO, Shapiro RA, Smith MA (2007) Neuronal cell cycle re□entry mediates Alzheimer disease□type changes. *Biochim Biophys Acta* **1772**, 467-472.

Navarrete-Reyes AP, Soto-Pérez de Celis E, Hurria A (2016) Cancer and Aging: a complex biological association. *Rev Invest Clin.* **68**,17-24.

Oliveira VC, Carrara RC, Simoes DL, Saggioro FP, Carlotti CG Jr, Covas DT, Neder L (2010) Sudan Black B treatment reduces autofluorescence and improves resolution of in situ hybridization specific fluorescent signals of brain sections. *Histol Histopathol* **258**, 1017-1024.

Panegyres PK, Zafiris-Toufexis K (2002) Polymorphisms in the tau gene in sporadic frontotemporal dementia and other neurodegenerative disorders. *Eur J Neurol* **9**, 485-489.

Pegoraro G, Misteli T (2009) The central role of chromatin maintenance in aging. *Aging (Albany NY)* **1**, 1017-1022.

Peng JC, Karpen GH (2007) H3K9 methylation and RNA interference regulate nucleolar organization and repeated DNA stability. *Nat Cell Biol* **9**, 25-35.

Povlishock JT, Buki A, Koiziumi H, Stone J, Okonkwo DO (1999) Initiating mechanisms involved in the pathobiology of traumatically induced axonal injury and interventions targeted at blunting their progression. *Acta Neurochir Suppl* **73**, 15-20.

Qi H, Cantrelle FX, Benhelli-Mokrani H, Smet-Nocca C, Buée L, Lippens G, Bonnefoy E, Galas MC, Landrieu I (2015) Nuclear magnetic resonance spectroscopy characterization of interaction of Tau with DNA and its regulation by phosphorylation. *Biochemistry* **54**:1525-1533.

Rossi G, Dalprà L, Crosti F, Lissoni S, Sciacca FL, Catania M, Di Fede G, Mangieri M, Giaccone G, Croci D, Tagliavini F (2008) A new function of microtubule-associated protein tau: involvement in chromosome stability. *Cell Cycle* **7**, 1788-1794.

Rouzier R, Rajan R, Wagner P, Hess KR, Gold DL, Stec J, Ayers M, Ross JS, Zhang P, Buchholz TA, Kuerer H, Green M, Arun B, Hortobagyi GN, Symmans WF, Pusztai L (2005) Microtubule-associated protein tau: a marker of paclitaxel sensitivity in breast cancer. *Proc Natl Acad Sci USA* **102**, 8315-8320.

Sanij E, Hannan RD (2009) The role of UBF in regulating the structure and dynamics of transcriptionally active rDNA chromatin. *Epigenetics* **4**, 374-382. DOI: 10.4161/epi.4.6.9449

Scuderi S, D'Amico, AG, Castorina A, Federico C, Marrazzo G, Drago F, Bucolo C, D'Agata, V. (2014) Davunetide (NAP) protects the retina against early diabetic injury by reducing apoptotic death. *J Mol Neurosci* **54**, 395-404.

Serrano M, Blasco MA (2007) Cancer and ageing: convergent and divergent mechanisms. Nat *Rev Mol Cell Biol* **8**, 715-522.

Sinclair DA, Guarente L (1997) Extrachromosomal rDNA circles a cause of aging in yeast. *Cell* **91**, 1033-1042.

Sjöberg MK, Shestakova E, Mansuroglu Z, Maccioni RB, Bonnefoy E (2006) Tau protein binds to pericentromeric DNA: a putative role for nuclear tau in nucleolar organization. *J Cell Sci* **119**, 2025-2034.

Smoter M, Bodnar L, Grala B, Stec R, Zieniuk K, Kozlowski W, Szczylik C (2013) Tau protein as a potential predictive marker in epithelial ovarian cancer patients treated with paclitaxel/platinum first-line chemotherapy. *J Exp Clin Cancer Res* **32**, 25.

Souter S, Lee G (2009) Microtubule-associated protein Tau in human prostate cancer cells: isoforms, phosphorylation and interactions. *J Cell Bioch* **108**, 555-564.

Takada H, Kurisaki A (2015) Emerging roles of nucleolar and ribosomal proteins in cancer, development, and aging. *Cell Mol Life Sci* **72**, 4015–4025. DOI: 10.1007/s00018-015-1984-1.

