

Premature centromere division of the X chromosome in neurons in Alzheimer's disease

Biljana Spremo-Potparević,* Lada Živković,* Ninoslav Djelić,† Bosiljka Plećaš-Solarović,* Mark A. Smith‡ and Vladan Bajić§

*Department of Physiology, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia

†Department of Biology, Faculty of Veterinary Medicine, University of Belgrade, Belgrade, Serbia

‡Department of Pathology, Case Western Reserve University, Cleveland, Ohio, USA

§Institute of Biomedical Research, Galenika a.d., Belgrade, Serbia

Abstract

Premature centromere division (PCD) represents a loss of control over the sequential separation and segregation of chromosome centromeres. Although first described in aging women, PCD on the X chromosome (PCD,X) is markedly elevated in peripheral blood lymphocytes of individuals suffering from Alzheimer disease (AD). The present study evaluated PCD,X, using a fluorescent *in situ* hybridization method, in interphase nuclei of frontal cerebral cortex neurons from sporadic AD patients and age-matched controls. The average frequency of PCD,X in AD patients ($8.60 \pm 1.20\%$) was almost three times higher ($p < 0.01$) than in the control group

(2.96 ± 1.20). However, consistent with previous studies, no mitotic cells were found in neurons in either AD or control brain, suggesting an intrinsic inability of post-mitotic neurons to divide. In view of the fact that it has been well-documented that neurons in AD can re-enter into the cell division cycle, the findings presented here of increased PCD advance the hypothesis that deregulation of the cell cycle may contribute to neuronal degeneration and subsequent cognitive deficits in AD. **Keywords:** Alzheimer's disease, cell cycle, fluorescent *in situ* hybridization, frontal cerebral cortex, premature centromere division.

J. Neurochem. (2008) **106**, 2218–2223.

Alzheimer disease (AD) is the most common cause of senile dementia (Smith 1998) and represents a complex and progressive neurodegenerative disorder of the human brain. The great majority of patients are classified as sporadic (90–95%) with predominating degenerative brain disorder in old age (Schellenberg 1995; Cruts *et al.* 1996; Blacker and Tanzi 1998; Hoyer 2006). The cause of sporadic AD is still unknown, however, age, or more specifically age-related alterations, are likely key. In relation to the latter regard, among the first genetic lesions found to be specifically associated with aging were aberrations in chromosome number and/or structure, primarily the sex chromosomes (Wojda *et al.* 2006).

One chromosomal alteration, premature centromere division (PCD), a phenomenon representing the loss of control over the sequential separation and segregation of chromosome centromeres, is characterized by distinctive and easily recognizable separation of chromatids occurring earlier than usual (Fitzgerald *et al.* 1986; Mehes and Buhler 1995; Spremo-Potparević *et al.* 2000; Spremo-Potparević *et al.* 2004). Conditions which express PCD include Robert's syndrome, Down's syndrome, neoplasias, and exposure to

toxic chemicals (Major *et al.* 1999) and PCD can affect many chromosomes. PCD, as a potential cause of improper chromosome segregation, is one of the genetic mechanisms related to increased aneuploidy, and many studies have shown a significant increase in chromosome loss in peripheral blood lymphocytes and a high percentage of PCD, in both men and women of advanced age (Ward *et al.* 1979; Migliore *et al.* 1999; Wojda *et al.* 2006; Živković *et al.* 2006). The most frequently investigated chromosomes in AD patients are the sex chromosomes because of their frequent aneuploidy rates

Received May 13, 2008; revised manuscript received June 27, 2008; accepted June 27, 2008.

Address correspondence and reprint requests to Biljana Spremo-Potparević Department of Biology and Human Genetics, Institute of Physiology, Faculty of Pharmacy University of Belgrade, Vojvode Stepe 450, 11000 Belgrade, Serbia. E-mail: bilja22@pharmacy.bg.ac.yu; Mark A. Smith, Department of Pathology, Case Western Reserve University, 2103 Cornell Road, Cleveland, OH 44106, USA. E-mail: mark.smith@case.edu

Abbreviations used: AD, Alzheimer's disease; DAPI, diamindole phenylindole; FISH, fluorescent *in situ* hybridization; H&E, hematoxylin and eosin; PCD, premature centromere division; PCD,X, premature centromere division on the X chromosome.

