Alzheimer Aβ Peptide Induces Chromosome Mis-Segregation and Aneuploidy, Including Trisomy 21: Requirement for Tau and APP

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Both sporadic and familial Alzheimer’s disease (AD) patients exhibit increased chromosome aneuploidy, particularly trisomy 21, in neurons and other cells. Significantly, trisomy 21/Down syndrome patients develop early onset AD pathology. We investigated the mechanism underlying mosaic chromosome aneuploidy in AD and report that FAD mutations in the Alzheimer Amyloid Precursor Protein gene, APP, induce chromosome mis-segregation and aneuploidy in transgenic mice and in transfected cells. Furthermore, adding synthetic Aβ peptide, the pathogenic product of APP, to cultured cells causes rapid and robust chromosome mis-segregation leading to aneuploid, including trisomy 21, daughters, which is prevented by LiCl addition or Ca2+ chelation and is replicated in tau KO cells, implicating GSK-3β, calpain, and Tau-dependent microtubule transport in the aneugenic activity of Aβ. Furthermore, APP KO cells are resistant to the aneugenic activity of Aβ, as they have been shown previously to be resistant to Aβ-induced tau phosphorylation and cell toxicity. These results indicate that Aβ-induced microtubule dysfunction leads to aneuploid neurons and may thereby contribute to the pathogenesis of AD.

INTRODUCTION

Developing early diagnoses and successful treatments for Alzheimer’s disease (AD) will be greatly aided by a clear understanding of all steps in the pathogenic pathway that leads to amyloid deposition, neurofibrillary tangle formation, inflammation, and neurodegeneration in the brain. Although most AD is “sporadic,” a large proportion is at least partly “familial” in that patients develop the disease by inheriting a mutant gene or a risk-enhancing genetic polymorphism. Autosomal dominant mutations, accounting for ~5% of AD, have been described in three genes, and their analysis has provided especially important insights into the AD pathogenic pathway (Glenner and Wong, 1984; Hardy and Selkoe, 2002). One of these genes encodes the amyloid precursor protein (APP) from which the key amyloid component, the Aβ peptide, is derived by proteolysis. Although mutations in the APP gene itself account for <1% of AD, they provided the proof that APP and Aβ are central to the disease process.

Most autosomal dominantly inherited familial Alzheimer’s disease (FAD) is caused by mutations in two presenilin genes, most commonly PS-1. The PS proteins must therefore also occupy a key place in the AD pathogenic pathway together with APP and the Aβ peptide. The role of the presenilins in AD pathology was clarified when they were found to form the enzymatic core of the γ-secretase complex that cleaves APP in its transmembrane region and generates the C-terminus of the Aβ peptide (Wolfe, 2003).

Several lines of evidence indicate that both sporadic and familiar AD patients, including those carrying APP and PS mutations, are abnormal in one or more aspects of the cell cycle (for reviews, see Obrenovich et al., 2003; Potter, 2004, 2008). For example, Down syndrome (DS) patients, who carry three copies of chromosome 21 in all of their cells due to meiotic chromosome mis-segregation in one of (usually) their mother’s germ cells, invariably develop AD-like pathology by the age 30–40 (Olson and Shaw, 1969; Glenner and Wong, 1984; Epstein, 1990). This and other findings led us to propose that over a lifetime, defective mitoses lead to the accumulation of aneuploid cells throughout the body, including the brain. When such chromosome mis-segregation generates trisomy 21 cells, the extra copy of the APP gene on chromosome 21 contributes to the development of Alzheimer neuropathology and dementia (Potter, 1991). The microtubule (MT) disfunction likely responsible for the aneuploidy in AD patients could also affect other aspects of cell physiology, especially in neurons.