Tan MG, Chua WT, Eisiri MM, Smith AD, Vinters HV, Lai MK (2010) Genome wide profiling of altered gene expression in the neocortex of Alzheimer's disease. *J Neurosci Res* **88**, 1157-1169.

Thurston VC, Pena P, Pestell R, Binder LI (1997) Nucleolar localization of the Microtubule-Associated Protein Tau in neuroblastomas using sense and anti-sense transfection strategies. *Cell Motility and the Cytoskeleton* **38**, 100–110.

Thurston VC, Zinkowski RP, Binder LI (1996) Tau as a nucleolar protein in human nonneural cells *in vitro* and *in vivo*. *Chromosoma* **105**, 20-30.

Vasudevaraju P, Guerrero E, Hegde ML, Collen TB, Britton GB, Rao KS (2012) New evidence on α-synuclein and Tau binding to conformation and sequence specific GC rich DNA: relevance to neurological disorders. *J Pharm Bioallied Sci* **4**, 112-117.

Violet M, Delattre L, Tardivel M, Sultan A, Chauderlier A, Caillierez R, Talahari S, Nesslany F, Lefebvre B, Bonnefoy E, Buée L, Galas MC (2014) A major role for Tau in neuronal DNA and RNA protection *in vivo* under physiological and hyperthermic conditions. *Front Cell Neurosci* **8**:84.

Von Bohlen und Halbach O. (2011) Immunohistological markers for proliferative events, gliogenesis, and neurogenesis within the adult hippocampus *Cell Tissue Res* **345**, 1-19. DOI: 10.1007/s00441-011-1196-4.

Wei Y, Qu MH, Wang XS, Chen L, Wang DL, Liu Y, Hua Q, He RQ (2008) Binding to the minor groove of the double-strand, tau protein prevents DNA from damage by peroxidation. *PLoS One* **3**, e2600.

Zheng-Fischhöfer Q, Biernat J, Mandelkow E-M, Illenberger S, Godemann R, Eckhard Mandelkow E (1998) Sequential phosphorylation of Tau by glycogen synthase kinase-3β and protein kinase A at Thr212 and Ser214 generates the Alzheimer-specific epitope of antibody AT100 and requires a paired-helical-filament-like conformation. *Eur J Biochem* **252**, 542-552.

Zhu X, Raina AK, Smith MA (1999) Cell cycle events in neurons. Proliferation or death? *Am J Pathol* **155**, 327□329.

Figure legends

Figure 1. AT100 epitope in pyramidal neurons from the CA1 region at different ages. Representative pyramidal neurons from the CA1 region of young (20-40 years) (A, D), adult (40-60 years) (B, E), and senile (more than 60 years) (C, F, G) brains, respectively. Nu: nucleolus. Scale bar in A, B, C: 100 µm: in D, E, F, G: 10 µm. (H) Total and relative number of the positive AT100 cells. (I) Relative quantification of the AT100 stain normalized respect to the young group. Data are expressed as the mean ± standard error of the mean (SEM) of three (young and adult) or six (senile) cases per group. Young and adult groups are always statistically different respect to senile (*** $P<0.001$).

Figure 2. Co-localization of AT100 epitope with the nucleolar marker UBF in pyramidal neurons from the CA1 region at different ages and in AD. Representative pyramidal neurons from the CA1 region of young (20-40 years) (A), adult (40-60 years) (B), senile (more than 60 years) (C), ADI (D) and ADIV (E) brains, respectively. Images show dual-color immunofluorescences to detect tau epitope AT100 (green signals) and nucleolar marker Upstream Binding transcription Factor RNA polymerase I (UBF) (red signals). White arrows indicate the co-localization of AT100 and UBF in the nucleolus. Yellow arrows indicate two affected cells from ADIV with neurofibrillary tangles (NFT) and a degraded nucleus. In the ADIV panel, the disappearance of UBF from the nuclei and its localization in the cytoplasm, decorating NFTs, is evident, as previously shown (**Hernandez-Ortega et al., 2015**). Quantitative data were in **Fig. 3**. Images were obtained with 630x magnification. A-E: merged signals (AT100+UBF) and DAPI staining (blue). F-

J: AT100 signals (green signals). K-O: UBF signals (red signals). P-T: DAPI staining (blue). Scale bar, shown in panel T: 20 µm.