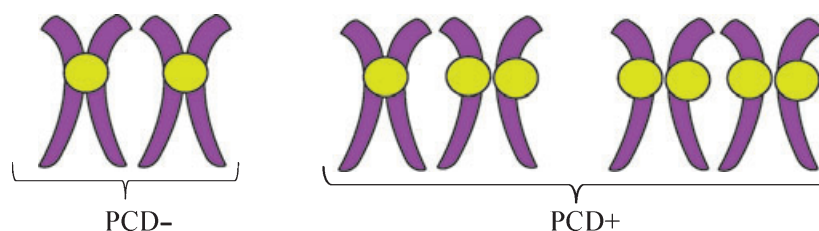


Fig. 1 Schematic representation of FISH visualization of PCD using a specific probe to the centromeric region of the X chromosomes, DXZ α (●). Normal cells (PCD-) show two distinct signals, whereas premature centromere division (PCD+) results in three or four signals.

which are correlated with age (Fitzgerald and McEwan 1977; Bajnoczky and Mehes 1988; Mosch *et al.* 2007).

Ectopic neuronal cell cycle re-entry has been well-documented in the brain of AD patients as well as in mouse models of AD (McShea *et al.* 1997, 2007; Herrup and Arendt 2002; Yang *et al.* 2006; Arendt and Bruckner 2007). In addition, shortened telomeres (Franco *et al.* 2006), neuronal binucleation events (Zhu *et al.* 2008), and increased aneuploidy (Geller and Potter 1999) provide further evidence for chromosomal instability in AD. While we have previously shown that the X chromosome is susceptible to the phenomenon of PCD in peripheral blood lymphocytes in AD patients (Spremo-Potparevic *et al.* 2004), whether this is also evident in the brain is unknown. As highly differentiated cells, neurons are not thought to undergo cell division. Therefore, in female cerebral cortex, it was expected to obtain two dot-like signals in each nucleus, i.e., one dot per each centromere of X chromosome. However, if PCD of X chromosome is present, bipartite signals will appear in one or both X centromeres, resulting in 3 or 4 dot signals per nucleus (Fig. 1).

Materials and methods

Subjects

Frontal cortical brain tissue was collected at autopsy from five sporadic female AD patients (ages 74–79 years), and five age-matched female controls (ages 73–78 years) following approved protocols and with written family consent. Figure 2 includes a further description of the cases used. In all cases, a pathohistological cross section of tissue from the frontal cerebral cortex was used for diagnosis according to established criteria.

Slide preparation

Brain tissue was routinely formalin fixed, paraffin embedded, and sectioned at 4 μ m. Slides were dewaxed in xylol for 30 min, dehydrated in absolute ethanol for 5 min and air-dried at 22°C. Slides were then treated with 0.2 N HCl for 20 min, deionized H₂O for 3 min and 2x SSC (1 x SSC: 0.15 M NaCl and 0.15 M sodium citrate pH 7.0) 1 and 10 min, respectively. Protease treatment was performed with pepsin (4 mg/mL) for 10 min at 37°C, followed by washing in 2 x SSC for 10 min. The slides were fixed in 10% formalin for 10 min, washed in 2 x SSC for 10 min and air-dried. Adjacent serial sections from each case were stained with hematoxylin and eosin (H&E). Cellular size and morphology, as

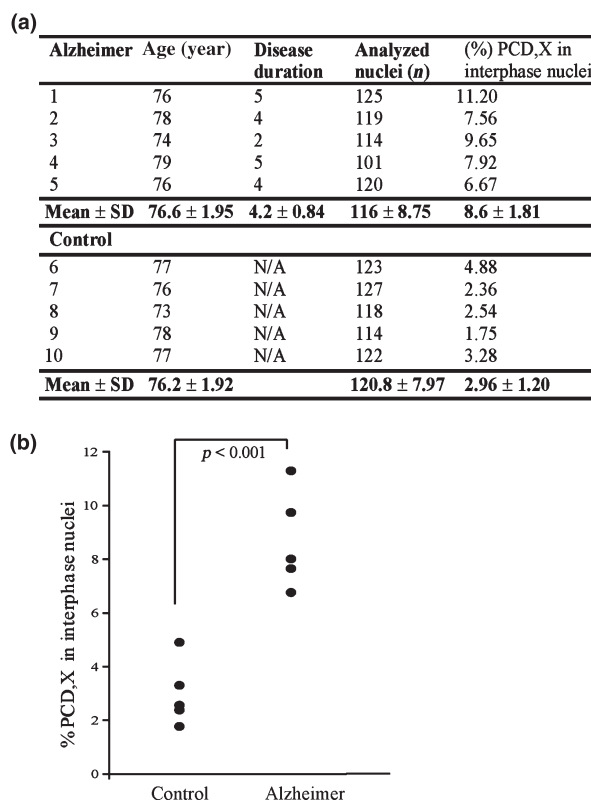


Fig. 2 (a) Clinical characteristics and quantification of PCD,X in five AD female patients and five female controls. In all patients, as well as in controls, at least 100 interphase nuclei from frontal cerebral cortex neurons were analyzed. (b) The percentage of nuclei displaying PCD,X is significantly higher in the AD cases analyzed when compared with age-matched controls ($p < 0.01$; Mann–Whitney).

well as nuclear size, were used to confirm the location of the pyramidal neurons in the adjacent sections for application of fluorescent *in situ* hybridization (FISH) for analysis of PCD on the X chromosome (PCD,X) in the neuronal nuclei.

