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Rola autofagii i starzenia w chemooporności raka płuca: analiza *in vitro*, *in vivo* i materiału klinicznego

Abstract

Chemotherapy is one of the most commonly used treatments for advanced lung cancer. However, often the therapy does not bring satisfactory results and after the treatment is completed, the therapy resistant cancer recurs. One of the possible mechanism for this occurrence is the development of Therapy Induced Senescence (TIS). Senescent cells show a number of characteristic features, such as: an increase in the activity of the specific enzyme SA-β-galactosidase, the Senescence Associated Secretory Phenotype (SASP), polyploidy or cell cycle arrest. The last one is beneficial for the treatment of cancer patients, however, recent studies suggest that TIS cells may revert to proliferative activity, leading to relapse of the neoplastic disease. The tumor microenvironment, characterized by hypoxia, is of key importance in the context of studying the cellular response to oncological therapies. Cancer cells can adapt to the hypoxia through various mechanisms including metabolic modifications and autophagy modulation, but the exact relationship between these mechanisms and TIS under hypoxic conditions remains unclear. Therefore, the aim of the study was to investigate the role of hypoxia in TIS development of lung cancer cells in response to chemotherapy and the influence of anaerobic metabolism or autophagy modulation in this process.

For this purpose, lung cancer cells were cultured under normoxic (~ 19% O2) or hypoxic (1% O2) conditions and received chemotherapy. The drugs most often used in the treatment of patients were applied: cisplatin, which damages DNA and therapeutics that disrupt the microtubules dynamics - docetaxel and vinorelbine. At the end of the experiment, TIS markers were tested.

The obtained results showed, that hypoxic conditions increased resistance to cisplatin by escaping from cellular senescence, which was not observed after microtubule poisons. The cells treated with cisplatin under hypoxia showed a reduced activity of SA-β-galactosidase and a decreased proportion of cells in the G2/M phase of the cell cycle compared to normoxic conditions. Moreover, hypoxia increased proliferative activity and the percentage of cells in the G0/G1 phase. In searching for the molecular mechanism responsible for this effect, it was found that hypoxia caused downregulation of the pro-senescence signaling associated with the p53-p21 pathway, and also induced epithelial to mesenchymal transition.

To study the effect of modulation of anaerobic metabolism or autophagy on the observed cisplatin resistance in hypoxia related to these processes genes silencing or pharmacological

inhibitors were used. Inhibition of the enzyme responsible for anaerobic metabolism (LDH) did not change the hypoxia effect on senescence escaping during cisplatin treatment. In contrast, autophagy inhibition through the use of a pharmacological inhibitor, hydroxychloroquine (HCQ), proved to be effective for this purpose. In the short term, HCQ reduces the proliferation of hypoxic lung cancer cells treated with cisplatin. In addition, NGS analysis showed, that among the down-regulated genes under the influence of HCQ revealed an overrepresentation of genes related to the terms of the cell cycle, DNA replication, and DNA repair pathways in KEGG base, which may be mechanisms to sensitize cells to platinum drug treatment. On the other hand, upregulated genes were overrepresented in terms related to infection response, immune system and inflammatory response, which could potentially play a role in tumor progression.

An attempt to evaluate the effect of HCQ on senescent cells treated with cisplatin in an *in vivo* murine model was unsuccessful due to the rapid growth of tumors prior to HCQ administration. This may be related to the occurrence of hypoxia in induced tumors and the observed *in vitro* resistance to cisplatin in hypoxia, however, in-depth research is required to confirm this hypothesis.

Since hypoxia proved to be a key factor in senescence escaping in ciplatin-treated lung cancer cells, it is reasonable to test hypoxia markers before administering this drug. Therefore, their expression was studied in samples from patients with non-small cell lung cancer. In some patients, different expression of hypoxia-induced factors was demonstrated in both tested samples, indicating the lung tumor heterogeneity. Moreover, the increased expression of HIF genes partially corresponded to the increase of stem markers, suggesting the role of hypoxia in promoting the stem phenotype of lung cancer cells.

In summary, hypoxia promotes the escape of cancer cells from cisplatin-induced senescence, which can be overcome by autophagy inhibition with HCQ. Thus, HCQ may be a potential therapeutic to reduce cancer relapses associated with senescence escaping in the response to platinum drugs in hypoxic tumor sites. However, the long-term effect of such therapy requires testing in preclinical and clinical trials.