Figure 3. Quantification of the positive AT100 pyramidal neurons from the CA1 region. The total and relative number of AT100 positive cells were shown. Data are expressed as the mean ± standard error of the mean (SEM) of three (young, adult, ADI and ADIV) or six (senile) cases per group. Young, adult and ADIV groups are statistically different respect to senile (*** $P<0.001$). ADI respect to senile is not statistically significant (NS: not significant).

Figure 4. Immunohistochemistry analysis of AT100 epitope of tau protein in granular neurons from the dentate gyrus (DG) at different ages. Granular neurons from the DG of fetus (8-12 weeks) (A, D), young (20-40 years) (B, E), and senile (more than 60 years) (C, F) brains. There is no cytoplasmic AT100 signal in the analyzed cells. DG: dentate gyrus; inm: inner nuclear membrane. Scale bar in A, B, C: 100 µm; in D, E, F: 10 µm. (G) Total and relative number of the positive AT100 cells. (H) Relative quantification of the AT100 stain normalized respect to the young group. Data are expressed as the mean ± standard error of the mean (SEM) of three (fetus and young) or six (senile) cases per group. Fetus and young groups are always statistically different respect to senile (*** $P<0.001$).

Figure 5. AT100 epitope in non-neoplastic colorectal mucosa, and neoplastic cells from adenocarcinoma. Epithelial cells from intestinal mucosa of young (20-40 years) (A, D), senile (more than 60 years) (D, E) and neoplastic tissue (C, F), respectively. The

AT100 isoform of the tau protein was localized in low and high amounts in the cytoplasm (Cy) and in the nuclei (n), in young and senile cells, respectively. In neoplastic cells, AT100 largely decreases in both the cytoplasm and nuclei. The immunosignal is more evident in the internal side of the nuclear membrane (inm) in the young, senile and neoplastic cells. Scale bar in A, B, C: 10 µm; in D, E, F: 5 µm. (G) Quantification analysis of the AT100 stain in the cell nuclei normalized to the young group. Data are represented as mean ± standard error (SEM). Young and neoplastic groups are statistically different respect to senile (*** $P<0.001$).

Figure 6. AT100 epitope in non-neoplastic and neoplastic breast cells. AT100 was localized in the nuclei of non-neoplastic cells and two types of breast carcinoma (grades I and III). In neoplastic cells, AT100 largely decreases in the nuclei of the more severe grade of carcinoma with respect to the less severe. Scale bar in A, D, G: 100 µm; in B, C, E, F, H, I: 10 µm. (J) Quantification analysis of the AT100 stain in the cell nuclei normalized to the healthy breast group. Data are represented as mean ± standard error (SEM). Healthy breast and BC-III groups are statistically different respect to BC-I (*** $P<0.001$).

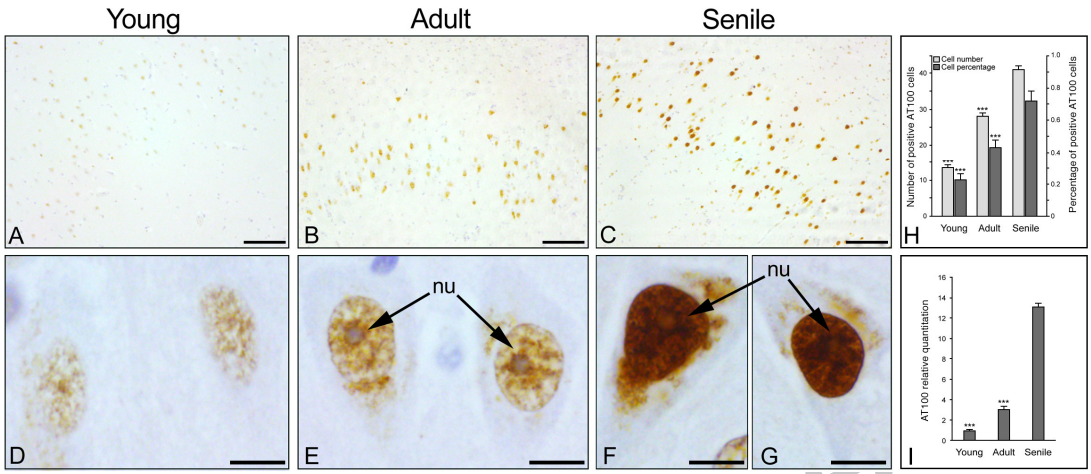
Supplementary Table 1: Summary of the cases used.

