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Human tau expression reduces adult neurogenesis in a mouse model of tauopathy

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ABSTRACT

Accumulation of hyperphosphorylated and aggregated microtubule-associated protein tau (MAPT) is a central feature of a class of neurodegenerative diseases termed tauopathies. Notably, there is increasing evidence that tauopathies, including Alzheimer's disease, are also characterized by a reduction in neurogenesis, the birth of adult neurons. However, the exact relationship between hyperphosphorylation and aggregation of MAPT and neurogenic deficits remains unclear, including whether this is an early- or late-stage disease marker. In the present study, we used the genomic-based hTau mouse model of tauopathy to examine the temporal and spatial regulation of adult neurogenesis during the course of the disease. Surprisingly, hTau mice exhibited reductions in adult neurogenesis in 2 different brain regions by as early as 2 months of age, before the development of robust MAPT pathology in this model. This reduction was found to be due to reduced proliferation and not because of enhanced apoptosis in the hippocampus. At these same time points, hTau mice also exhibited altered MAPT phosphorylation with neurogenic precursors. To examine whether the effects of MAPT on neurogenesis were cell autonomous, neurospheres prepared from hTau animals were examined in vitro, revealing a growth deficit when compared with non-transgenic neurosphere cultures. Taken together, these studies provide evidence that altered adult neurogenesis is a robust and early marker of altered, cell-autonomous function of MAPT in the hTau mouse mode of tauopathy and that altered adult neurogenesis should be examined as a potential marker and therapeutic target for human tauopathies.

1. Introduction

In the mammalian central nervous system, there are 2 areas in which adult neurogenesis, the creation of new neurons during adulthood, takes place—the subgranular zone within the hippocampal formation and the subventricular zone lining the lateral ventricles (Altman, 1962; Eriksson et al., 1998). These newly created neurons, derived from neuronal stem cells, are thought to incorporate into hippocampal circuits from the subgranular zone and the olfactory bulb from the subventricular zone and are known to be critical for certain types of learning and memory (Arruda-Carvalho et al., 2011; Deng et al., 2010; Nakashiba et al., 2012; Whitman and Greer, 2009).

Recent evidence has implicated adult neurogenesis as playing a potential role in the pathophysiology of several neurodegenerative diseases, including Parkinson's disease, Alzheimer's disease, and frontotemporal dementia (DeCarolis and Eisch, 2010; Gendron and Petrucelli, 2009; Ghosal et al., 2010; Hamilton et al., 2010; Hou and Hong, 2008). In particular, there is increasing evidence for reduced adult neurogenesis in patients diagnosed with tauopathies (Lazarov and Marr, 2010; Thompson et al., 2008; Winner et al., 2011). Tauopathies are a class of neurodegenerative disease characterized by the pathological phosphorylation and aggregation of the microtubule-associated protein tau (MAPT), which is thought to cause neurotoxicity. Notably, the mechanism of neurotoxicity in tauopathies as well as the pathological form of MAPT is unknown, and there is no known treatment or cure.

The present studies sought to examine the effects of pathological MAPT on adult neurogenesis in the genomic-based hTau mouse model of tauopathy (Andorfer et al., 2003, 2005; Polydoro et al., 2009). The hTau mouse expresses all 6 isoforms of wild-type human MAPT at the physiological level in the absence of endogenous mouse MAPT. The hTau mice develop an age-progressive tauopathy,

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with abnormal MAPT phosphorylation beginning at around 3 months of age, tau aggregation initiating at 10 months of age, and behavioral abnormalities at 14 months of age.

The present study found that alterations in adult neurogenesis in the hTau mouse are present by 2 months of age and continue to be affected up until 12 months of age. These reductions in adult neurogenesis are because of reductions in proliferation of the neural progenitors within the neurogenic niche. Furthermore, tau phosphorylation was found specifically in the adult neurogenic niche in the hTau mouse at 2 months, before tau phosphorylation in other areas. Finally, it has been found that proliferation of neural stem cells is decreased because of the direct action of hTau.

2. Materials and methods

2.1. Animals

The original hTau mice generated by Peter Davies et al. used a human *MAPT* transgene (Andorfer et al., 2003) (Jackson Laboratory #005491) in a *Mapt*$^{-/-}$ knockout mouse (Jackson Laboratory #004779) that was predicted to express a short, truncated form of MAPT. To eliminate this potential confound, the human MAPT transgenic strain was mated to a separate *Mapt*$^{-/-}$ knockout mouse (Jackson Laboratory #007251) that was predicted to be a functional null allele. All mice were of an inbred C58BL/6J genetic background. Experimental protocols were performed in accordance with the US National Institutes of Health guidelines on animal care and were approved by the Cleveland Clinic Institutional Animal Care and Use Committee.

