Scientific goal and hypotheses.

Besides the well-defined role of p53 antitumor protein in inhibition of cell cycle and activation of apoptosis, p53-dependent mechanisms are also involved in the innate immunity and inflammation. However, these immunity-related functions of p53 are much less studied and defined. Our project is based on hypothesis that co-treatment of cells with actinomycin D and nutlin-3a (A+N, experimental drug combination) can strongly upregulate a large proportion of p53-regulated genes, which are poorly stimulated in other commonly employed experimental conditions. This creates opportunity to identify p53-regulated genes, which have not been so far identified as such. The knowledge about the accurate mechanisms of cancer cell destruction by immune cells can be of particular importance in the development of efficient immunotherapy of cancer. Our previous experiments revealed that the expression of numerous genes was elevated at least 10-fold in A549 lung cancer cells exposed A+N or to camptothecin (CPT), which is an anticancer drug and another strong activator of p53. Great proportion of these genes code for proteins of innate immunity - the first line of defense against infections or cancers. This system relies on the recognition of characteristic part of a stressed cell by specialized receptors on effector cells. Very important part of innate immunity are naturally occurring cytotoxic lymphocytes (called natural killer cells - NK) with intrinsic anti-tumor or antiviral properties. They express various activating receptors, which recognize the specific activating markers on target cells. After binding to the stressed target cells the NK lymphocytes kill them. Our preliminary results showed that CPT as well as A+N augmented (even 100-fold) in various cancer cell lines the expression of *SLAMF7*, which codes for the marker on stressed cells, which activates the cytotoxic action of NK lymphocytes. The general goal of this project is the identification of novel, p53-regulated genes and preliminary biological characterization of these, with virtually unknown function (e.g. KLRG2, hypothetically - another activating ligand). Our specific goal is to test two hypotheses. First, the cytotoxic activity of drugs is mediated in part by upregulation of the "kill me" signals (e.g. SLAMF7) on cancer cells, what marks them for the destruction mediated by NK lymphocytes. Second, SLAMF7 and other "kill me" signals are induced by activated p53, consequently, in cancer cells with a mutated p53 gene, the "kill me" signals can not be efficiently induced by drugs and these cells are less sensitive to destruction by NK lymphocytes.

Methodology

We plan to find out if *SLAMF7* and *KLRG2* (and possibly other genes) are activated by p53 protein. In order to do this we are going to clone gene fragments containing putative p53 binding sites and using the reporter tests, we are going to find out if the cloned DNA fragments respond to p53 produced from expression plasmid. Moreover, we will test if in cells with p53 gene knocked-out by CRISPR/Cas9 method, the studied genes can be upregulated by treatment with camptothecin or A+N. Subsequently, in a panel of 11 human cancer cell lines and in normal cells, we will explore the degree of p53 activation and the level of gene expression (e.g. *SLAMF7*, *KLRG2*) in control growth conditions and following treatment with selected anticancer drugs. Next, using lactate dehydrogenase assay which monitors the viability of cells, we will test whether cancer cell lines differ in sensitivity to the killing mediated by a model NK cell line (NK-92), whether chemotherapeutics sensitize selected cancer cell lines to the destruction mediated by NK-92 cells and whether knockout of *SLAMF7*, *KLRG2* or p53 reduces their sensitivity to the elimination by NK cells.

Expected impact

This timely project will primarily have impact on the growing field of experimental immunotherapy of cancer. Specifically, we will better understand poorly studied mechanism of indirect cell killing by p53, which involves making cancer cells better targets for destructive activity of NK lymphocytes. Tumors develop various immune-evasion strategies. The repression of activating markers for NK cells is one of them. Restoration, by therapeutic agents, of the expression of activating markers on cancer cells is a strategic goal of NK-based immunotherapy of cancer. Our project may help to design better strategies to boost natural killer cell-based immunotherapy with anticancer agents. Moreover, our study will identify new facets of functioning of p53 tumor suppressor protein, which still keeps many secrets.