mgr Justyna Zińczuk

Ocena ekspresji białek regulatorowych cyklu komórkowego w zmianach przedrakowych zewnątrzwydzielniczej części trzustki

Streszczenie

Unfavorable statistics relating to the outcome of patients with pancreatic ductal adenocarcinoma caused a noticeable increase in the research focused on the biology of this cancer, what in turn contributed to prove that it develops from precursor lesions, the most common of which is pancreatic intraepithelial neoplasia (PanIN). The presence of numerous genetic changes such as telomere shortening, activation of oncogenic *KRAS* and *c-erbB-2* or inactivation of tumor suppressor genes *CDKN2A/INK4A, TP53, DPC4/Smad4* and *BRCA2* occurring in both intraepithelial neoplasia and invasive panctreatic cancer confirms the aforementioned assumptions. As an example - the inactivation of p16 gene or mutation of TP53 gene disturb the proper functioning of the cell cycle leading to uncontrolled cell proliferation and consequently the process of malignant transformation. Among the many crucial for those processes proteins is PCNA, which plays an active role in the regulation of the cell cycle through participation in DNA replication, repair damaged DNA and gene transcription. In the S phase of the cell cycle, PCNA binds to the cyclin A-CDK2, directing cyclin A-CDK2 complex to bind with proteins involved in DNA replication. PCNA concentration increases in the final stage of G1 phase, reaches a maximum in S phase, and then decreases during the G2 phase until completely disappears during mitosis and G0 phase. An important mediator of the regulatory PCNA actions might be p21 protein, which, through binding to the proliferation cell nuclear antigen, inhibits DNA elongation by the pol δ or pol ε. Interestingly, p21 protein binds to the cyclin D-CDK 4/6 leading to Rb phosphorylation inhibition and cell cycle arrest in G1 phase. It is worth noting that p21 is present in normal human fibroblasts in the G0 phase while the decline in its expression stimulates the cell to enter back into the cell cycle and DNA synthesis. In turn, p21 overexpression is observed in G1, S and G2 phases of cell cycle. Described protein might be activated in response to DNA damage by p53 protein that mediates cell cycle arrest at phase G1/S, allowing DNA repair. It might also activate GADD45 resulting in cell cycle arrest in G2/M. If damage cannot be repaired, p53 induces programmed cell death - apoptosis. Besides cell cycle regulation, p53 is involved in gene transcription, DNA synthesis and repair, cells differentiation, angiogenesis and senescence. Another example of negative PCNA regulator is cyclin D1, which belongs to

the family of cyclins regulating G1 phase, and is responsible for protein kinase CDK4 and CDK6 activation, with which it forms complexes required to initiation of DNA replication in S phase. During the cell cycle, concentration of cyclin D1 increases from the beginning of G1 phase to the checkpoint at the interface of the G1/S phases and then subsequently decreases. Cyclin degradation is essential for the initiation of replication, as its overexpression prevents the entrance to S phase. If cell proliferation continues, the level of cyclin D1 increases again in the G2 phase and is permanently maintained, even during mitosis. An excellent marker for the fraction of dividing cells is the Ki67 - non-histone protein binding to DNA in nuclear matrix. Ki67 protein is present in all active phases of the cell cycle (G1, S, G2 and M), yet its function in the regulation of cell cycle is still unknown, except for the fact that its lack inhibits cell division. An important role in the cell cycle regulation plays also p16 protein which inhibits cyclin-dependent kinase CDK4/6 counteracting Rb phosphorylation. Consequently Rb protein remains bound to the E2F transcription factor, thereby inhibiting transcription of E2F target genes expression of which is essential for the transition from G1 to S phase, and arresting the cell cycle at this stage.

This study aims at the evaluation of cell cycle regulatory proteins expression: cyclin D1, Ki 67, PCNA, p16, p21 and p53 in normal pancreatic ducts and in the tissue of patients suffering from pancreatic intraepithelial neoplasia. The expression analysis of examined proteins has been based on degree of pancreatic intraepithelial neoplasia and chosen clinical and histopathological parameters such as patients' age and sex, type of primary disease or the location of lesions in the pancreas. Moreover, the expression of cell cycle regulatory proteins in pancreatic intraepithelial neoplasia has been compared with expression in normal pancreatic ducts to verify whether there are correlations between examined parameters.

The study involved a group consisting of 70 patients who underwent surgery due to pathological changes in the pancreas (ductal adenocarcinoma, cysts, pancreatitis) in years 2006-2014 in the 2nd Clinical Department of General and Gastroenterological Surgery at University Hospital in Bialystok. The material has been been processed and subjected to histological evaluation in the Department of General Pathology at Medical University of Bialystok. Paraffin blocks with embedded tissue were cut into 5-μm thick sections and stained with hematoxylin-eosin (H+E). Histopathological analysis included diagnosis of primary disease, but also possible presence and degree of pancreatic intraepithelial neoplasia. Immunohistochemical analyses were carried out with the use of monoclonal and polyclonal antibodies against cyclin D1, Ki-67, PCNA, p16, p21 and p53. Respective steps of polymer method (blocking endogenous peroxidase activity, blocking other proteins capable of binding

an antibody, incubation with primary antibody, incubation with secondary antibody, incubation with Novolink Polymer) has been followed by DAB solution application as a visualization system of antigen-antibody complex. The results were subjected to statistical analysis using Spearman's rank correlation test. Correlations between proteins expression depending on PanIn degree were tasted with the use of Mann-Whitney's test. The level of statistical significance describe values $p < 0.05$. Gaps in the data were removed in pairs.

Statistical analysis revealed that pancreatic intraepithelial neoplasia more often affects women and patients aged over 60 years. Significant relationship has been also demonstrated between the degree of pancreatic intraepithelial neoplasia and the expression of cell cycle regulatory proteins - cyclin D1, Ki67, PCNA, p16, p21 and p53. Taking into consideration following markers: cyclin D1, Ki67, PCNA, p21 and p53, was observed positive correlation between their increased expression level and PanIN degree, whereas, opposite results have been obtained within p16 expression. The expression level of cyclin D1 protein, Ki67, PCNA and p21 within patients aged >60 years was significantly higher as compared to those who have not reached this age. Furthermore, expression of proteins Ki67 and PCNA was stronger among males than among females. Statistically significant relation has been also observed between p53 expression and lesions' location in the body of the pancreas. p16 and PCNA expression analysis showed a statistically significant dependence on the type of primary disease, and more precisely - the highest PCNA expression was observed in pancreatic ductal adenocarcinoma, while p16 in pancreatic cysts. Statistically significant difference in the expression level of all examined proteins was observed while comparing normal pancreatic ducts and PanIN. Additionally, expression levels of tested cell cycle regulatory proteins (cyclin D1, Ki-67, PCNA, p16, p21 and p53) significantly correlate with each other.

The results described above led to a more accurate understanding of the cell cycle deregulations and uncontrolled cellular proliferation which are crucial in development of pancreatic ductal adenocarcinoma and pancreatic intraepithelial neoplasia. Further the advancement of knowledge in terms of cell cycle deregulations may help to determine pathomechanisms contributing to the formation of premalignant lesions or malignant transformation in the pancreas, improve early diagnosis and implementation of appropriate treatment for the prevention of transformation into malignancy. All these aspects contribute to the achievement of a measurable effect seen in the prolonged survival of patients suffering from pancreatic ductal adenocarcinoma.