The chromosome mis-segregation/MT disfunction hypothesis of AD makes several easily-testable predictions (Potter, 1991). For example, AD patients should be mosaic for trisomy 21, and, indeed, we found trisomy 21 and other aneuploid cells in primary skin fibroblast cultures from patients with both the familial (early age of onset) and sporadic (late age of onset) forms of the disease (Potter et al., 1995; Geller and Potter, 1999). Trisomy 21 cells have also been observed among peripheral blood lymphocytes, buccal cells, and brain neurons from sporadic AD patients and among lymphocytes of mothers who, at a young age, gave birth to a DS child and are prone themselves to develop AD later in life (Schupf et al., 1994; Migliore et al., 1999, 2006; Yang et al.,...
Suzuki et al., segregation and aneuploidy, including trisomy 21 (Boeras overexpression or mutation of PS-1 leads to chromosome mis-segregation. All assays, tissues, and cells yielded the same results and allowed the conclusion that PS-1 gene on the cell cycle. Analysis of the PS-1–transfected cells showed that endogenous PS-1 and APP and some of their mutant forms of presenilin failed to induce chromosome hyperphosphorylated during mitosis (Pope et al., 2003; Zitnik et al., 2000; Johnsingh et al., 1994; Preuss et al., 1995).

Furthermore, immunocytochemical and FRET results have shown that endogenous PS-1 and APP and some of their interacting proteins reside in cell structures involved in mitosis, such as the nuclear membrane, centrosomes, or kinetochore (Zimmermann et al., 1988; Li et al., 1997; Honda et al., 2000; Johnsgingh et al., 2000; Kimura et al., 2001; Tezapsidis et al., 2003; Zitnik et al., 2006; Nizzari et al., 2007) and become hyperphosphorylated during mitosis (Pope et al., 1994; Suzuki et al., 1994; Preuss et al., 1995).

Previously, we used transgenic and knockin mice and transfected cells in culture to test directly the effect of the PS-1 gene on the cell cycle. All assays, tissues, and cells yielded the same results and allowed the conclusion that overexpression or mutation of PS-1 leads to chromosome mis-segregation and aneuploidy, including trisomy 21 (Boeras et al., 2008; Potter, 2008). Analysis of the PS-1–transfected cells by immunocytochemistry revealed numerous abnormalities in the mitotic spindle apparatus, including improper MT arrays and lagging chromosomes. Finally, dominant negative mutant forms of presenilin failed to induce chromosome mis-segregation, showing that presenilin/γ-secretase is directly involved in the mutant PS-1–induced cell cycle and chromosome segregation defects.

During the course of these studies, we noted that the percentage of cells with abnormal chromosome complements that arose in the PS-1–transfected cultures was often higher than the measured transfection efficiency. This fact strongly suggested that the PS-1 effect on chromosome mis-segregation was not restricted to the PS-1–expressing cells, but also extended to adjacent, nontransfected cells (i.e., was non-cell autonomous) and thus might be induced by a secreted molecule. Coupling this observation to the fact that γ-secretase activity was essential for the PS-1–induced chromosome mis-segregation and to the previous finding that patients carrying FAD mutations in APP also developed trisomy 21 mosaicism led us to hypothesize that secreted Aβ peptide itself might induce cell cycle defects including chromosome mis-segregation (Boeras et al., 2008).

In this article we test whether 1) expression of an FAD mutant APP gene in transgenic mice or in transfected cells leads to chromosome mis-segregation, particularly in brain neurons, 2) exposing cells in culture to Aβ peptide itself leads to chromosome mis-segregation, 3) Ca\(^{2+}\) chelation or exposure to LiCl (two treatments that have been shown to obviate Aβ toxicity by inhibition of calpain and GSK-3β, respectively) prevent Aβ from inducing chromosome mis-segregation, and finally whether 4) knocking out the MT also causes chromosome mis-segregation.

**MATERIALS AND METHODS**

**Mice**

Transgenic mice expressing human APP with the V717F APP mutation (19–21 months) or knocked out for APP (3 months) and their nontransgenic littermates were used. Tau+/− mice and Tau−/− mice and their nontransgenic Tau+/+ controls were 6–7 mo old (Jackson Labs). All mice used in this study had C57BL/6 background strain.

**Primary Cells**

Mouse primary splenocytes were prepared for metaphase chromosome analysis and fluorescence in situ hybridization (FISH; Boeras et al., 2008). Mouse brains were harvested, the meninges and cerebella were removed, and cells were obtained by triturating brain pieces in ice-cold 1× PBS (Cellgro, Manassas, VA) –10 ml with fire-polished Pasteur pipettes of different pore sizes. The cell suspension was fixed in cold 3:1 anhydrous methanol/acetic acid fixative and kept on ice for at least 30 min. Fixed brain cells were stored at −20°C before any downstream assay was performed.

