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CHROMOSOME INSTABILITY IN ALZHEIMER'S DISEASE

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Abstract - Alzheimer's disease (AD), as the most common form of dementia, has for many years attracted the attention of researchers around the world, primarily because of the problems of reliable diagnostic methods that could help in the early detection of this devastating disease. One of the important aspects of genetic research related to AD is the analysis of chromosome instability which includes: aneuploidies of different chromosomes, telomere shortening and the phenomenon of premature centromere division (PCD). The aim of this study was to describe specific biomarkers in different types of cells as potential parameters for the diagnosis of AD in order to promptly recognize pre-symptomatic stages and prevent the development of disease and/or slow down its progression.

Key words: Alzheimer's disease, chromosomes, aneuploidy, telomeres, centromere

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INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia. It is a set of irreversible, neurodegenerative alterations leading to a progressive loss of memory and intellectual abilities. In AD we can differentiate two basic forms of the disease: sporadic and familial (hereditary). Over 90% of all cases of AD have the sporadic form that occurs after the 65th year of age. Only 5-10% of patients have a familial form of AD that is characterized by a much earlier onset between 40-50 years of age. The inherited form of the disease with early symptoms is caused by autosomal dominant mutations in the genes for the synthesis of amyloid precursor protein (APP) on chromosome 21, presenilin 1 on chromosome 14 and presenilin 2 on chromosome 1 (St. George et al., 1987; Schellenberg et al., 1992; Cruts et al., 1996).

AD pathology is associated with the loss of neurons and synapses in the cerebral cortex and specific subcortical brain regions. Histological indicators of these changes are amyloid plaques resulting from the accumulation of extracellular fibrils composed of amyloid beta protein, and neurofibrilar tangles formed from tau protein (Hardy and Selkoe, 2002). One of the biggest problems associated with AD is the lack of reliable diagnostic methods to confirm AD during the life of the patient and also the lack of methods by which we could highlight some of the presymptomatic states in these patients which could consequently reduce the risk of developing AD.

In the numerous scientific studies related to AD there are different directions of research. The largest volume of research is related to the peptide amyloid beta (A-beta) and tau protein. Also, considerable research is directed towards studies of genetic changes that are directly related to the disease. In this paper the focus will be on chromosome instability in AD with special emphasis on: (1) the significance of aneuploidy in the process of neurodegeneration in AD,

(2) the importance of telomere shortening, and (3) the phenomenon of premature centromere division (PCD) of individual chromosomes in patients with AD. Tests for chromosomal instability in AD, as well as many other studies, are aimed at finding specific biomarkers that could present possible diagnostic parameters of AD in order to influence neurodegenerative processes by slowing down the progress of the disease in these patients.

Aneuploidy in Alzheimer's disease

An adult human body is made at least of 10^{14} cells and 210 different types of cell. They all originate from the first diploid cell (zygote); the end-result is 10^{16} mitotic divisions which is about 45 cell generations (Iourov et al., 2010). It is hard to imagine that all these cells share the same genome. Genomic variations in somatic cells are constantly present as a consequence of 10^4-10^5 DNA lesions per day. Most of the lesions are corrected by repair mechanisms, but at the same time these processes represent one of the exogenous sources of change in the human genome and may be a cause of different pathological processes (Jackson and Bartek, 2009). During the numerous cell divisions in an organism, aneuploidy, i.e., changes in the chromosome number, occurs very often. Chromosome instability, including aneuploidy, is considered as one of the mechanisms of aging at the cellular level (Yurov et al., 2009; Finkel et al., 2007). On the other hand, numerous authors point to the fact that aneuploidy is a relevant factor in neurodegenerative changes in AD (Potter, 1991; Potter, 2010; Kormann-Bortolotto et al., 1993; Migliore et al., 1997; Žekanovski and Wojda, 2009). Early studies have shown that the median frequency of aneuploidy in the neurons of healthy adults is about 10%, which corresponds to a frequency of 0.1-0.7% per chromosome pair (Iourov et al., 2006). In a fetal brain this frequency is 2-3 times higher and probably is a consequence of numerous mitotic divisions, while apoptosis is the mechanism by which the organism eradicates aneuploid cells (Yurov et al., 2007). Also, it has been shown that aneuploid neurons in healthy adults are functionally active due to changes in genomic expression when compared to euploid neurons (Kingsbury et al., 2005). In the brains of AD subjects the number of aneuploid neurons exceeds 20% (Mosch, 2007). However, in the pyramidal neurons of the hippocampus of AD patients aneuploidy is present in 3-4% of cells (Yang et al., 2001).

