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Rola autofagii i starzenia w chemooporności raka nerki: analiza *in vitro* i *in vivo*

Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne

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4. Abstract

A Renal cell carcinomas (RCCs) account for approximately 90% of all renal cancers. In 90% of RCC cases, there is a lack of functionality of the pVHL protein, a negative regulator of hypoxia inducible factors (HIF). Its absence leads to a state of pseudohypoxia. Since RCCs show a poor response to most standard systemic therapies, there is a strong need for the development of new therapeutic strategies. It is suspected that cancer cells that have developed an aging phenotype in response to therapy (therapy-induced senescence, TIS) may be responsible for the development of chemoresistance. TIS cells are irreversibly inhibited in the cell cycle and show characteristic morphological, biochemical, and molecular changes. TIS cells, despite the irreversible blockade of the cell cycle, are capable of generating daughter cells. It is suspected that autophagy may play an important role in the survival of old cells, as well as in the activation of their division. Its modulation seems to be a promising strategy in senolytic therapies. Therefore, this study aimed to determine whether the inhibition of autophagy would sensitize old renal cell carcinoma cells to cell death induction and/or affect the resumption of their proliferative potential and production of daughter cells under normoxic and hypoxic conditions.

In the first part of the experiment, human renal cell carcinoma cells with mutated pVHL and mouse cells with normal pVHL were treated with chemotherapeutics under normoxic (~19% O2) and hypoxic (1% O2) conditions. For the induction of senescence, a drug that blocks the mitotic division - vinblastine, and drugs with genotoxic properties - gemcitabine and 5-fluorouracil were used. When the experiments were completed, analyses of senescence markers expression were performed.

It has been shown that vinblastine has the strongest dose-dependent pro-senescence effect in kidney cancer cells, and its effect is independent of hypoxia when pVHL is mutated. Under the influence of treatment, most of the cells in the culture expressed activity of SA-β-Galactosidase and secreted SASP factors. Accumulations of enlarged, granular and polyploid cells were also observed in the cultures. The cells showed lower proliferation associated with cell cycle blockade. The induction of senescence was confirmed by significantly higher expression of p21 and p53 proteins. NGS analyses confirmed that DNA replication and cytokinesis are blocked as a result of senescence induction. In addition, NGS analyses have shown that in senescent cells, pathways related to neuronal development: axon guidance and neuroactive ligand-

receptor interaction were activated, which induction has not been described in context of senescence induction before.

In the second stage of the research, experiments were carried out to assess the effect of early and late autophagy inhibition in cells undergoing TIS. Early autophagy was inhibited in the cells before exposure to VIN and 5-FU. To this end, single genes encoding the ATG5, ATG7, Becn1, and ULK1 proteins were silenced in human kidney cancer cells by siRNA. The analysis showed that the inhibition of early autophagy does not significantly affect the induction of aging. In turn, pharmacological inhibition of late autophagy with HCQ was associated with the induction of cell death and blockade of the cell cycle. These changes were observed in untreated and VIN-treated cells. This indicates that HCQ is not a senolytic compound. In addition, it was observed that under the influence of autophagy inhibition, there is an escape from senesence, and hypoxia accelerated the generation of daughter cells. Cells generation was most likely the result of amitotic divisions. These changes were accompanied by the inhibition of autophagic flow, increased lysosomal activity, and increased SASP secretion. In addition, NGS analyses identified 67 genes whose expression differed between normoxia and hypoxia in cells escaping senescence. These genes may play a key role in regulating the generation of daughter cells. Experiments in a syngeneic mouse model have shown that aging can be effectively induced using chemotherapy *in vivo*. Molecular analyzes have shown that inhibition of autophagy with HCQ, in the perspective of short-term therapy, does not affect tumor growth and the expression of markers of epithelial-mesenchymal transition, stemness, immune response, and hypoxia. However, the use of HCQ promoted the production of IL-8 and osteopontin in tumors, which correlated with a decrease in the expression of the cell cycle inhibitor, p16, and a higher expression of the antioxidant response protein, GpX1. These changes suggest that the inhibition of autophagy may have contributed to escape from senescence in vivo. These results, however, require confirmation in experiments on larger research groups.

In conclusion, kidney cancer cells undergo cellular senescence under the influence of cytotoxic drugs, regardless of aerobic conditions. Inhibition of late autophagy leads to escape from aging, and hypoxia accelerates the generation of daughter cells. These results indicate that the inhibition of autophagy may have a negative effect in long-term therapies, but this needs to be confirmed in further studies.