**Cell Line**

The hTERT-HME1 cell line is a primary human mammary epithelial cell line that permanently expresses the telomerase reverse transcriptase (Clontech, Palo Alto, CA) and has a stable karyotype (Iang et al., 1999). Cells were maintained in supplemented mammary epithelium basal medium (MEMB, Lonza, Hopkinton, MA) and passaged every 2–3 d according to the supplier’s recommendations. All experiments were conducted with the hTERT cells passages 3–6.

**Plasmids**

Plasmids constructed by inserting an FAD combination mutant N1-APP K595N/M596L (Swedish) and V717I APP V717I into pAG3 vector were gifts of Dr. Chad Dickey (University of South Florida, Tampa) and Todd Golde (Mayo Clinic, Jacksonville). NucleoBond Plasmid Purification kit (BD Biosciences, San Diego, CA) was used for nucleic acid purification.

**Transient Transfections**

One day before the transfection, the hTERT cells (1–1.5 × 10^5 cells/2 ml) were plated in a six-well plate and grown in supplemented MEBM. A FuGene6 (Roche Applied Science, Indianapolis, IN) DNA complex was prepared according to the supplier’s recommendations using a ratio of Fugene 6 to DNA of 3 μL/μg and was applied to the cells. At 48 h after transfection, cells were either harvested immediately for FISH or treated with 37 ng/ml colcemid for 6–7 h before the harvest, collected, fixed, and scored for aneuploidy in metaphase chromosome spreads.

**Peptides and Salts**

Synthetic Aβ1-40, Aβ1-42, and Aβ42-1 from either BioSource International (Camarillo, CA) or American Peptide Company (Sunnyvale, CA) were reuspended in sterile HPLC water at room temperature, aliquoted, and stored at −80°C before use. Other control peptides were custom made by either Sigma Genosys (St. Louis, MO) or Bio-Synthesis (Lewisville, TX), and were designed by random scrambling of the Aβ1-42 or Aβ1-42 sequence (NH2-VEEQQKLYV-FADYGVSNK-OH and NH2-ADFVGSVINIGKLELKMVGQVGVHGIAE-VHDFYSFADHEARG-OH), respectively. Similarly, LiCl (Fisher Scientific, Pittsburgh, PA) and BAPTA (Invitrogen, Carlsbad, CA) salts were reuspended in HPLC water.

**In Vitro Incubation of the Primary Cells and Cell Lines with Aβ Peptides, BAPTA, and LiCl**

For each experiment, a fresh aliquot of the various Aβ peptides, BAPTA, or LiCl was used and thawed on ice to avoid repeated thaw-freeze cycles and possible changes in the structure of the molecules. Primary mouse splenocytes were stimulated to divide in concanavalin A (Con-A) containing media for 44 h in either 100-mm dishes (7.5 × 10^5 cells/10 ml growth media) or in six-well plates (4–5 × 10^5 cells/3 ml growth media). The cells were treated with Aβ peptide for 44–48 h and prepared according to established cytogenetic procedures described below. In concurrence experiments, the Tau+/+ splenocytes were pretreated...
with either 1 μM of BAPTA for 3 min before the peptides were added or with 2.5 mM of LiCl for the last 7 h of Aβ peptide incubation.

Similarly, 24 h before AβJ treatment, a low passage of the hTERT-HME1 cells was seeded and cultured in either six-well dish (1–1.5 × 10^5 cells/1 ml) or T-150 cm2 flask (1 × 10^6 cells/10 ml) in T-75 cm2 culture dish (4–5 × 10^5 cells/55 cm^2). For the last 10–12 h of peptide exposure, the cells were treated with 33 ng/ml colcemid, harvested, fixed, and scored for aneuploidy by either karyotype analysis or FISH.