Application of FISH for analysis of PCD on the X chromosome in interphase nuclei

The probe specific for the X centromeric α repetitive sequences, locus DXZ (DXZ α) was used (Cooke and Hindley 1979). The probe was labeled with biotin-16 dUTP in nick-translation reaction using a bio-nick labeling system (Gibco-BRL, Paisley, UK). For each slide, 200 ng of the probe was mixed in 16 μ L of hybridization buffer,

consisting of 50% formamide, 10% dextran-sulfate, 1% sodium dodecyl sulfate, 1× Denhardt's, 2× SSC and 0.04 M Sodium phosphate pH 7.0, denatured for 10 min at 68°C, and applied to slides.

Hybridization and detection

After overnight hybridization at 37°C, detection was performed essentially as previously described (Verbić *et al.* 2000). Briefly, the biotinylated probe was detected with Fluorescein Avidin DCS (Vector Laboratories, Peterborough, UK) and Biotinylated anti-avidin D (Vector Laboratories). For amplification of signals, three layers of Fluorescein Avidin DCS were applied. The slides were mounted in 0.4 µg/mL diamindino phenylindole (DAPI) and 0.4 µg/mL propidium iodide, counterstained in Vectashield Antifade Buffer (Vector Laboratories). The slides, blinded as to diagnosis, were viewed under an Olympus BX 50 (Olympus Optical Co., GmBH, Hamburg, Germany) epifluorescent microscope with an appropriate filter combination for detecting fluorescein (Spectrum Green) and DAPI and analyzed using Cytovision 3.1 (Applied Imaging Corporation, Santa Clara, CA, USA).

Centromere analysis

FISH analysis of PCD on the X chromosome was analyzed in interphase nuclei from neurons of the frontal cerebral cortex using the FISH centromere assay (Fig. 1). In addition to nuclear size, the location of all neurons within each field was determined by direct comparison with the adjacent H&E stained sections. Only nuclei contained within neurons were evaluated by FISH on adjacent sections. In both groups, AD patients and controls, at least 100 interphase nuclei were analyzed per patient.

Statistical analysis

Statistical analysis was performed by Mann–Whitney test using the Statgraph 4.2 software.

Results

After analyzing neuronal nuclei from all AD and control samples, the average frequency of PCD,X in AD group was found to be $8.60 \pm 1.81\%$, whereas, in a group of five age-matched female controls, the average frequency of PCD,X was found to be $2.96 \pm 1.20\%$ (Fig. 2). In both AD and control cases, in all analyzed nuclei, PCD,X was present on only one of two X chromosomes, resulting in three dots. No cell showed four dots. The bipartite signal of X chromosome where PCD was verified was scored as PCD+ (Fig. 3a and c), while the X chromosome where PCD was not present was scored as PCD- (Fig. 3a and b). No mitotic neuronal cells were found in either AD or control brain (Fig. 3a).

The presented results show almost three times higher incidence ($p < 0.01$) of PCD,X in the neurons of the frontal lobe cortex in AD patients than in age-matched controls.

Discussion

A long standing dogma in neuroscience is that neurons in the adult CNS are in the terminal stage of differentiation.

However, over the last decade, accumulating evidence indicates that neurons may be capable of re-entering the cell division cycle under pathological conditions (Arendt *et al.* 1995, 1996, 1998; Vincent *et al.* 1996, 1997; McShea *et al.* 1997, 2007; Nagy and Esiri 1997; Nagy *et al.* 1998, 2000; Raina *et al.* 2004) and in rare instances display binucleation (Zhu *et al.* 2008). This capability likely depends on extracellular signals, i.e., on the balance between mitogenic stimuli and differentiating factors (Hengst and Reed 1996; Lavoie *et al.* 1996; McShea *et al.* 1999; Nagy *et al.* 2000; Zhu *et al.* 2004), and various mitogenic signals cause cell cycle re-entry of neurons in the CNS of AD patients, including loss of synaptic connections (Nagy *et al.* 2000) and cerebral hypoxia (Smith *et al.* 1999). Furthermore, there is evidence that amyloid-β protein is mitogenic in cultured neurons (Schubert *et al.* 1989; McDonald *et al.* 1998; Pyo *et al.* 1998). Interestingly, AD affects twice as many women as men, indicating that hormonal factors may also play an important role in the loss of the differentiated phenotype in neurons (Bernal and Nunez 1995; Singer *et al.* 1998; Denver *et al.* 1999; Perez-Juste and Aranda 1999; Pike 1999; Webber *et al.* 2006, 2007). Additionally, genetic influences are also involved since mutations in the presenilin 1 gene, resulting in abnormal presenilin function, have been found to lead to chromosome missegregation (Boeras *et al.* 2008). The present study, by employing a novel method that enables a direct visual proof of centromere division, further suggests that neurons of cerebral cortex begin to re-enter into the cell division cycle.

