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IMMUNOHISTOCHEMICAL STUDY OF CASPASE-3 EXPRESSION IN COLORECTAL ADENOCARCINOMA

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Malignant transformation develops against the background of enhanced cell proliferation, mediated by activation of oncogenes and inactivation of tumor-suppressor genes, and also because of mutations in genes, which control apoptosis [1]. Well known, that malignant tumors are characterized by the low apoptotic activity level [2], but there are some exceptions. According to current literature, the breast cancer [3], the squamous cell carcinoma of oral cavity [4] are characterized by the high caspase-3 expression level. It is noteworthy that these carcinomas are more unfavorable in prognostic sense [2-4].

The current data that characterize the immunohistochemical expression of caspase-3 in colorectal adenocarcinoma are variable. It is considered that the cancer cells of colorectal adenocarcinoma are predominantly characterized by the low caspase-3 expression level [5-8], but also we found the data, that suggest a reverse [9]. Furthermore, we managed to find only the one study on the immunohistochemical expression of caspase-3 by the stromal cells of colorectal adenocarcinoma [10].

Aim. To study the caspase-3 expression levels in the tumor cells and the stromal cells of colorectal adenocarcinoma.

Materials and methods. Pathomorphological and immunohistochemical studies of the operational material from 30 patients with colorectal adenocarcinoma (I-IV clinical stages) were conducted.

The microstructure of the cancer was evaluated in paraffin sections, that were stained by hematoxylin and eosin. IHC study was performed using the monoclonal antibodies Mo a-Hu Caspase Ab-3 (Clone 3CSP03, «Thermo Scientific», USA) and visualization system DAKO EnVision+ with diaminobenzidine («DAKO», Denmark). The results of the study were evaluated in Axioplan 2 microscope («Carl Zeiss», Germany), microsections were photographed by digital camera «Canon EOS 1000D» (Japan) with an increasing of x200 in 5 fields of view.

The expression of caspase-3 in the tumor cells and the stromal cells of

colorectal adenocarcinoma was determined by the digital morphometry method [11]. In this way, the expression level of caspase-3 was expressed in the conventional units of the optical density (CUOD) and was graduated on 4 levels: lack of expression – 0-20 CUOD, the low expression level of caspase-3 – 21-50 CUOD, the moderate – 51-100 CUOD and the high expression level of caspase-3 – more than 100 CUOD.

Statistical processing of the results was performed on a personal computer using program «STATISTICA® for Windows 6.0» (StatSoft Inc., License № AXXR712D833214FAN5). The median (Me), the lower and the upper quartiles (Q_1; Q_3) were calculated. Comparison was performed using Mann-Whitney U-test. The study of correlations between the studied parameters was performed using Spearman's rank correlation test. The results were considered as statistically significant when $p<0,05$.

Results and discussion. Immunohistochemical studies results showed that the nuclear-cytoplasmic expression of caspase-3 was determined in the tumor cells and the stromal cells. The median of caspase-3 expression by the tumor cells of colorectal adenocarcinoma is 28,72 CUOD (15,64; 76,71), by the stromal cells – 37,78 CUOD (26,27; 54,60). The difference between these indices is not statistically significant ($p> 0,05$). Therefore, it was established that the tumor cells, as well as the stromal cells of colorectal adenocarcinoma, are characterized by the low apoptotic activity levels.

The correlation analysis showed that there is a direct strong correlation between the caspase-3 expression levels in the cancer cells and the stromal cells ($r=0,78$). This result indicates a close association between the apoptotic activity of the cell elements of the tumor parenchyma and the tumor stroma.

Correlations between the caspase-3 expression levels, depth of the tumor invasion, presence of regional metastases, distant metastases, clinical stage of the disease and degree of histological differentiation of the tumor have not been revealed.

These data are consistent with the current literature: the most studies describe the low caspase-3 expression level in colorectal adenocarcinoma, as well as the absence of correlations between the expression of this marker and the main clinico-morphological characteristics of the tumor [5-8, 12]. An exception is the study by J. Alcaide et al. (2013), in which it was reported that metastatic colorectal adenocarcinoma is significantly more apoptotic [9].

The question of the caspase-3 prognostic value for the patients with colorectal adenocarcinoma is actively discussed. J. Alcaide et al. (2013) [9], L. Flanagan et al. (2016) [12] reported that caspase-3 can be used as a prognostic marker: the high level of its expression is associated with a poor prognosis. P. Noble et al. (2015) [10] in their study came to the different conclusion: the high level of caspase-3 expression in colorectal adenocarcinoma is associated with a favorable prognosis. Moreover, in this study, the association between the high caspase-3 expression level and a favorable prognosis was established for the cancer cells and also for the stromal cells.

Conclusion.

1. Colorectal adenocarcinoma is characterized by the low levels of apoptosis of the tumor cells and the stromal cells, that is assessed by the caspase-3 immunohistochemical expression levels (Me=28,72 CUOD (15,64; 76,71) and Me=37,78 CUOD (26,27; 54,60), respectively).

2. The direct strong correlation between the caspase-3 expression levels in the cancer cells and the stromal cells (r=0,78) indicates a close association between the apoptotic activity of these cells elements.

3. The absence of correlations between the caspase-3 expression levels and the main clinico-morphological characteristics of the tumor indicates that the isolated use of the marker has no predictive value.

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DYNAMICS OF MORPHOLOGICAL PARAMETERS OF THE THYROID GLAND IN HYPERTENSIVE SHR RATS UPON ADMINISTRATION OF PROPYLTHIOURACIL

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Background. The main clinical manifestations of thyroid gland (TG) diseases are associated with the influence of thyroid hormones on the cardiovascular system. At the same time, hypothyroidism as well as hyperthyroidism increase the risk of arterial hypertension. Based on this, the development of an experimental model that combines thyroid pathology and hypertension is relevant. From this point of view, the hypertensive SHR rats is a valuable object of study, since the course of arterial hypertension in these animals corresponds to the signs of human hypertension.

Objective. Study of the dynamics of the morphological parameters of the thyroid gland in SHR rats with prolonged administration of propylthiouracil (PTU).

Methods. Female SHR rats were used in the experiments; drinking water contained a 0.1% solution of PTU. TG was taken on the 17^{th}, 25^{th}, 31^{st}, 39^{th} and 47^{th} days of the experiment, subjected to histological examination and staining with hematoxylin / eosin according to standard methods. We analyzed the average height of the follicular epithelium, the average follicle area, the nuclear cytoplasmic ratio (NCR) of thyrocytes, the ratio of the number of thyrocytes to fibroblasts per 50 μm^2 (T/FB).

Results. It was established that the height of the epithelium, NCR, and the area of the follicles change irregularly during the intake of PTU: at first, fluctuations of