**Metaphase Chromosome Analysis**

After colcemid treatment, cells were harvested according to standard cytogenetic methods as described (Boeras et al., 2008). Genus 2.81 software (Applied Imaging, San Jose, CA) and the Metafer 3.31 Slide Scanning System (MetaSystems, Altlussheim, Germany) with Isis 5.2 (ver. 2007; MetaSystems) was used for metaphase spreads capture and chromosome analysis. At least 80 mouse splenocyte metaphases and 45 hTERT-HME1 cells metaphase spreads were analyzed per each sample.

**FISH**

A bacterial artificial chromosome (BAC) containing a mouse chromosome 16–specific sequence (a gift from Dr. Bruce Lamb at Case Western Reserve University) was labeled by nick translation (Abbott, Vysis, Downer’s Grove, IL) as described elsewhere (Kulnane et al., 2002; Boeras et al., 2008) with modifications. Specifically, 1 mM of either Spectrum Green dUTP (Abbott, Vysis) or Spectrum Orange dUTP (Enzo Life Science, Bedford, MA) was used to label 1 μg of BAC DNA. The preincubated (37°C over night) BAC probe was used for FISH of mouse primary cells. Labeled BAC probe and mouse brain cells dehydrated in ethanol solutions (70, 80, and 90%) were denatured at 74°C for 4 min and hybridized at 37°C for 22 h in the HyBrite (Vysis) hybridization chamber followed by additional 20–22 h of hybridization at 38°C. Excess probe was removed by three consecutive washes in 0.4 × SSC at 37°C for 4 min, 2 × SSC/0.1% NP40 at room temperature for 3 min, and 4 × SSC/0.1% NP40 for 2 min. DAPI II (Abbott, Vysis) or Vectashield (Vector Laboratories, Burlingame, CA) counterstain was used to stain nuclear DNA. Mouse spleen cells were subject to similar in situ protocol except they required shorter hybridization time and less thorough washing.

Interphase FISH of hTERT-HME1 cells was performed using the LSI TEL/AML1 ES Dual Color Translocation Probe (Abbott, Vysis). Hybridizations were done according to Vysis recommendations for LSI probes in the HyBrite hybridization chamber (Vysis) and counterstained with DAPI II.

**Image Acquisition and Analysis**

Hybridization signals were scored according to Vysis guidelines using either a Nikon Eclipse E1000 microscope (Melville, NY) with a 4912 CCIR high-performance Cohu CCD camera (San Diego, CA) and Genus 2.81 software or under 49 DAPI, 38 HE Green Fluorescent, and 36752 red signals (Genus 2.81 software or under 49 DAPI, 38 HE Green Fluorescent, and 43HE Red Zeiss filters with a Zeiss Imager.M1 Axio fluorescence microscope. The Abbott/Vysis (http://www.abbottmolecular.com/DualColor Enumeration.aspx) guidelines were followed meticulously. Particularly important is that, as required, closely adjacent double spots, double spots linked by a light fluorescent thread, and defused signals were counted as only single spots (i.e., one chromosome) and not two spots (i.e., two chromosomes).

**Antibodies**

For immunocytochemistry and immunofluorescence we used anti-α-tubulin (Sigma, clone B-5-1-2, 1:500; Mx X Neuronal Nuclei (NeuN) AlexaFlour 488 (Miliipore, Bedford, MA), 1:100; and Alexa Flour 488 rabbit α-mouse IgG (Invitrogen, Molecular Probes, Eugene, OR), 1:1000.

**Immunocytochemistry after FISH**

Immediately after hybridization, brain cells were incubated in 1 × PBS for 10 min. The slides were blocked in 10% goat serum/0.1% Triton X-100 × 1 PBS solution for 1 h. Conjugated Mx X Neuronal Nuclei AlexaFlour 488 (Millipore) was diluted 1:100 in 1% BSA/0.1% Triton X-100 × 1 PBS and applied to the slides overnight to stain for neurons. After final washes, coverslips were mounted onto slides with DAPI II (Abbott, Vysis).

**Statistical Analysis**

Paired Student’s t test was used to compare the aneuploidy induced by different peptide and inhibitor treatments and plasmid transfections in multiple experiments and to compare the levels of aneuploidy in mouse primary cell line with and without a human FAD transgene. At least 10–12 mice were analyzed for each graph and five to seven transfections/treatments of each plasmid/peptide were scored for aneuploidy.