Using the FISH method, the presence of PCD,X was verified in frontal cerebral cortex cells of all analyzed individuals. For this study, female subjects were analyzed, because in earlier studies, PCD, X was found in both male and in female lymphocytes, yet was only significantly different between AD and control in female population (Spremo-Potparevic *et al.* 2004). In sporadic AD patients, the frequency of PCD,X was significantly higher than in control group. To generate PCD+ signals, the cell must first transit from G₀ to G₁ phase of the cell cycle, complete DNA replication (S phase) and go further to G₂ phase. Only a chromosome that has completed replication can generate two signals from one centromere, i.e., each chromatid from chromosome with PCD behaves like a separate chromosome (Fig. 1).

Although our results corroborate DNA replication in the neurons of the frontal cerebral cortex (Mosch *et al.* 2007), in no cases were mitotic cells evident. Therefore, it is conceivable that neurons do not pass the G₂-M transition but rather, after G₂ phase, neurons may undergo cell death (Zhu *et al.* 1999). One mechanism that may drive cell death is the expression of the cell cycle-dependent kinase cdc2 which, when activated, promotes phosphorylation of BCL2-antagonist of cell death (Zhu *et al.* 1999; Konishi *et al.* 2002). Of note, cdc2 is expressed at higher levels in AD and is

