## Project summary

Research goals within our proposed project аre carried out in the following direction: 1. Analysis of cell cycle disorders in Alzheimer's disease (AD) by determining the causes that lead to the re-entry of the neuron into the cell cycle and the loss of the ability of the same cell to be divided in the process of mitosis . Special attention is devoted to the study of the phenomenon of premature centromere division (PCD), by which we may monitor the phase of the cell cycle, therefore PCD could be a potential cytogenetic biomarker in AD. The experimental part of this study includs: - cytogenetic analysis of PCD on metaphase chromosomes of peripheral blood lymphocytes in AD and control subjects in relation to sex and age; - cytogenetic monitoring of PCD, X chromosomes at different stages of the cell cycle, by fluorescence in situ hybridization (FISH) in different types of cells in AD subjects. The FISH method, serves to detect the PCD phenomenon in the interphase nucleus and in the metaphase, which gives precedence to this method in comparison to classical cytogenetic analysis; 2. Monitoring the expression of CDK11 proteins in pyramidal neurons of the hippocampus of AD subjects, by immunohistochemical methods. CDK11 is one of the protein kinases that regulates G2 / M transition and regulates centromere stability. The aim of the experiment is to evaluate the level of expression of this kinase in neurons of AD subjects relative to control. 3. examination and comparison of the degree of DNA damage of peripheral blood lymphocytes in young, old and AD subjects, using the Comet test. - PCD phenomena on peripheral blood lymphocytes in patients with AD, using classical cytogenetics and FISH methods, were also tested. The correlation between these two parameters of genomic instability in peripheral cells is observed.The second direction of research within the proposed project included the assessment of the genotoxic effects of various hormones, primarily adrenaline, estrogen and thyroxin in young healthy, old healthy and AD subjects, as well as the effect of antioxidants on possible reductions in DNA damage caused by these hormones and other oxidative stress inducers. Oxidative stress parameters are monitored by antioxidant defense enzymes (SOD, catalase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase), non-enzymatic components (reduced and oxidized glutathione), and the formation of reactive nitrogen species (via the nitrite content of the ELISA test). The third phase of the study in the proposed project is focused on monitoring the expression of Hsp70, Hsp90, p53 and PCNA proteins, oncogenic and anti-oncogene products. Hsp70 is a protein that has a regulatory role in the processes of DNA replication and the progression of the cell cycle from G1 to S phase. Hsp90 stabilizes the cyclin-dependent kinase in the late G1 phase, the S and G2 phase of the cell cycle. The tumor suppressor protein p53, the "genome keeper," has the primary role of as a transcription factor that prevents the replication of damaged DNA. It also has the role as an antioxidant by regulating the transcription of the antioxidant enzyme gene, thereby indirectly reducing the level of free radicals in the cell. p53 also regulates the breakdown of the cell cycle in the G2 / M phase. The experimental part of this study involved: - monitoring the expression of the said protein on samples of malignant tissue taken from patients at different stages of the disease, in order to determine the malignant potential of a particular type of tumor. This part of research was aimed at examining the difference in the immunoexpression of the said protein in relation to the stage of the disease, as well as in relation to the degree of tumor differentiation.