**RESULTS**

**Expression of FAD Mutant APP Causes Chromosome Mis-Segregation in Transgenic Mice**

To understand the mechanism by which chromosome aneuploidy arises in AD, we asked whether the expression of FAD-mutant APP disrupts the cell cycle and causes chromosome mis-segregation. First, metaphase chromosome analysis was used to compare the chromosome complement of FAD-APP–expressing transgenic mice (PDAPP) and age-matched normal mice. Primary splenocytes were chosen for this analysis because they can be induced to divide in culture, a requirement for metaphase analysis, and the transgene promoter is active in the spleen. The dividing cells were arrested at metaphase by colcemid treatment and the chromosomes stained and counted. The data showed twice the level of aneuploidy (i.e., 25% of cells with both less or more than the normal mouse complement of 40 chromosomes) in the FAD-APP animals compared with nontransgenic animals (Figure 1A). There was no increase in the number of tetraploid/polyploid cells (not shown).

FISH allows for aneuploidy for particular chromosomes to be assessed at all phases of the cell cycle. A BAC carrying a 300-kb fragment of mouse chromosome 16 (Kulnane et al., 2002) was labeled with spectrum green dUTP (Abbott) by nick translation and used as a hybridization probe to tag chromosome 16 in transgenic and normal mouse splenocytes (Figure 1B). Expression of the mutant APP transgene induced a many-fold increase in both trisomy and monosomy 16 (Figure 1, C and D).
Chromosome Aneuploidy in Neurons from FAD-APP Transgenic Mice

The effect of an FAD mutation (V717F) in the APP gene on the chromosome complement of brain cells was examined using DNA FISH, which can be used to count chromosomes in both the nondividing neurons and cycling cells such as glia. Whole brains from PS-1 transgenic and nontransgenic mice were processed to yield suspensions of mixed primary cells. The cells were fixed to slides and hybridized with the mouse chromosome 16 BAC probe labeled with Orange-dUTP. The hybridization efficiency was ~90%, with most cells being disomic, i.e., exhibiting two signals (Figure 2A). By colabeling with Neu-N antibody, we found that ~6.5% of neurons in APP transgenic mice were trisomic for chromosome 16 compared with 1.5% of neurons in nontransgenic mice brain (Figure 2B). There was no increase in aneuploidy in nonneuronal cells (Neu-N negative; Figure 2C). These robust results with one chromosome probe indicate that even more tgAPP neurons are likely to be aneuploid, just as 5% trisomy 16 among spleen cells correlated to 25% total aneuploidy in metaphase spreads (which cannot be generated for postmitotic neurons).

Small numbers of tetraploid neurons have been reported in AD humans and mice and interpreted as being due to adult neurons reentering the cell cycle and completing DNA replication, but failing to complete cell division (Yang et al., 2001, 2006; Obrenovich et al., 2003; Mosch et al., 2007; Zhu et al., 2008). We observed no increase in tetrasomy 16 in disaggregated cells from APP mice brains compared with nontransgenic brains (Figure 2D); see also Iourov et al. (2009), who also report finding human AD cortical neurons with trisomy 21 but very few neuronal or nonneuronal cells showing tetrasomy or polyploidy.

These and previous results show that expression of FAD mutant forms of either APP or PS-1 in transgenic mouse models of AD disrupts normal mitosis, leading to chromosome mis-segregation and the development of aneuploid cells in both the brain and the periphery. The presence of aneuploid neurons in both APP and PS-1 transgenic mice indicates that both classes of FAD-causing mutations cause cell cycle and chromosome mis-segregation defects in neuronal precursor cells.

Expression of FAD Mutant APP Causes Chromosome Mis-Segregation and Aneuploidy in Transfected Cells

To confirm that the aneuploidy observed in FAD-APP transgenic mice was caused directly by the expression of the mutated gene, we asked whether mutant APP could also induce aneuploidy after transient expression in mammalian cells in culture. To assure a low background level of cell cycle defects such as aneuploidy, we used as a transfection recipient the hTERT-HME1 cell line, a (karyotypically stable) primary human cell line transfected with a telomerase reverse transcriptase gene to confer immortality (Jiang et al., 1999; Morales et al., 1999).

