

glandular hyperplasia in reproduction period (mean percentage of reactive cells – Labeling Index-LI $14,8 \pm 0,4\%$), menopause period (LI $18,2 \pm 0,8\%$), atypical hyperplasia (LI $15 \pm 0,5\%$) and well differentiated carcinoma (LI $22,6 \pm 0,3\%$). All specimen of glandular hyperplasia were negative for p53 expression. P21 expression was observed in 50% cases of glandular hyperplasia, but LI was low (<7) P16 expression was lower in hyperplastic endometrium of reproduction period (LI $30 \pm 0,3\%$), increased in menopause (LI $41 \pm 0,6\%$), decreased in atypical hyperplasia (LI $33,2 \pm 0,2\%$) and high differentiated carcinoma (LI $14,9 \pm 0,2\%$). It was observed over expression of both p53 and p21 (LI $91,0 \pm 0,9\%$ and $28,5 \pm 3,2\%$ accordingly), lowering of expression p16 (LI $28,0 \pm 0,3\%$) in one case of atypical hyperplasia, other specimen of atypical hyperplasia were negative for p53 and p21 expression. In groupe of patients with high differentiated adenocarcinoma was determined increasing expression of p53 (LI $35,9 \pm 0,8\%$), p21 (LI $7,8 \pm 0,2\%$) and decreasing expression of p16 (LI $14,9 \pm 0,2\%$).

Conclusion: The most of cases of hyperplastic endometrium were characterized high proliferative activity, which was increased in patients with glandular hyperplasia in menopause and atypical hyperplasia. Patients with high proliferative potential, high expression of Ki-67, p53 p21 genes and lowering expression of p16 form high group of risk for malignant transformation and require especial dynamic observation.

P21

Nucleolar organizer regions (NOR's) of chromosome as marker of endometrial proliferation

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Objective: The evaluation of NOR's in patients with glandular hyperplasia (GH) and endometrial cancer (EC).

Material and methods: Cultivated peripheral blood lymphocytes (PBL) and the surgical material of 106 patients with GH and EC were used. The NOR's analysis in metaphase chromosomes of PBL and histological sections of endometrium, stained with AgNO₃ according Howell and Black was performed. DNA content in endometrial epithelial cells was carried out using scanner microcytospectrophotometer MCSU-2MT.

Results: The estimation of PBL NOR's revealed the significant increasing of NOR's level of expression in 13, 21 and 22 chromosomes and elevated association index in EC patients in comparison with the same in GH patients. The biggest frequency of 13 and 21 chromosomes association was determined in cancer patients.

The study of NOR's in endometrial interphase cells revealed reliable increasing of mean quantity AgNOR's in tumour cells in comparison with GH of endometrium. It was determined progressive increasing of expression of NOR's in high-, middle- and low differentiated carcinomas (LI= $2,91 \pm 0,27\%$; $4,63 \pm 0,41\%$; $8,57 \pm 0,4\%$, accordingly) in patients with endometrial cancer. The analysis of cell number with more than 5 AgNOR's, that reflects the cell fraction in S-phase of cell cycle demonstrated the increasing of proliferating epithelial cells population in the line from high- to low-differentiated EC. The last correlated with increasing level of Ki-67 expression (LI= $22,6 \pm 0,3\%$ in high- in comparison with $47,6 \pm 0,4\%$ in

low differentiated carcinomas) and p53 (LI= $35,9 \pm 0,8\%$ in high- compared with $57,4 \pm 0,6\%$ in low differentiated carcinomas) and with elevated aneuploidy and predomination of nearly-triploid (56,6%) cells in patients with low level differentiated cancer. The correlation between mitotic activity and mean quantity of AgNOR's in nuclei in endometrial tumour cells was determined ($r=0,63$).

Conclusion: The obtained data give evidence that the level of expression of ribosome cystrons correlates with structural peculiarities of endometrium and can be a cytogenetic marker of genome instability. Activity AgNOR's with indicator expression of Ki-67 and p53 are objective criterions of proliferative potencial and can be used for differential diagnostics of glandular hyperplasia and endometrial cancer and as an additional marker of malignancy degree of tumour process in uterus.

P22

Cysteine proteases as biomarkers in murine tumors sensitive and resistant to cyclophosphamide

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The expression, activity and concentration of cysteine proteases in tumor tissue and serum can be used as prognostic and diagnostic biomarkers in several types of human cancer (Kos et al., 2002).

Aim: to evaluate the cysteine proteases as biomarkers in murine tumors, sensitive or resistant to cyclophosphamide (CPA) treatment.

Methods: DBA/2 mice with leucosis P-388 and L1210 (less aggressive) and CBA mice with lymphosarcoma LS, sensitive or resistant to cyclophosphamide (CP) were treated by CP (25, 30, 50 and 100 mg/kg) or/and new immunomodulator sulfoethylated glucan (SEG, Institute of Chemistry, Slovak AS, Bratislava, 10, 25, 50 mg/kg, single or three times). Cysteine protease activity was measured by fluorometrical method (Barrett, Kirshke, 1980).

Results: In murine leukosis P-388 as compare to leucosis L1210 (less aggressive) solid tumor development was characterized by lower cathepsins B, L and D activity in tumor tissue. The effective treatment by CPA and SEG was followed by tumor growth suppression and increased activity of proteases studied. In comparison to sensitive variant the resistant variant of lymphosarcoma LS was characterized by aggressive development, faster tumor growth, poor condition of mice and their shorter survival. Activity of cathepsins B, L and D in tumor tissue in resistant type was significantly lower comparatively to susceptible type of lymphosarcoma LS, during combined therapy activity of proteases increased. Preliminary to CPA administration of SEG enhanced therapeutic action of CPA both susceptible and resistant type of lymphosarcoma LS. In the both cases the significant synergistic effect of SEG was revealed at the lowest dose applied (10 mg/kg). The results obtained imply a possibility of application of low doses of SEG during combined tumor therapy with cytostatics. Similar doses SEG were effective in combination with CPA as preventive (preliminary administration of SEG). Moreover, SEG (10, 25 mg/kg) alone revealed antitumor activity in lymphosarcoma LS