

The influence of SIRT1 deacetylase and epigenetic changes on the biological activity of vitamin D in lung cancer cells

Vitamin D is already known, not only from its influence on calcium homeostasis, but also from the regulation of immune response and anticancer activity. Vitamin D by means of VDR regulates a number of genes, including genes responsible for the regulation of cellular proliferation, differentiation, angiogenesis and immunomodulatory activities on both innate and adaptive immune responses. A number of studies showed that vitamin D reveals antiproliferative activity against many types of cancer cells and the data of epidemiologic studies suggest that low vitamin D status is correlated with increased risk of cancer. However, not all cancer cell types are sensitive to anticancer activity of vitamin D.

The transcription of genes on the genome level is regulated by DNA methylation and histones modifications, which determine which genes are activated and which genes are repressed. For example histone acetylation enables the opening of the structure of the chromatin and next gene transcription activation. Steady-state acetylation levels result from the balance between the opposing activities of enzymes called histone acetyltransferases (HATs) and histone deacetylases (HDACs). In recent years, the role of histone deacetylases in the development of cancer turned out to be of increasing importance. SIRT1 is a NAD-dependent histone deacetylase that is responsible for deacetylation of histones H3K9Ac and H4K16Ac. According to the literature, the decrease in SIRT1 activity seems to be prevalent in smoking-associated lung adenocarcinoma. It was showed that cigarette smoke, the main etiological factor of lung cancer, causes the acetylation/deacetylation imbalance of histones and probably non-histone proteins.

There are two main gene mutations driving lung cancer: *EGFR* and *KRAS*, that are correlated with the history of tobacco smoking in a given patient. The *KRAS* mutations are more common in smokers, while *EGFR* mutations are associated with never smokers. *EGFR* mutant positive lung cancer cells were showed to be more sensitive to antiproliferative activity of vitamin D compared to *KRAS* mutant cells. Our hypothesis is that maybe there is a different acetylation/deacetylation status of *EGFR* and *KRAS* mutant lung cancer cells, and it has an impact on the biological activity of vitamin D in these cells.

In our studies we plan to test antiproliferative activity of calcitriol in combination with SIRT1 activator or inhibitor on the lung cancer cells that differ in *EGFR* and *KRAS* mutation status, and next to evaluate the differences in gene expression by means of RNA-seq after SIRT1 activation or inhibition in these cells and treatment with calcitriol. Based on the results from the previous steps we will look for the differences in expression of genes dependent on vitamin D and/or SIRT1 activity, differences in VDR binding to DNA in cells with *EGFR* and *KRAS* mutations with or without SIRT1 silencing treated or non-treated with calcitriol. We also plan to analyze the influence of SIRT1 activity on the ability of VDR to bind to retinoid X receptor RXR, corepressors NCoR1 or coactivators NCoA1 of gene transcription and the localization of VDR binding with SIRT1 (nucleus vs. cytoplasm).

Research in this project will allow the assessment of the impact of selected epigenetic changes on the differential sensitivity of lung cancer cells with different *EGFR* and *KRAS* mutations status, to antitumor activity of vitamin D. Understanding the epigenetic mechanisms affecting the anti-cancer activity of vitamin D in lung cancer would justify further research to searching for new therapies based on the influence on epigenetic changes and vitamin D supplementation in the treatment and / or prophylaxis of lung cancer. In addition, the research of the presented project may contribute to the next step in the direction of broadening our knowledge about these two molecular subtypes of lung cancer, i.e. with the *EGFR* and *KRAS* mutations.