Parallel cultures of hTERT cells were transiently transfected with plasmids expressing a human APP gene carrying both the NL-APP K595N/M596L (Swedish) and V642I (London) mutations or the V717I APP mutation. The empty vectors served as controls. FISH with a double-labeled probe showed that expression of mutant APP induced chromosome mis-segregation and the development of aneuploidy for both chromosome 21 and 12 (Figure 3, A–C), but did not induce an increase in tetraploid cells (Figure 3D). Together, these results show that the aneugenic activity of mutant APP expression likely affects all chromosomes randomly and therefore probably alters an essential aspect of normal mitosis. Mutant APP did not induce aborted cell cycles in which DNA replication occurs without a subsequent cell division in these short-term experiments.

AD-causing Peptides Induce Chromosome Mis-Segregation and Aneuploidy

Because overexpression or mutation in either PS-1 or APP cause cell cycle defects and chromosome mis-segregation, we hypothesized that the product of APP cleavage by PS-1—the Aβ peptide itself—might be the effector molecule responsible for disrupting the mitotic spindle (Boeras et al., 2008). We tested...
this hypothesis by directly exposing cells in culture to Aβ and various control peptides for 48 h and counting chromosomes by karyotype analysis and by FISH. Both Aβ1-40 and Aβ1-42 induced significant chromosome mis-segregation and aneuploidy in cultured hTERT cells within 48 h whereas the Aβ42-1 reverse peptide, the Aβ1-42 scrambled peptide and the Aβ12-28 scrambled peptide had no significant effect on untreated cells (Figure 4A,B), a result confirmed by FISH analysis of chromosomes 21 and 12 (Figure 5, A–C).

**Mechanism of Aβ-induced Chromosome Mis-Segregation: Roles for Tau and APP**

To investigate the mechanism by which Aβ, and by implication mutant APP and PS, cause cells to undergo chromosome mis-segregation and yield aneuploid daughters, we assumed that its aneugenic function was related to the peptide’s other toxic activities, especially those related to MT-based transport, which is, of course, required for proper chromosome segregation. The data of Figure 6, A and B, show that pretreating normal splenocytes with BAPTA, which chelates extracellular Ca\(^{2+}\) and thus inhibits, for instance, calpain activation, or with LiCl, which inhibits GSK-3β, prevents Aβ from inducing chromosome mis-segregation. BAPTA and LiCl have previously been shown to inhibit Aβ toxicity (Lee et al., 2000; Takashima 2006).

Numerous studies, both in vitro and in vivo, have shown that Aβ (and FAD mutant PS-1 and APP) induces increased phosphorylation of the MT-associated protein Tau, the main component of the intraneuronal paired helical filaments and neurofibrillary tangles of AD (Lee 1996; Pigino et al., 2001; Small and Duff, 2008). Indeed, the toxicity of Aβ depends on the presence of Tau (Rapoport et al., 2002; Roberson et al., 2007). Interestingly, activated calpain cleaves Tau, inactivating it, and yielding a toxic fragment, and GSK-3β phosphorylates Tau at AD-PHF-relevant sites and is itself involved in chromosome segregation (Lee 1996; Wakefield et al., 2003; Park and Ferreira, 2005). We therefore investigated the role of Tau in chromosome segregation. Splenocytes were prepared from normal, Tau+/−, and full Tau−/− knockout mice, allowed to grow for 48 h in the presence and absence of Aβ, and analyzed for chromosome aneuploidy. As shown in Figure 7, A and B, knocking out one, or even more effective, both copies of Tau led to increased aneuploidy. Addition of Aβ only induced a clear increase in chromosome mis-segregation in normal cells, indicating that the aneugenic effect of Aβ requires and disrupts normal, Tau-stabilized, MT function.
Finally, Aβ-induced cellular toxicity has been shown to require the presence of full-length APP as a receptor on the surface of the target cell, perhaps to aid in the uptake of exogenous Aβ or to undergo induced endocytosis and processing to generate intracellular Aβ (Lorenzo et al., 2000; Shaked et al., 2006; Sola Vigo et al., 2009). We have tested whether the effect of Aβ on chromosome segregation in treated cells similarly requires interaction with endogenous APP. Addition of Aβ 40 or 42 to APP−/− spleen cells fails to induce chromosome mis-segregation and aneuploidy over background, (Figure 8). This result further indicates that Aβ-induced chromosome mis-segregation is part of, and likely contributes to, the peptide’s toxic action.