Increased aneuploidy of chromosome 17 and 21 was also shown at the periphery, in cells of the buccal epithelium of AD patients (Thomas and Fenech, 2008). At the same time, Iourov et al. (2008) has applied a new molecular cytogenetic technique and analyzed interphase chromosomes by a specific multicolor banding (ICS-MCB) in order to evaluate the aneuploidy of chromosome 21 in different parts of the brains of AD patients and corresponding controls. In all investigated parts of the brain, i.e. the cerebral cortex, hippocampus and cerebellum, the percentage of aneuploidy of chromosome 21 in AD was significantly higher than in controls. In the control samples the average frequency of aneuploidy of chromosome 21 was 0.7%, but in the samples of AD brains the aneuploidy of chromosome 21 was present in over 20% of cells (Iourov et al., 2008).

The relationship between Down's syndrome (DS) and AD was described two decades ago by Potter et al. (1991). In DS patients around the age of 30 rapid changes to the brain occur, which histologically cannot be distinguished from those found in AD patients (Wisniewski et al., 1988; Petronis, 1999; Stanton and Coetzee, 2004; Granic et al., 2010). Since it is known that the gene for APP is located on chromosome 21 on the position q21, it is considered that chromosome 21 trisomy can lead to an increased synthesis of this protein and accordingly to increased production of the beta amyloid peptide that is responsible for pathological changes in the brain (Oyama et al., 1994; Petterson and Costa, 2005). The consequence of chromosome segregation disturbance is found to affect chromosome 21 resulting in its trisomy (Potter, 2008). There is more and more evidence that AD is characterized by alterations of the cell cycle in post-mitotic neurons, which consequently result in genomic variability primarily represented by aneuploidies.

Telomere shortening in Alzheimer's disease

Telomeres are repetitive, non-coding sequences of DNA located on the ends of chromosomes. Telomeres shorten after each cell division due to the incomplete synthesis of the lagging strand of DNA, per replication process. The shortest telomeres are found in cells that are mitotically very active, such as skin and gastrointestinal epithelial cells and white blood cells. In human newborn cells, telomere length is about 15-20 kb and it is gradually reduced over a lifetime. So we can say that telomere length represents a biological counter for the number of cell divisions, reflecting the replicative history of each cell (Shay and Wright, 2007).

Unlike most somatic cells, the constant telomere length in stem cells, male gametes and cancer cells is maintained by the presence of the enzyme telomerase. Telomerase is composed of two essential components: a catalytic subunit with a function of a reverse transcriptase (TERT) (Nakamura et al., 1997) and an RNA telomerase subunit (Feng et al., 1995). The mechanism of telomerase action is in the adding of TTAGGG repetitive sequence at the ends of chromosomes which compensates for the erosion of telomeres which happens in its absence (Blackburn 1992; Greider et al., 1985; Cristofaro and Lingner, 2006). Most human differentiated cells do not express telomerase This causes the cell to lose a part of the telomere at each division. The consequences of these kinds of processes in cells can be termed as replicative aging. Therefore, one of the many theories of aging is based on telomere shortening. Panossian et al. (2003) analyzed telomere length in the cells of the immune system in AD patients and showed that the telomeres of T lymphocytes in AD patients are shorter than in the corresponding control group. Lukens et al. (2009) made a study measuring telomere length in peripheral blood leukocytes (PBL) in AD patients and in an appropriately age-matched control. They also questioned the length of telomeres in cerebellar neurons. The results of this study revealed that the telomeres of the PBL in AD patients were significantly shorter than in the corresponding controls, but the differences in telomere length in the cerebellar neurons of AD patients and controls were not statistically significant. It is not clear whether the latter result reflects the status of telomeres in the nerve cells of other brain areas, but the authors believe that the telomere length may be a marker of changes in different tissues of AD patients.