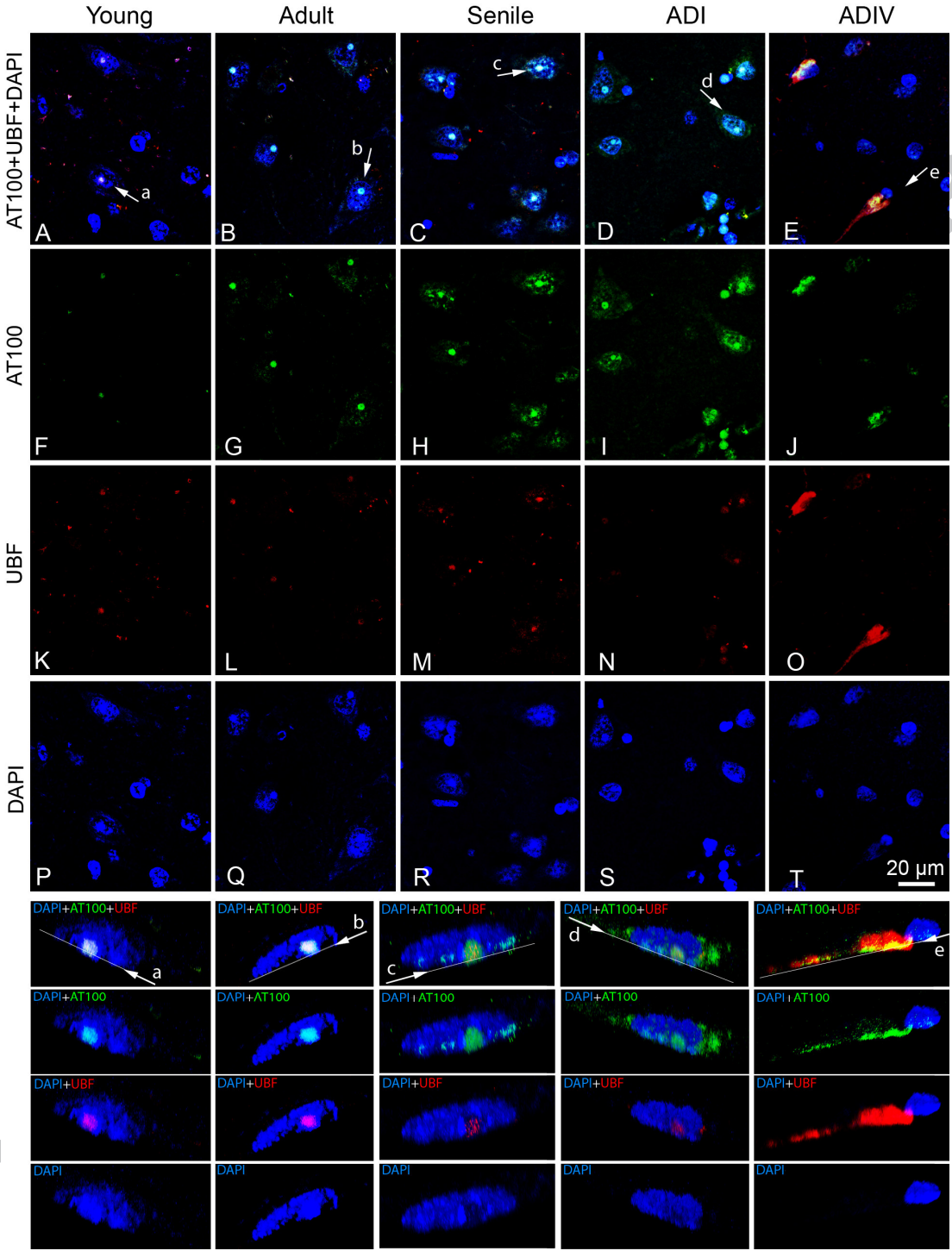
Case n.	Tissue type	Diagnosis	Age (years)	Cause of death
1	Brain	ND	21	Non natural
2	Brain	ND	27	Non natural
3	Brain	ND	32	Non natural
4	Brain	ND	45	Non natural
5	2 Brain	ND	58	Non natural
6	2 Brain	ND	63	Non natural
7	Brain	ND	65	Non natural
8	3 Brain	ND	68	Non natural
9	3 Brain	ADI	> 70	AD
10	3 Brain	ADIV	> 70	AD
11	3 Brain	ND	8-12 weeks	Miscarriage
12	3 Breast	BC grade I	50 - 65	Alive
13	4 Breast	BC grade III	50 - 65	Alive
14	Colon	Acute appendicitis	23	Alive
15	Colon	Follicular hyperplasia lymphoid	31	Alive
16	Colon	Adenoma	40	Alive
17	Colon	Adenocarcinoma	54	Alive
18	2 Colon	Adenocarcinoma	68	Alive
19	3 Colon	Follicular hyperplasia lymphoid	68	Alive
20	Colon	Diverticulitis	79	Alive