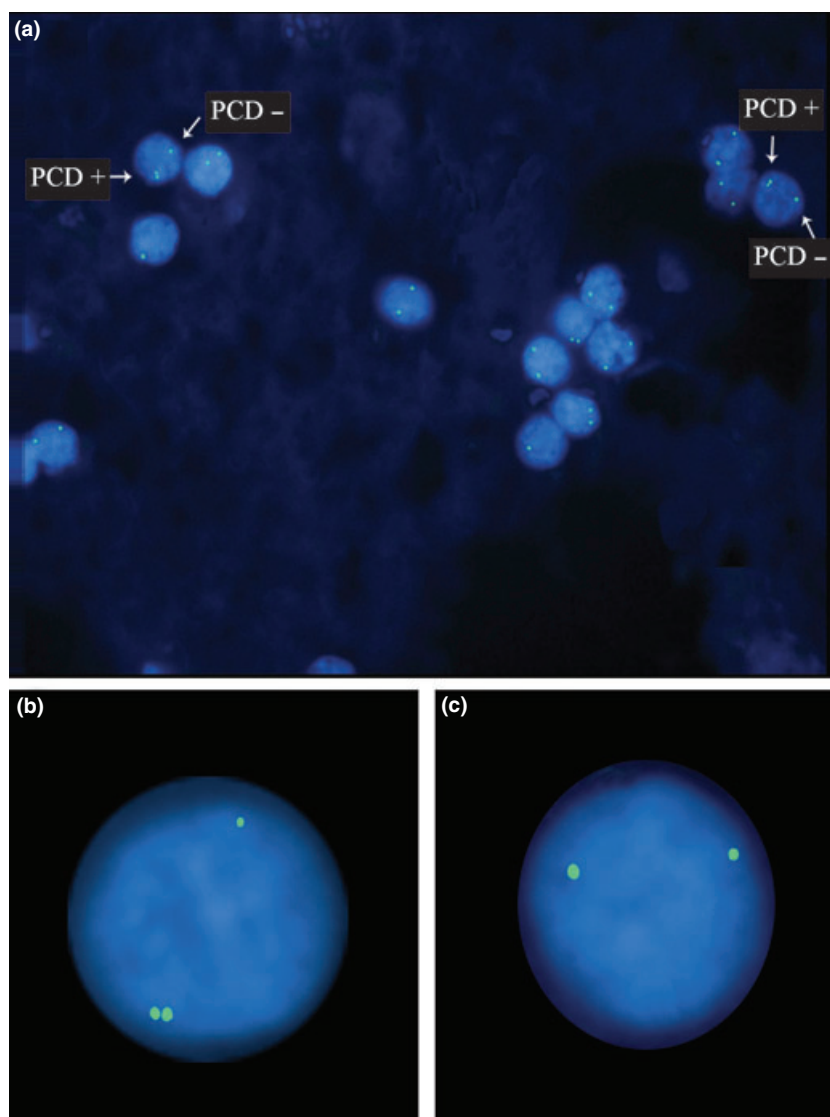


Fig. 3 Configurations of fluorescent hybridization signals identifying the X chromosome specific α satellite loci (DXZ α) in interphase nuclei of neuronal cells of female AD patients. (a) Interphase nuclei of neurons from the frontal cerebral cortex of a female AD patient. Original magnification 1000 \times . Arrows show premature centromere division of one X chromosome (PCD+), and normal centromere of the other X chromosome in the same nucleus (PCD-). Other nuclei have two dot signals, one for each X chromosome, which represent normal centromeres of both X chromosomes (PCD-). At higher magnification (b), AD female patient nucleus with one dot like signal for one X chromosome (PCD-), and one bipartite signal for the other X chromosome (PCD+); (c) AD female patient nucleus with two dot like signals, each for one X chromosome (PCD-).

localized within glia and neurofibrillary tangles (Vincent *et al.* 1997).

One of the first results based on FISH method for the analysis of centromere regions of chromosomes 18 and 21 in hippocampal interphase nuclei, pointed to an ultimate cell death as a consequence of genetic misbalance caused by a 'supposed' tetraploid state of their genome (Yang *et al.* 2001). However, considering the data generated here, these findings may represent interphase PCD, also observable as two dot-like signals per each analyzed chromosome, rather than tetraploidy. Using another novel slide-based cytometry method, others suggested that some neurons in AD can pass through a functional interphase with a complete DNA replication (Mosch *et al.* 2007). Here we also demonstrated that only one of two X chromosomes generates PCD+ signals and the question arises which of the two X chromosomes undergoes PCD. Soon after the discovery of PCD,X in

peripheral blood lymphocytes of elderly women, autoradiographic studies revealed that PCD predominantly occurs on a partially inactive X chromosome (Fitzgerald and McEwan 1977; Galloway and Buckton 1978; Abruzzo *et al.* 1985). Therefore, one significant consequence of inactivation of excess centromeres is a differential pattern of replication versus separation when compared to the active centromere. Inactivation destabilizes the time pattern of centromere replication between two X chromosomes, leading to genome instability, i.e., aneuploidy (Litmanovitch *et al.* 1998). Inactivated centromeres exhibit early replication and, interestingly, PCD (Litmanovitch *et al.* 1998). In fact, a dysregulated centromere segregation has been hypothesized as one pathway leading to the neurodegeneration in diseases such as AD (Bajic *et al.* 2008).

The interaction between cell cycle re-entry and other disease parameters such as oxidative stress (Barlow *et al.*

1999; Nunomura *et al.* 1999) is likely critical in the development of the disease phenotype (Zhu *et al.* 2004). In this regard, it is of note that mutations associated with the familial forms of AD are not only associated with alterations in oxidative stress (Nunomura *et al.* 2004) but also cell cycle alterations (Prat *et al.* 2002).

In conclusion, the results of this work provide compelling evidence for re-entry into the cell cycle of cerebral cortical neurons leading to PCD in the interphase of the cell cycle immediately after replication. This pattern of genome instability can be viewed as a disorder of the hierarchical control of the sequence of centromere separation and segregation. Based on the findings of PCD in the neurons as well as peripheral blood lymphocytes (Spremo-Potparević *et al.* 2004), PCD,X may be a possible cytogenetic biomarker in patients with AD.

Acknowledgments

We are grateful to all patients' families. The work was supported by the Serbian Ministry of Science (grant #143018) and by the National Institutes of Health (AG028679 and AG031364).