The Nobel Prize for medicine and physiology in 2009 was awarded to two researchers from the U.S., Carol Greider and Jank Szostak, and a scientist from Australia, Elizabeth Blackburn, for the discovery of the role of telomere shortening and its implications in the aging process and cancer. As for the activity of telomerase, aging cells and its malignant transformation are two sides of the same coin. Inhibition of telomerase in cancerous cells could be an end-point for researchers in cancer biology, while the expression of telomerase in normal cells would mean an extension of a healthy life expectancy. This view could have significance for diseases related to aging processes, such as the sporadic form of AD. Study of the gene for the enzyme telomerase will have several important applications in future medical research and cell engineering (Shay and Wright, 2007).

Premature centromere division (PCD) and Alzheimer's disease

Centromeres in chromosomes are the regions that hold the sister chromatids together during the period from completion of replication to early anaphase of mitosis and the first meiotic division. Centromeres have a fundamental role in the proper segregation of chromosomes during cell division, establishing the connection with microtubules of the mitotic spindle and in chromosome movement toward the poles. PCD can be defined as a phenomenon of an earlier than usual centromere separation of chromosomes. This phenomenon was first described in the metaphase X chromosome in healthy, older women (Fitzgerald and McEwan, 1977). Later it was shown that there is a correlation between the presence of PCD and X chromosome aneuploidy (Nakagome et al., 1984; Stone and Sandberg, 1995).

In the PBL of AD patients there is a higher percentage of PCD on chromosomes than in control groups (Migliore et al., 1997), which is especially true for the X chromosome (Spremo-Potparević et al., 2004) and chromosome 18 (Živković et al. 2006). Using the method of fluorescent *in situ* hybridization (FISH) for centromere regions of the these chromosomes it was shown that in sporadic AD patients PCD already occurs in the interphase of the cell cycle, immediately after the replication process in the PBL (Spremo-Potparević et al., 2004, Živković et al., 2006). These authors pointed out an increase in the level of hyperploidy and hipoploidy in AD patients, which may be a consequence of chromosome instability and PCD on the interphase chromosomes. In the interphase neurons of the cerebral cortex of sporadic AD patients, the presence of PCD X chromosome was corroborated by the FISH method (Spremo-Potparević et al., 2008). All the aforementioned findings could point to the loss of spatial and temporal control of centromere separation and segregation, which results in impaired microtubule organization and errors in the sister chromatid segregation of chromosomes. Cyclin dependent kinase 11 (CDK 11) may have an important role in these processes, particularly in the progression of mitosis and maintenance of sister chromatid cohesion (Bajic et al., 2008; 2011). The presence of PCD in the interphase chromosomes of the cerebral cortex neurons of AD patients is proof that these cells perform DNA replication and confirms that neurons in AD actually enter into a new cell cycle, because only replicated chromosomes can exhibit the phenomenon of PCD. It was also shown that postmitotic neurons do not have the ability to divide (Currais et al., 2009).

PCD in neurons can be considered as evidence of the deregulation of the cell cycle that leads to neurodegeneration and eventually to cell death. It is likely that the described events are one of the important mechanisms of the rapid neurodegenerative process in the brains of patients with AD.

CONCLUSIONS

In patients affected by AD the presence of chromosome instability has been established in both cells of the central nervous system and different cell types in the periphery. Chromosome instability is reflected by the presence of numerous but specific changes. Aneuploidy of different chromosomes is present in peripheral blood cells a few years before the onset of the clinical symptoms of AD. Telomeres are significantly shorter in the PBL of AD patients compared to controls. The presence of the phenomenon of PCD in the cells of AD patients leads to a loss of hierarchy in the process of separation and segregation of chromosomes that is spatially and temporally determined. These facts can be crucial to finding specific biomarkers that will contribute to the timely and precise diagnosis of Alzheimer's disease.

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