

PROLIFERATIVE-APOPTOTIC PROCESSES IN TESTICULAR SEMINOMA

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Testicular tumors, though being equal up to 1 % out of all the male neoplasms all over the world, are the most frequent among white men within the period from puberty up to the age of 40 in industrially advanced countries [1]. At that, testicular germ cell tumors (TGCT) amount to more than 90% of all the testicular tumors and seminoma amounts about 50% of them [2]. Objectives: to study the proliferative-apoptotic processes in testicular seminoma.

Materials and Methods. Material was collected in Pathological Anatomy Department of Kharkiv Regional Clinical Centre of Urology and Nephrology named after Shapoval V.I. We analyzed 13 cases of testicular seminoma which were obtained with orchifuniculectomy. Immunohistochemical (IHC) examination was performed by indirect immunoperoxidase reaction. For estimation of proliferative-apoptotic processes the following primary antibodies were used: Mo α -Hu Ki-67 (Monoclonal Antibody, clone MIB-1, «DAKO», Denmark), Rb α -Hu Bax (Polyclonal Antibody, «Thermo Fisher Scientific Inc.», USA), Mo α -Hu Bcl-2 (Monoclonal Antibody, clone 100/D5, «Thermo Fisher Scientific Inc.», USA) and Mo α -Hu p53 (Monoclonal Antibody, clone DO-7, «DAKO», Denmark). The reaction was visualized using Ultra Vision Quanto Detection Systems HRP Polymer («Thermo Fisher Scientific Inc.», USA). For the most vivid comparison of IHC characteristics all observations were divided into groups depending on the degree of tumor progression. Thus, guided by WHO and pathological pTNM classifications [3], the following groups were formed: group «1» – tumors of this group corresponded to the stages $T_1N_0S_{0-2}$ (n=4), group «2» – $T_2N_{1-3}S_{0-2}$ (n=5), group «3» – $T_3N_{1-3}S_{0-2}$ (n=3) and group «4» – $T_{2-3}N_{0-3}S_{0-2}$ with the presence of distant metastasis (n=1). IHC stained histological sections of seminomas were recorded using an Olympus BX-41TF microscope (Japan) and a digital camera Olympus C3040-ADU (Japan). For the morphometric measurement of the relative area of immunopositive structures determined in % the received photos were processed in Matlab software using standard digital image processing tools. Counting of mentioned parameter was performed in 10-30 randomly selected fields of vision of the microscope Olympus BX-41TF at magnification $\times 200$ ($3,12 \times 10^{-7}$ m²) in each case. All values are expressed as means and standard error of the mean for statistical analysis. Spearman's rank correlation coefficient (r) was counted for measure of the strength of relationship between paired data. The accepted level of significance was $p \leq 0.05$.

Results. The average relative area (ARA) of Ki-67 expression in the observations of group «1» was low and comprised $1.17 \pm 0.07\%$. At that, expression of Ki-67 was identified predominantly in tumor cells, but was also present in cells of the immune infiltrate. In the group «2» ARA of Ki-67 expression ($1.56 \pm 0.13\%$) was not significantly different from the same in the group «1» ($p > 0.05$). As well as in the previous group, the expression of Ki-67 was predominantly located in tumor cells. In the group «3» the ARA of Ki-67 expression ($1.91 \pm 0.05\%$) was greater than the same index in the group «1» ($p < 0.05$) and did not differ significantly from it in the group «2». In a single observation of the group «4» the ARA of Ki-67 expression was $1.91 \pm 0.1\%$.

In the group «1» ARA of Bax expression was $2.74 \pm 0.1\%$ and in the group «2» and group «3» – $5.35 \pm 0.07\%$ and $6.0 \pm 0.06\%$ respectively. Comparison of these values showed increasing of ARA of Bax expression in the groups «2» and «3» relative to the same index in group «1» ($p < 0.05$ and $p < 0.05$ respectively). Besides, the ARA of Bax expression in the group «3» was greater than the same in the group «2» ($p < 0.05$). In a single observation

of group «4», the ARA of Bax expression was $5.93 \pm 0.1\%$. Expression of Bax was identified predominantly in the cytoplasm of tumor cells and in single cells of immune infiltrate. As regards the anti-apoptosis marker bcl-2, it was detected only in single tumors: in one of five observations in the group «2», in one of three observations in the group «3» and in a single case of group «4». Immunopositive staining was detected only in the cytoplasm of single cells of the immune infiltrate. Expression of bcl-2 was absent in tumor cells. Expression of p53 was absent in all observations of the group «1» and in two of five cases of the group «2» (respectively, in three, there was a positive staining of single tumor cells). In groups «3» and «4», all observations were characterized by a positive reaction with p53, but only in single tumor cells. Statistical analysis showed a high positive correlation between the relative area of Ki-67 and relative area of Bax and p-53 expression ($r=+0.83$ and $r=+0.70$ respectively). Also moderate positive correlation between the relative area of Bax and p-53 expression ($r=+0.62$) was identified. Valid correlation between the relative area of Bcl-2 and other investigated markers was absent. Prospects for further research. Further investigation of proliferative-apoptotic processes in other germ cell tumors is planned with the detection of differences between them.

References:

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DOI: 10.29256/v.03.01.2019.escbm74

GENETIC PROFILE OF THE RISK OF DEVELOPING FATAL CASES IN PREMATURE NEWBORNS WITH SEVERE INTRAVENTRICULAR HEMORRHAGES

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Intraventricular hemorrhage (IVH) is one of the main causes of infant mortality. According to data from the University of Southern California, the mortality rate of newborns with intraventricular hemorrhage III and IV degrees is 18-40% [4], initial studies indicated that mortality in premature newborns with intraventricular hemorrhage III degree of severity till 30% and till 60% in children with intraventricular hemorrhage IV degree [2, 3], the same data are confirmed by Ahn S.Y. from et al. [1]. According to the analysis of the mortality rate of prematurely born children with severe IVH in the Poltava region for 2009- 2016 years, we found that the mortality rate remains constant [5]. In our opinion, this situation can be explained by an increase in the survival of newborns with body weight at birth <1000 g, due to improved diagnostic protocols, the presence of combined pathology in premature infants. The purpose of the work was to determine the profile of the risk of developing fatal cases in premature newborns with severe IVH, taking into account the impact statement of polymorphous variants of the PAC and eNOS genes.

Materials and Methods. To study the effect of the polymorphism of the studied genes on the development of severe IVH and their negative effects, the following genetic models were used: the dominant model (DD DI vs. II) of the ACE gene, the dominant model (CA AA vs. AA) of the AGT2R1 gene, the dominant model (aa ab vs. bb) eNOS gene and their combinations. The material for conducting genetic studies was 0.25 ml of blood of newborns, which was taken on the 6th-10th day of life after