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„Expression of selected factors associated with genomic instability in pancreatic ductal adenocarcinoma: assessment of prognostic value and impact on chemoresistance of cancer cells"

Research conducted in recent years has demonstrated that genomic instability (GIN) plays a key role in the pathogenesis and progression of pancreatic cancer. In light of these findings and the lack of factors enabling effective prediction of disease course, the present doctoral dissertation aimed to identify new prognostic markers for pancreatic cancer. Four proteins associated with GIN that exhibit deregulated expression in pancreatic ductal adenocarcinoma (PDAC) were selected for analysis: cyclin F, RRM2, SPDL1, and SKA3. The study was divided into two main stages. The first stage utilized transcriptomic data collected as part of the TCGA project, as well as tissue samples obtained from PDAC patients. This phase aimed to assess the expression of cyclin F, RRM2, SPDL1 and SKA3 (at mRNA and protein levels) in the context of their prognostic value in pancreatic cancer and their association with selected clinicopathological features of patients. The second stage involved *in vitro* experiments conducted on the human pancreatic cancer cell line PANC-1. This phase aimed to evaluate the impact of altered *SPDL1* gene expression on the biological properties of the cells and their response to selected anticancer drugs.

It has been shown that elevated mRNA expression of all analyzed factors constitutes an independent negative prognostic indicator in pancreatic adenocarcinoma. Additionally, the combined analysis of *SPDL1*, *CCNF*, and *RRM2* mRNA expression was found to be more effective at predicting patient survival than assessing each gene individually. The analysis of the relationship between the protein levels of Cyclin F, RRM2, SPDL1, and SKA3 and patient survival in PDAC revealed that high immunoexpression of SPDL1 and SKA3 is an independent predictor of favorable prognosis for patients with PDAC in terms of overall survival, and in the case of SKA3, also disease-free survival.

In vitro analyses revealed that silencing *SPDL1* gene expression in the PANC-1 cell line reduces cell proliferation and colony-forming capacity while stimulating their migration ability. This genetic modification also significantly affected cell morphology, resulting in a population of large multinucleated cells and micronuclei, along with numerous mitotic abnormalities. These changes were often accompanied by elevated γH2AX expression within the nuclei. In analyzed cells, increased expression of factors associated with the biological

aggressiveness of cancer cells (including *CDH2*, *VIM*, *MMP9*, *CD44*) was also detected. These observations suggest that reduced *SPDL1* expression disrupts chromosome segregation and leads to DNA breaks, contributing to GIN in pancreatic cancer cells. Moreover, with regard to the first stage of the research, these abnormalities suggest that the positive prognostic value of the SPDL1 protein is likely linked to counteracting GIN, as well as the invasion and migration of pancreatic cancer cells.

In the subsequent stages of the *in vitro* research, the effect of altered *SPDL1* gene expression on the cytotoxicity of selected anticancer drugs against PANC-1 cells was evaluated. The results showed that paclitaxel and gemcitabine administered at clinically achievable doses significantly reduced the survival of PANC-1-SPDL1↓ cells by inducing apoptosis. It was concluded that the increased chemosensitivity to these drugs may result from the increased GIN of the analyzed cells.

The results presented in the doctoral dissertation suggest that the expression of all analyzed transcripts, as well as the SPDL1 and SKA3 proteins, should be considered potential prognostic factors in pancreatic cancer that require validation in multicenter studies involving larger patient cohorts. Additionally, the *in vitro* studies demonstrated that reducing SPDL1 expression enhances the genetic instability of PDAC cells, which may explain their increased chemosensitivity to certain anticancer drugs.