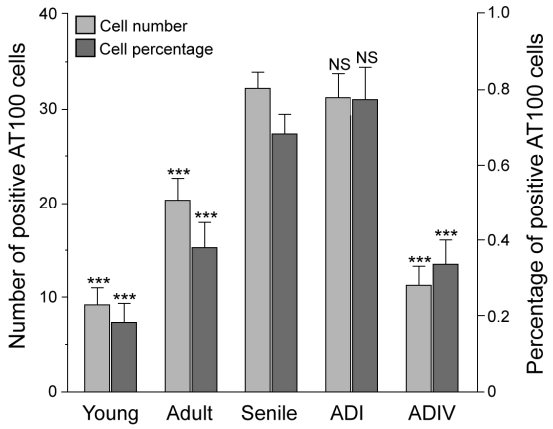
ND: not determined. AD, ADI, ADIV: Alzheimer's diseases stages of Braak and Braak; BC: breast cancer

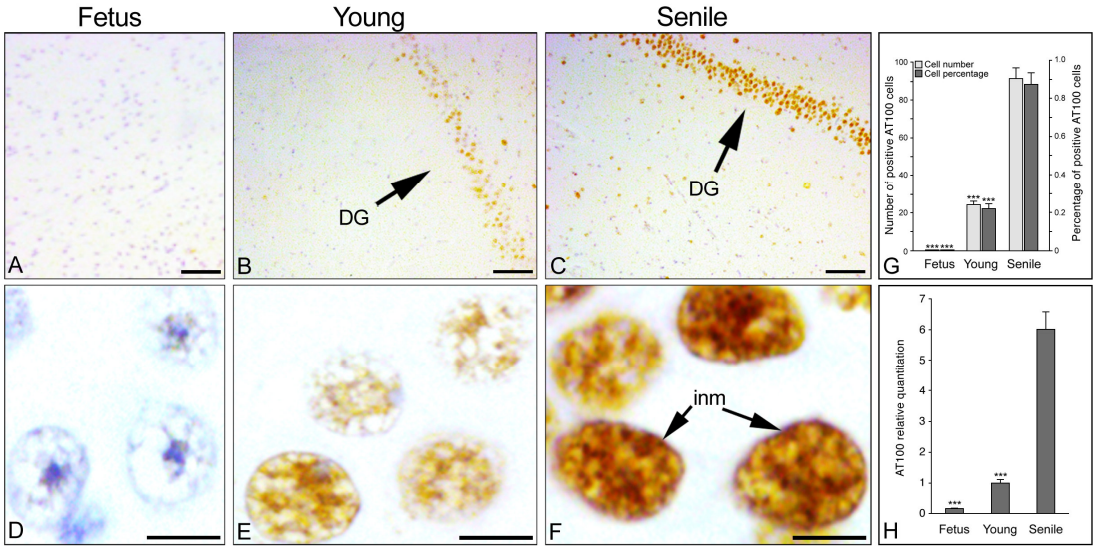
Abbreviations:

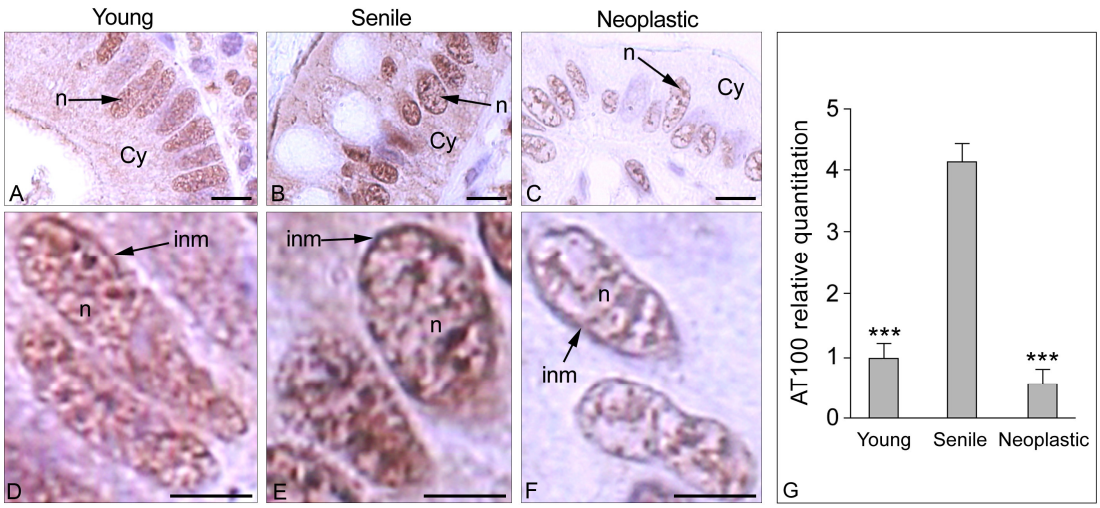
AD:	Alzheimer's disease
ADI-ADIV	Stages of AD following Braak and Braak (1991) classification
AT100:	Tau epitope with phosphorylated Thr212/Ser214 residues
CA1:	Cornus ammonis 1
CLSM:	Confocal laser scanning microscopy
DAPI:	4′,6-diamidino-2-phenylindole
DG:	Dentate gyrus
FITC:	Fluorescein isothiocyanate
MAPT :	Microtubule-associated protein tau gene
MTBD:	Microtubule-binding domain in tau protein
PRD:	Proline-rich domain in tau protein
TRITC:	Tetramethylrhodamine isothiocyanate
UBF:	Upstream binding factor

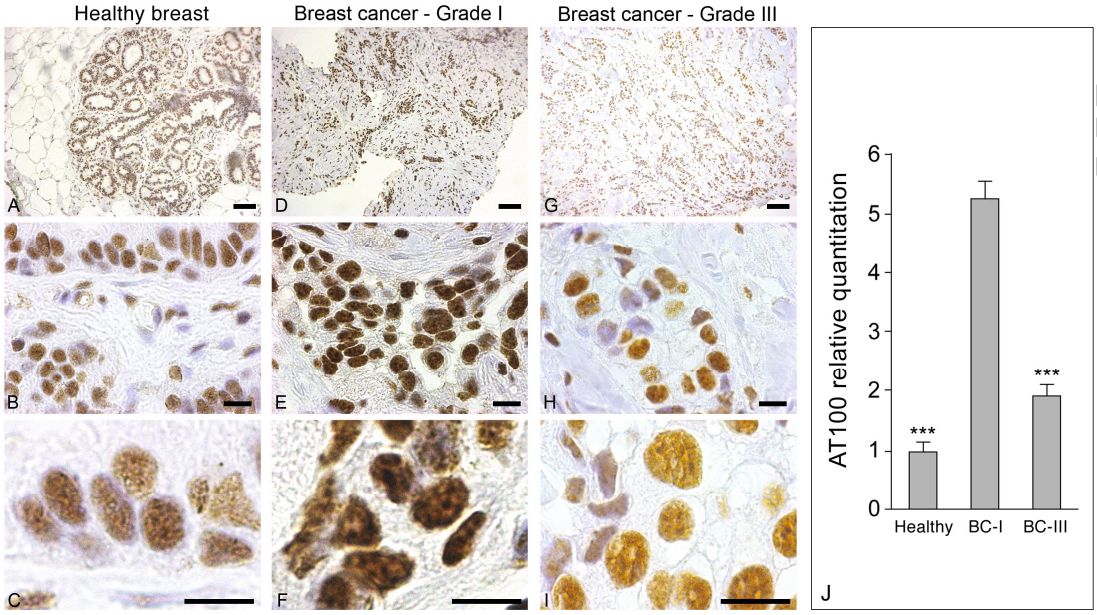












Highlights

AT100 nuclear tau progressively increases in the cell nuclei during aging

Phosphorylated AT100 nuclear tau is a putative molecular marker of cell aging

AT100 nuclear tau was detected in the nucleolus of pyramidal cells from CA1 region

AT100 nuclear tau disappears from pyramidal cells of CA1 region during AD progression