References

- Abruzzo M. A., Mayer M. and Jacobs P. A. (1985) Aging and aneuploidy: evidence for the preferential involvement of the inactive X chromosome. *Cytogenet. Cell Genet.* **39**, 275–278.
- Arendt T. and Bruckner M. K. (2007) Linking cell-cycle dysfunction in Alzheimer's disease to a failure of synaptic plasticity. *Biochim. Biophys. Acta* **1772**, 413–421.
- Arendt T., Holzer M., Grossmann A., Zedlick D. and Bruckner M. K. (1995) Increased expression and subcellular translocation of the mitogen activated protein kinase kinase and mitogen-activated protein kinase in Alzheimer's disease. *Neuroscience* **68**, 5–18.
- Arendt T., Rodel L., Gartner U. and Holzer M. (1996) Expression of the cyclin-dependent kinase inhibitor p16 in Alzheimer's disease. *Neuroreport* **7**, 3047–3049.
- Arendt T., Holzer M. and Gartner U. (1998) Neuronal expression of cyclin dependent kinase inhibitors of the INK4 family in Alzheimer's disease. *J. Neural Transm.* **105**, 949–960.
- Bajic V. P., Spremo-Potparević B., Zivkovic L., Djelic N. and Smith M. A. (2008) Is the time dimension of the cell cycle re-entry in AD regulated by centromere cohesion dynamics? *Biosci. Hypotheses* doi: 10.1016/j.bihy.2008.03.006.
- Bajnoczky K. and Mehes K. (1988) Parental centromere separation sequence and aneuploidy in the offspring. *Hum. Genet.* **78**, 286–288.
- Barlow C., Dennery P. A., Shigenaga M. K., Smith M. A., Morrow J. D., Roberts II L. J., Wynshaw-Boris A. and Levine R. L. (1999) Loss of the ataxia-telangiectasia gene product causes oxidative damage in target organs. *Proc. Natl Acad. Sci. USA*, **96**, 9915–9919.
- Bernal J. and Nunez J. (1995) Thyroid hormones and brain development. *Eur. J. Endocrinol.* **133**, 390–398.
- Blacker D. and Tanzi R. E. (1998) The genetics of Alzheimer disease: current status and future prospects. *Arch. Neurol.* **55**, 294–296.
- Boeras D. I., Granic A., Padmanabhan J., Crespo N. C., Rojiani A. M. and Potter H. (2008) Alzheimer's presenilin 1 causes chromosome missegregation and aneuploidy. *Neurobiol. Aging* **29**, 319–328.
- Cooke H. J. and Hindley J. (1979) Cloning of human satellite III DNA: different components are on different chromosomes. *Nucleic Acids Res.* **6**, 3177–3197.
- Cruts M., Hendriks L. and Van Broeckhoven C. (1996) The presenilin genes: a new gene family involved in Alzheimer disease pathology. *Hum. Mol. Genet.* **5** Spec No, 1449–1455.
- Denver R. J., Ouellet L., Furling D., Kobayashi A., Fujii-Kuriyama Y. and Puymirat J. (1999) Basic transcription element-binding protein (BTEB) is a thyroid hormone-regulated gene in the developing central nervous system. Evidence for a role in neurite outgrowth. *J. Biol. Chem.* **274**, 23128–23134.
- Fitzgerald P. H. and McEwan C. M. (1977) Total aneuploidy and age-related sex chromosome aneuploidy in cultured lymphocytes of normal men and women. *Hum. Genet.* **39**, 329–337.
- Fitzgerald P. H., Archer S. A. and Morris C. M. (1986) Evidence for the repeated primary non-disjunction of chromosome 21 as a result of premature centromere division (PCD). *Hum. Genet.* **72**, 58–62.
- Franco S., Blasco M. A., Siedlak S. L., Harris P. L. R., Moreira P. I., Pery G. and Smith M. A. (2006) Telomeres and telomerase in Alzheimer's disease: epiphenomena or a new focus for therapeutic strategy? *Alzheimer's Dementia* **2**, 164–168.
- Galloway S. M. and Buckton K. E. (1978) Aneuploidy and ageing: chromosome studies on a random sample of the population using G-banding. *Cytogenet. Cell Genet.* **20**, 78–95.
- Geller L. N. and Potter H. (1999) Chromosome missegregation and trisomy 21 mosaicism in Alzheimer's disease. *Neurobiol. Dis.* **6**, 167–179.
- Hengst L. and Reed S. I. (1996) Translational control of p27Kip1 accumulation during the cell cycle. *Science* **271**, 1861–1864.
- Herrup K. and Arendt T. (2002) Re-expression of cell cycle proteins induces neuronal cell death during Alzheimer's disease. *J. Alzheimer's Dis.* **4**, 243–247.
- Hoyer S. (2006) The aging brain: the risk factor for sporadic Alzheimer's disease (SAD). Cellular and molecular aspects, in *Frontiers in Alzheimer's Disease Research* (Welsh E. M., ed.), pp. 179–212. Nova Science Publishers, Inc., New York.
- Konishi Y., Lehtinen M., Donovan N. and Bonni A. (2002) Cdc2 phosphorylation of BAD links the cell cycle to the cell death machinery. *Mol. Cell*, **9**, 1005–1016.
- Lavoie J. N., Rivard N., L'Allemain G. and Pouyssegur J. (1996) A temporal and biochemical link between growth factor-activated MAP kinases, cyclin D1 induction and cell cycle entry. *Prog. Cell Cycle Res.* **2**, 49–58.
- Litmanovitch T., Altaras M. M., Dotan A. and Avivi L. (1998) Asynchronous replication of homologous alpha-satellite DNA loci in man is associated with nondisjunction. *Cytogenet. Cell Genet.* **81**, 26–35.
- Major J., Jakab M. G. and Tompa A. (1999) The frequency of induced premature centromere division in human populations occupationally exposed to genotoxic chemicals. *Mutat. Res.* **445**, 241–249.
- McDonald D. R., Bamberger M. E., Combs C. K. and Landreth G. E. (1998) beta-Amyloid fibrils activate parallel mitogen-activated protein kinase pathways in microglia and THP1 monocytes. *J. Neurosci.* **18**, 4451–4460.
- McShea A., Harris P. L., Webster K. R., Wahl A. F. and Smith M. A. (1997) Abnormal expression of the cell cycle regulators P16 and CDK4 in Alzheimer's disease. *Am. J. Pathol.* **150**, 1933–1939.
- McShea A., Zelasko D. A., Gerst J. L. and Smith M. A. (1999) Signal transduction abnormalities in Alzheimer's disease: evidence of a pathogenic stimuli. *Brain Res.* **815**, 237–242.
- McShea A., Lee H. G., Petersen R. B., Casadesus G., Vincent I., Linford N. J., Funk J. O., Shapiro R. A. and Smith M. A. (2007) Neuronal cell cycle re-entry mediates Alzheimer disease-type changes. *Biochim. Biophys. Acta* **1772**, 467–472.