DISCUSSION

The preponderance of the data—from pathology, from genetics, from biochemistry, from cell biology, and from the mouse models designed to mimic the human disease—point to the Aβ peptide as playing a central role in the pathogenic pathway to AD (Hardy and Selkoe, 2002). For example, mutations in APP or PS affect either the sequence of Aβ, or the cleavage of the APP protein, so as to generate forms of Aβ that, under the essential catalytic influence of inflammatory proteins, specifically apolipoprotein E and antichymotrypsin, are more prone to first oligomerize and then polymerize and aggregate into the toxic amyloid deposits of AD (Wolfe, 2003; Potter et al., 2001). Both in vitro and in vivo studies have shown that oligomerized and/or polymerized Aβ is toxic to neurons (Yankner et al., 1989; Ma et al., 1994, 1996; Wisniewski et al., 1994; Kayed et al., 2003; Chromy et al., 2003; Townsend et al., 2006).

The importance of Aβ is also supported by the fact that DS patients, whose cells carry three copies of chromosome 21 (and of the APP gene) in all of their cells due to chromosome mis-segregation during meiosis produce increased levels of Aβ peptide and invariably develop AD pathology at an early age (Olson and Shaw 1969; Epstein, 1990, Potter, 1991, 2008). Even mosaic DS individuals, with only a small proportion of trisomy 21 cells, develop early dementia. Furthermore, FAD can be caused by a duplication of one APP gene on one chromosome, confirming that a mere 50% overproduction of APP/Aβ is sufficient to cause very early AD in the context of an otherwise normal human brain (Sleegers et al., 2006; Rovelet-Lecrux et al., 2006).

The data of this article, together with our earlier results, support the hypothesis that a potentially important step in the pathogenic pathway by which Aβ overproduction leads...
to AD is Aβ’s interference with the cytoskeleton, leading to chromosome mis-segregation during mitosis (Potter, 1991, 2008; see also Rassoulzadegan et al., 1998). More specifically, the Aβ product of the PS-γ-secretase enzyme acting on APP, at least in part by inactivating Tau, may contribute to AD by inducing the mis-segregation of chromosomes and the development of aneuploid, including trisomy 21, cells. Knocking out the Tau gene also causes chromosome mis-segregation and aneuploidy.

The mechanism by which Aβ induces cell cycle abnormalities evidently involves activation of GSK-3β and the influx of Ca2+, because blocking either of these two activities restores chromosome segregation to normal. In addition, Tau-stabilized MTs are a necessary substrate for the aneugenic activity of Aβ. These findings are linked, for Ca2+-induced cleavage and inactivation of Tau and GSK-3β for Aβ-induced phosphorylation and inactivation of Tau and both for Aβ toxicity (Park and Ferreira, 2005; Takashima, 2006). Ca2+ is also necessary for activation of kinesin-like MT motors through Ca2+-binding regulatory proteins (Vinogradova et al., 2009; Wang and Schwartz, 2009). Indeed we consider it likely that Aβ directly damages the MT system such that Tau disconnects from MTs, becomes prone to cleavage, and phosphorylation, further destabilizing MTs.

Aβ’s ability to inhibit MT function and cause chromosome mis-segregation will have major deleterious effects, particularly on neurons and on their precursors. Because neurogenesis occurs throughout life, especially following neuronal loss (Zhao et al., 2008), Aβ-induced chromosome mis-segregation will yield aneuploid, defective, neuronal precursor cells and should thus inhibit the production of fully functional replacement neurons, as found in FAD-tg mice and AD patients. For instance the trisomy 21 cells that accumulate in AD patients overexpress APP and Aβ, which imbalance should promote the disease as it does in mosaic trisomy 21 or APP-duplication individuals who are born normal but later develop early AD pathology and dementia. Indeed, trisomy 21 neurons are prone to apoptosis, PS-1 mutation or overexpression (and thus increased Aβ production) induces cell cycle defects in cultured cells, and mice harboring an FAD mutant human APP or PS-1 gene have reduced neurogenesis and other cell cycle defects (Wolozin et al., 1996; Busciglio and Yankner, 1997; Janicki and Monteiro, 1999; Chui et al., 1999; Feng et al., 2001; Wen et al., 2004; Wang et al., 2004; Chevallier et al., 2005; Boeras et al., 2008; Zhang et al., 2007; Verret et al., 2007; Boeras et al., 2008; Varvel et al., 2008). Any dividing precursor or mature cell, in culture or in vivo, which cannot undergo proper chromosome segregation will produce defective progeny prone to apoptosis.