## Sažetak projekta OI 173034

Istraživanja u okviru predloženog projekta se odvijaju u sledećem pravcu : 1. Analiza poremećaja ćelijskog ciklusa kod obolelih od Alchajmerove bolesti (AD) utvrđivanjem uzroka koji dovode do ponovnog ulaska neurona u ćelijski ciklus i gubitka sposobnosti iste ćelije da se podeli u procesu mitoze. Posebna pažnja je posvećena proučavanju fenomena prevremene centromerne deobe (PCD), potencijalni citogenetički biomarker kod AD. Eksperimentalni deo rada u okviru ovog dela istraživanja podrazumeva: - citogenetičku analizu PCD na metafaznim hromozomima limfocita periferne krvi kod AD i kontrolnih subjekata, u odnosu na pol i godine starosti; - citogenetičko praćenje PCD, X hromozoma u različitim fazama ćelijskog ciklusa, metodom fluorescentne in situ hibridizacije (FISH) u različitim vrstama ćelija kod AD subjekata. FISH metod, služi za detektovanje fenomena PCD u interfaznim jedrima i u metafazi, što daje prednost ovoj metodi u odnosu na klasičnu citogenetičku analizu; 2. praćenje ekspresije CDK11 proteina u piramidnim neuronima hipokampusa AD subjekata, imunohistohemijskim metodama. CDK11 je jedna od protein kinaza koja reguliše G2/M tranziciju i reguliše centromernu stabilnost. Cilj eksperimenta je da se evaluira nivo ekspresije ove kinaze u neuronima AD subjekata u odnosu na kontrolu. 3. ispitivanje i poređenje stepena oštećenja DNK limfocita perifene krvi kod mladih, starih i AD ispitanika, primenom Komet testa. – Takođe, ispitan je fenomena PCD na limfocitima periferne krvi kod osoba obolelih od AD, primenom klasičnih metoda citogenetike i FISH metode. Praćena je korelacija između ova dva parametara genomske nestabilnosti u perifernim ćelijama. Drugi pravac istraživanja u okviru predloženog projekta obuhvata procenu genotoksičnih efekata različitih hormona, pre svega adrenalina, estrogena i tiroksina kod mladih zdravih, starih zdravih i AD subjekata, kao i uticaj antioksidanasa na eventualna smanjenja DNK oštećenja izazvanih pomenutim hormonima i drugim induktorima oksidativnog stresa. Od parametara oksidativnog stresa praćeni su enzimi antioksidativne odbrane (SOD, katalaza, glutation peroksidaze, glutation reduktaze, glutation-S-transferaze), neenzimske komponente (redukovani i oksidovani glutation), kao i stvaranje reaktivnih azotovih vrsta (preko sadržaja nitrita ELISA testom).Treća faza istraživanja u predloženom projektu je praćenje ekspresije proteina Hsp70, Hsp90, p53 i PCNA, produkata onkogena i antionkogena. Hsp70 je protein koji ima regulatornu ulogu u procesima replikacije DNK i progresiji ćelijskog ciklusa iz G1 u S fazu. Hsp90 stabilizuje ciklin zavisne kinaze u kasnoj G1 fazi, S i G2 fazi ćelijskog ciklusa. Tumor supresorni protein p53, „čuvar genoma“, ima primarnu ulogu transkripcionog faktora koji sprečava replikaciju oštećene DNK. On takodje reguliše transkripciju gena za antioksidansne enzime i na taj način indirektno smanjuje nivo slobodnih radikala u ćeliji. p53 reguliše i prekid ćelijskog ciklusa u G2/M fazi. Eksperimentalni deo rada u okviru ovog dela istraživanja podrazumeva: - praćenje ekspresije pomenutih proteina na uzorcima malignog tkiva uzetih od pacijenata u različitim stadijumima bolesti, da bi se ustanovio maligni potencijal određene vrste tumora. Ovaj deo rada ima za cilj ispitivanje razlike u ekspresiji pomenutih proteina u odnosu na stadijum bolesti, kao i u odnosu na stepen diferencijacije tumora.

## Selected results/Odabrani rezultati

1. Alterations of the X Chromosome in Lymphocytes of Alzheimer's Disease Patients. [Spremo-Potparevic B](https://www.ncbi.nlm.nih.gov/pubmed/?term=Spremo-Potparevic%20B%5BAuthor%5D&cauthor=true&cauthor_uid=26502819), [Bajic V](https://www.ncbi.nlm.nih.gov/pubmed/?term=Bajic%20V%5BAuthor%5D&cauthor=true&cauthor_uid=26502819), [Perry G](https://www.ncbi.nlm.nih.gov/pubmed/?term=Perry%20G%5BAuthor%5D&cauthor=true&cauthor_uid=26502819), [Zivkovic L](https://www.ncbi.nlm.nih.gov/pubmed/?term=Zivkovic%20L%5BAuthor%5D&cauthor=true&cauthor_uid=26502819)1[Curr Alzheimer Res.](https://www.ncbi.nlm.nih.gov/pubmed/26502819) 2015;12(10):990-6.
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