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Rola PTEN w hipoksyjnym mikrośrodowisku raka nerki i czerniaka

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

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ABSTRACT

The role of PTEN in the hypoxic microenvironment of kidney cancer and melanoma

Understanding the molecular mechanisms involved in cancer progression and a comprehensive approach taking into account the entire tumor microenvironment (TME) is crucial for the search of effective anticancer treatment. One of the critical features of the TME of solid tumors is hypoxia - low, non-physiological oxygen tension. Hypoxia is the main factor causing pathological angiogenesis in tumors. Vessels abnormalities have a significant impact on cancer progression and determine the effectiveness of treatment. Normalization of vessels via the allosteric effector of hemoglobin – ITPP (myo-inositol trispyrophosphate), seems to be a promising therapeutic approach, as demonstrated, among others, in a melanoma model. In addition to compensation of tumor hypoxia, ITPP can also activate the tumor suppressor PTEN (phosphatase and tensin homolog). PTEN plays an important role in different cellular processes, including cell proliferation and metabolism. PTEN mutations or loss of PTEN function are observed in many types of cancer. The aim of this study was to verify the effectiveness of ITPP treatment in a murine model of kidney cancer and to determine the role of PTEN in the hypoxic tumor microenvironment.

Experiments were performed using murine models of kidney cancer (Renca) and melanoma (B16 F10). The effectiveness of ITPP in kidney cancer was tested *in vivo* – however, the positive effects of therapy, previously observed in melanoma, were not demonstrated. Therefore, research was conducted to determine the role of PTEN in both models characterized by a different response to ITPP treatment. The experiments aimed to assess the activity and function of PTEN in cancer cells in hypoxia, taking into account their effect on angiogenesis (*in vitro*). Additionally, using CRISPR/Cas9 mediated genome editing, murine melanoma and kidney cancer cell lines with *Pten* knockout were established, which allowed direct determination of the role of PTEN in tumor progression (*in vivo*, *in vitro*), as well as in response to standard anticancer treatment (*in vitro*). Moreover, in a kidney cancer model, the impact of hypoxia and *Pten* mutations on changes in miRNA expression were determined (by next-generation sequencing, NGS), *in vivo* and *in vitro*.

Both in melanoma and kidney cancer cells we observed hypoxia-dependent decrease in PTEN levels and a domination of the phosphorylated form (pPTEN). However, only in Renca hypoxia caused changes in p53/MDM2 pathway in the absence of pAKT accumulation, while in melanoma, a decrease in PTEN levels resulted in classic PI3K/AKT activation. Hypoxia also strongly stimulated the secretion of pro-angiogenic factors in the kidney cancer model, which resulted in changes in the activity and function of endothelial cells. Experiments performed using established *Pten* knockout cell lines showed no significant impact of PTEN dysfunction on tumor growth (*in vivo*) and cell proliferation (*in vitro*). However, both tested models showed different resistance to cisplatin treatment. Renca cells with *Pten* knockout (*Pten/KO*) were more resistant to treatment than *Pten/WT* cells. In turn, B16 F10 *Pten/KO* cells were more sensitive to cisplatin than *Pten/WT* cells. The observed differences may be related to the different effects of *Pten* knockdown on p53 expression and PAI-1 secretion in both tested models. Moreover, in Renca cells, *Pten* knockdown caused changes in the levels of markers characteristic for epithelial to mesenchymal transition (EMT). In kidney cancer model, a significant effect of hypoxia and *Pten* knockout on changes in miRNA expression were also observed. The main miRNA upregulated by hypoxia was miR-210, while the increase in miR-221 expression may be associated with hypoxia-dependent decrease of PTEN level. Among the miRNAs destabilized in *Pten* mutant cells, the oncomir miR-155 and miR-100 were upregulated.

To conclude, hypoxia is an important factor regulating PTEN activity in melanoma and renal cell carcinoma, but the outcome of PTEN modulation differs significantly in both tested models. These differences may play a key role in the response of cancer cells to standard anticancer treatment, and together with differences in pro-angiogenic potential, they may determine the ITPP-dependent vessels normalization. Deregulation of PTEN may also significantly modify the tumor microenvironment through changes in miRNA expression.