2.2. Antibodies

Mouse monoclonal antibodies AT8 (Pierce), NeuN (Millipore), and BrdU (Millipore) were used in conjunction with rabbit monoclonal antibodies Dcx (Cell Signaling), Ki67 (Abcam), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Cell Signaling).

2.3. Immunofluorescence

Mice were killed at various ages (2, 6, and 12 months) and perfused with phosphate-buffered saline at pH 7. After perfusion, the brain was removed and drop-fixed in 4% paraformaldehyde for a minimum of 24 hours and then transferred to 30% sucrose. Free-floating sections at 30 μm were processed for standard immunofluorescence staining:briefly, sections underwent antigen retrieval in sodium citrate and were blocked in diluted normal goat serum. Primary antibodies were used at the following dilutions: AT8 at 1:500, NeuN at 1:1000, Dcx at 1:400, and Ki67 at 1:500, and incubated overnight at 4 °C. Secondary antibodies (1:1000) conjugated to Alexa Fluor dyes (Invitrogen) were incubated at room temperature for 1 hour. Sections were then mounted in VECTASHIELD HardSet Reagent with DAPI (Vector) and coverslipped. All immunofluorescent imaging procedures were carried out using a confocal microscope. Quantification was performed in the dentate gyrus by counting Dcx and NeuN double-labeled cells in unprojected *z*-series stacks (figures show projected *z*-series stacks). Similarly, quantification was performed in the subventricular zone by counting Ki67-positive cells near the entrance to the rostral migratory stream in unprojected *z*-series stacks (figures show projected *z*-series stacks).

2.4. BrdU incorporation

Two-month-old mice were injected with bromodeoxyuridine (50 mg/kg body weight) and were killed 24 hours after injection. Perfused brains were then fixed and underwent HCl bath followed by standard immunofluorescence protocol. BrdU was detected through immunofluorescence staining using a confocal microscope. Quantification was performed on the dentate gyrus by counting BrdU and Dcx double-labeled cells.

2.5. Western blotting

Two-month-old mice were perfused with phosphate-buffered saline at pH 7. After perfusion, the brains were immediately removed and microdissected on ice to isolate the hippocampus, which was further homogenized in tissue protein extraction reagent with protease and phosphatase inhibitors. In addition, 20 μg of protein was loaded onto 4%–12% Bis-Tris gels, transferred onto polyvinylidene fluoride membrane, blocked in 5% milk, and incubated overnight with AT8 at 1:5000 and GAPDH at 1:10,000. This was followed by secondary antibodies (1:10,000) conjugated to IRDyes (LI-COR). Membranes were developed using an Odyssey imager.

2.6. Neurosphere assay

Neurospheres were generated as previously described (Mori et al., 2007). Briefly, mice were killed at either embryonic day 16 or postnatal day 60 (2 months old), and their brains were mechanically divided by trituration in Hank's balanced salt solution. After passing through a 40-μm filter, the cells were grown in suspension in Dulbecco's modified eagle medium/nutrient mixture F12 supplemented with B-27 minus vitamin A, epidermal growth factor, and fibroblast growth factor. Cells were grown in culture for more than 14 days, and were imaged on an upright bright-field microscope under standard light conditions.

2.7. Statistical analysis

Statistical significance of differences between groups was analyzed using unpaired 2-tailed Student's *t*-test. A *p*-value of <0.05 was considered significant. Data are shown with mean $\pm$ standard error mean (SEM).

3. Results

3.1. Adult neurogenesis is reduced in the dentate gyrus

Adult neurogenesis occurs in 2 primary locations within the mammalian central nervous system: the dentate gyrus of the hippocampus and the subventricular zone lining the lateral ventricles. To determine whether the hTau mouse model of tauopathy exhibited age- and pathology-related alterations in neurogenesis, hTau and non-transgenic controls were killed at 2-, 6-, and 12-month time points and the brain tissue was processed for immunohistochemistry. Notably, sagittal sections of the dentate gyrus show a qualitative decrease in the number of doublecortin (Dcx, a marker for neural progenitors and some daughter neurons) and NeuN (a marker for neuronal nuclei) double-positive cells at the 2- and 6-month time points (Fig. 1A), suggesting a decrease in the neurogenic population within the dentate gyrus. Quantification of the Dcx-NeuN positive cells revealed a reduction of 23% and 27% at the 2- and 6-month time points (Fig. 1B), respectively, when comparing the hTau mice with non-transgenic controls. Quantification of the 12-month time point was not possible because of reduced neurogenesis in both hTau and wild-type mice, as has been previously reported in aged C57BL/6J mice (Bondolfi et al., 2004; Kempermann et al., 1998; Rodriguez et al., 2008). These results show that adult neurogenesis is reduced in the dentate gyrus in the hTau mice as early as 2 months of age, which is maintained at the