- Mehes K. and Buhler E. M. (1995) Premature centromere division: a possible manifestation of chromosome instability. *Am. J. Med. Genet.* **56**, 76–79.
- Migliore L., Botto N., Scarpato R., Petrozzi L., Cipriani G. and Bonuccelli U. (1999) Preferential occurrence of chromosome 21 malsegregation in peripheral blood lymphocytes of Alzheimer disease patients. *Cytogenet. Cell Genet.* **87**, 41–46.
- Mosch B., Morawski M., Mittag A., Lenz D., Tarnok A. and Arendt T. (2007) Aneuploidy and DNA replication in the normal human brain and Alzheimer's disease. *J. Neurosci.* **27**, 6859–6867.
- Nagy Z. S. and Esiri M. M. (1997) Apoptosis-related protein expression in the hippocampus in Alzheimer's disease. *Neurobiol. Aging* **18**, 565–571.
- Nagy Z., Esiri M. M., Hindley N. J. *et al.* (1998) Accuracy of clinical operational diagnostic criteria for Alzheimer's disease in relation to different pathological diagnostic protocols. *Dement. Geriatr. Cogn. Disord.* **9**, 219–226.
- Nagy Z. S., Smith M. Z., Esiri M. M., Barnetson L. and Smith A. D. (2000) Hyperhomocysteinaemia in Alzheimer's disease and expression of cell cycle markers in the brain. *J. Neurol. Neurosurg. Psychiatry* **69**, 565–566.
- Numomura A., Perry G., Pappolla M. A., Wade R., Hirai K., Chiba S. and Smith M. A. (1999) RNA oxidation is a prominent feature of vulnerable neurons in Alzheimer's disease. *J. Neurosci.* **19**, 1959–1964.
- Numomura A., Chiba S., Lipka C. F., Cras P., Kalara R. N., Takeda A., Honda K., Smith M. A. and Perry G. (2004) Neuronal RNA oxidation is a prominent feature of familial Alzheimer's disease. *Neurobiol. Dis.* **17**, 108–113.
- Perez-Juste G. and Aranda A. (1999) Differentiation of neuroblastoma cells by phorbol esters and insulin-like growth factor 1 is associated with induction of retinoic acid receptor beta gene expression. *Oncogene* **18**, 5393–5402.
- Pike C. J. (1999) Estrogen modulates neuronal Bcl-xL expression and beta-amyloid-induced apoptosis: relevance to Alzheimer's disease. *J. Neurochem.* **72**, 1552–1563.
- Prat M. I., Adamo A. M., Gonzalez S. A. *et al.* (2002) Presenilin 1 overexpressions in Chinese hamster ovary (CHO) cells decreases the phosphorylation of retinoblastoma protein: relevance for neurodegeneration. *Neurosci. Lett.* **326**, 9–12.
- Pyo H., Jou I., Jung S., Hong S. and Joe E. H. (1998) Mitogen-activated protein kinases activated by lipopolysaccharide and beta-amyloid in cultured rat microglia. *Neuroreport* **9**, 871–874.
- Raina A. K., Zhu X. and Smith M. A. (2004) Alzheimer's disease and the cell cycle. *Acta Neurobiol. Exp. (Wars)* **64**, 107–112.
- Schellenberg G. D. (1995) Genetic dissection of Alzheimer disease, a heterogeneous disorder. *Proc. Natl Acad. Sci. USA*, **92**, 8552–8559.
- Schubert D., Cole G., Saitoh T. and Oltersdorf T. (1989) Amyloid beta protein precursor is a mitogen. *Biochem. Biophys. Res. Commun.* **162**, 83–88.
- Singer C. A., Rogers K. L. and Dorsa D. M. (1998) Modulation of Bcl-2 expression: a potential component of estrogen protection in NT2 neurons. *Neuroreport* **9**, 2565–2568.
