Research project objectives/Research hypothesis

The p53 protein is one of the most important factors regulating response to cellular stress like: DNA damage, hypoxia, oncogen activation or viral infection. The important role of p53 is its function as a tumor suppressor, regulator of antiviral response and cellular inflammation. The human p53 protein attracts a lot of attention from researchers because *TP53* mutations occur in more than half of human tumors, and mutations transmitted between parents and children (germline mutations) increase cancer risk, in women to almost 100%. Despite of the intensive research on *TP53* gene and its protein, there are still unidentified mechanisms concerning functioning of p53.

We observed, that two substances, which stimulate p53 in different ways: actinomycin D and nutlin-3a, when acting simultaneously (A + N) they induce synergistic activation of p53 in lung cancer cells (A549). As a result, a lot of genes regulated by p53 are synergistically activated. Probably, the synergism of these molecules is due to the fact that actinomycin D stimulates phosphorylating of p53 by various kinases, whereas nutlin-3a by blocking the negative regulator of p53, the MDM2 protein, helps the kinases in efficient phosphorylation of p53. One of the genes stimulated by A+N, *TREM2*, encodes cell surface receptor. Polymorphisms and mutations of *TREM2* are associated with Alzheimer's disease and other neurodegenerative disorders. High expression of *TREM2* in cancer cells is correlated with poor prognosis. After the analysis of biochemical and biological properties of the best known kinases, we hypothesized that the phosphorylation of p53 under the A + N treatment is performed by the glycogen synthase kinase (GSK-3). The enzyme participates not only in metabolism, but it also regulates many signaling pathways in cells. Following application of a specific inhibitor called CHIR-98014, which works on the two isoforms of the enzyme (GSK-3α, GSK-3β) which are encoded by separate genes (*GSK-3A*, *GSK-3B*), we observed that it inhibited the phosphorylation of p53 at position Ser46 and blocked the activation of the gene TREM2 or some other genes regulated by p53. With this in mind, we formulated two main research objectives. First, we will examine, which kinase (GSK-3α or GSK-3β) participates in p53 activation under A + N conditions. We aim to achieve this by observing the effects of silencing gene expression by genetic engineering. Secondly, by transcriptomic methods, we will examine, which genes are sensitive to the inhibitory effects of CHIR-98014 on p53. Thirdly, we will look for the biological effect of silencing expression of *GSK3A*, *GSK3B* and *TREM2*. We hypothesize, that the knock-down of one of these genes and / or treating cells A + N and / or CHIR-98014 sensitizes the cells to receptor-initiated apoptotic death.

Research methodology

The first part of the project will consist of measuring the level of gene expression by transcriptome sequencing (RNA-Seq) in non-stressed A549 cells (control) and in cells exposed to A + N, A + N in the presence of GSK-3 inhibitor (CHIR -9814) and in cells treated with the inhibitor alone. The changes in gene expression will be compared with the use of bioinformatic methods between individual experimental conditions (6 comparisons). The sequencing and basic bioinformatic analysis will be outsourced to a specialized company. In this way, we will identify genes, whose activation by A + N is weakened by CHIR-98014. The knock-down of *GSK-3A* and *GSK-3B* genes will be made using commercially available lentiviruses that produce silencing shRNA molecules. The A549 cell line with knocked-down *TREM2* is already at our disposal. The changes in gene expression identified by RNA-Seq were confirmed by qRT-PCR, while the expression of proteins encoded by selected genes and p53 phosphorylation will be analyzed by Western blotting. Other biological tests such as apoptosis detection, cell cycle analysis, proliferation essay, etc. will be performed according to routine procedures such as using flow cytometry. The main conclusions will be verified using other types of the cell lines to see if the findings are applicable more universally.

Expected impact of the research project on the development of science, civilization and society

The most important result of the project would be a better understanding of the functional relationship between the GSK-3, p53 and TREM2 signaling pathways that participates in regulation of inflammation and apoptosis. The imbalance of this processes is the cause of cancer-related mortality, neurodegenerative diseases and certain infections. Also, we will be able to better understand the functioning of GSK-3 kinase, which is involved in the regulation of astonishingly many signaling pathways. Although our research model is not directly related to Alzheimer's disease, a better understanding of the regulation of TREM2 expression may shed new light on the origins of this disorder, which is becoming a social problem within the aging European population.