Aβ damage to MT function will also lead to intracellular trafficking defects that will particularly affect mature neurons (see for example Stokin et al., 2005; Lazarov et al., 2007). Motor proteins needed for proper interaction of MTs with kinetochores or other cargo require Ca2+-, and stability of MTs in neurons is reduced by Ca2+-dependent cleavage of Tau or by GSK-3β-dependent phosphorylation of Tau, both of which are induced by Aβ, as discussed above.

A direct connection between P-Tau and chromosome mis-segregation is reinforced by the recent finding that the P301L mutation in Tau that causes frontal temporal dementia (FTD) also causes Tau hyperphosphorylation, chromosome mis-segregation, and aneuploidy (Rossi et al., 2008). Just as we showed for AD, the finding of FTD-related chromosome defects confirms the presence of Tau, MT disfunction, and chromosome mis-segregation on the causal path to neurodegeneration in these two related disorders. Interestingly, Pin1, which modulates Tau phosphorylation, also causes chromosome mis-segregation, aneuploidy, and oncogenic transformation in transfected cells (Suizu et al., 2006).

Figure 7. Knocking out Tau replicates/replaces ability of Aβ to induce chromosome mis-segregation. Spleen cells from normal (WT) and Tau+/− and Tau−/− mice were cultured ± Aβ1-42 and Aβ1-40 for 48 h, and the resulting chromosome aneuploidy was assessed (A and B). Tau+/− and −/− cells displayed a higher-than-normal inherent level of aneuploidy, consistent with the requirement for Tau in the MT function in the mitotic spindle. Aβ’s ability to induce chromosome mis-segregation was greatly attenuated (i.e., replaced) in Tau+/− and Tau−/− cells.

Figure 8. Knocking out APP in the target cells prevents Aβ from inducing further chromosome mis-segregation and aneuploidy. Spleen cells from APP−/− mice were cultured ± Aβ1-42, Aβ1-40, and scrambled Aβ1-42 for 48 h, and the resulting chromosome 16 aneuploidy was assessed. Aβ induced aneuploidy in the normal (NON) cells (p = 0.01), but failed (p = 0.4) to increase aneuploidy over background in the APPKO cells. Interestingly, like the Tau−/− cells examined in Figure 7, APP−/− cells also exhibited higher levels of aneuploidy compared with nontransgenic cells (p < 0.05).
The fact that both AD and FTD are associated with advancing age and that aging leads to increased aneuploidy even in normal neuronal precursors and other cells (Geller and Potter, 1999; Rehen et al., 2001; Thomas and Fench, 2008; Granic and Potter, unpublished data), further supports the hypothesis that slow development of such aneuploidy contributes to age-related neurodegenerative disease (Potter, 1991; Li et al., 1997). Indeed two other common age-promoted diseases, cancer and cardiovascular disease, are also characterized and likely promoted by chromosome mis-segregation and aneuploidy (Duesberg, 1999; Potter et al., 2008).

In sum, the data of this article and previous results show that the Aβ peptide found at increased levels in both sporadic and familial AD interferes with mitosis and chromosome segregation, thus leading to trisomy 21 mosaicism and other chromosome aneuploidy. The implication of the results is that MT disruption leading to cell cycle, chromosome mis-segregation, and other cytoskeletal defects in neuronal precursor cells may underlie many of the neurotoxic aspects of AD. The findings also suggests that novel approaches to diagnosis and treatment directed at detecting and preventing disruption of MT function and/or the development of chromosome aneuploidy with age may be successful against AD and possibly other age-associated disorders.

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