- Smith M. A. (1998) Alzheimer disease. *Int. Rev. Neurobiol.* **42**, 1–54.
- Smith M. Z., Nagy Z. and Esiri M. M. (1999) Cell cycle-related protein expression in vascular dementia and Alzheimer's disease. *Neurosci. Lett.* **271**, 45–48.
- Spremo-Potparevic B., Zivkovic L., Djelic N. and Bajic V. (2004) Analysis of premature centromere division (PCD) of the X chromosome in Alzheimer patients through the cell cycle. *Exp. Gerontol.* **39**, 849–854.
- Spremo-Potparević B., Verbić V. and Stevanović M. (2000) Experimental model for studying Premature Centromere Division (PCD) in all phases of the cell cycle. *Balkan J. Med. Genet.* **3**, 29–34.
- Verbić V., Grujić D., Sokolović M. and Stevanović M. (2000) Detection of chromosome 21 aneuploidy by fluorescent in situ hybridization. *Arch. Biol. Sci. Belgrade* **52**, 15–20.
- Vincent I., Rosado M. and Davies P. (1996) Mitotic mechanisms in Alzheimer's disease? *J. Cell Biol.* **132**, 413–425.
- Vincent I., Jicha G., Rosado M. and Dickson D. W. (1997) Aberrant expression of mitotic cdc2/cyclin B1 kinase in degenerating neurons of Alzheimer's disease brain. *J. Neurosci.* **17**, 3588–3598.
- Ward B. E., Cook R. H., Robinson A. and Austin J. H. (1979) Increased aneuploidy in Alzheimer disease. *Am. J. Med. Genet.* **3**, 137–144.
- Webber K. M., Casadesus G., Zhu X., Obrenovich M. E., Atwood C. S., Perry G., Bowen R. L. and Smith M. A. (2006) The cell cycle and hormonal fluxes in Alzheimer disease: a novel therapeutic target. *Curr. Pharm. Des.* **12**, 691–697.
- Webber K. M., Perry G., Smith M. A. and Casadesus G. (2007) The contribution of luteinizing hormone to Alzheimer disease pathogenesis. *Clin. Med. Res.* **5**, 177–183.
- Wojda A., Zietkiewicz E., Mossakowska M., Pawlowski W., Skrzypczak K. and Witt M. (2006) Correlation between the level of cytogenetic aberrations in cultured human lymphocytes and the age and gender of donors. *J. Gerontol. A Biol. Sci. Med. Sci.* **61**, 763–772.
- Yang Y., Geldmacher D. S. and Herrup K. (2001) DNA replication precedes neuronal cell death in Alzheimer's disease. *J. Neurosci.* **21**, 2661–2668.
- Yang Y., Varvel N. H., Lamb B. T. and Herrup K. (2006) Ectopic cell cycle events link human Alzheimer's disease and amyloid precursor protein transgenic mouse models. *J. Neurosci.* **26**, 775–784.
- Zhu X., Raina A. K. and Smith M. A. (1999) Cell cycle events in neurons. Proliferation or death?. *Am. J. Pathol.* **155**, 327–329.
- Zhu X., Raina A. K., Perry G. and Smith M. A. (2004) Alzheimer's disease: the two-hit hypothesis. *Lancet Neurol.* **3**, 219–226.
- Zhu X., Siedlak S. L., Wang Y., Perry G., Castellani R. J., Cohen M. L. and Smith M. A. (2008) Neuronal binucleation in Alzheimer disease hippocampus. *Neuropathol. Appl. Neurobiol.* **34**, 457–465.
- Zivkovic L., Spremo-Potparevic B., Djelic N. and Bajic V. (2006) Analysis of premature centromere division (PCD) of the chromosome 18 in peripheral blood lymphocytes in Alzheimer disease patients. *Mech. Ageing Dev.* **127**, 892–896.