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p53 and bcl-xl - apoptotic markers predicting axillary recurrence following axillary radiotherapy in carcinoma of breast

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Introduction: Management of node-positive axilla from breast cancer remains controversial, axillary radiotherapy, axillary clearance being the two options. Specific molecular markers in axilla and primary tumour that mediate radioresistance could influence choice of treatment.

Aim: To assess the expression of apoptosis proteins as markers for axillary recurrence following axillary radiotherapy.

Methods: Ten patients who developed axillary recurrence following axillary radiotherapy were compared with 10 matched controls who remained disease-free at 5 years. All underwent a wide local excision (WLE)/mastectomy followed by radiotherapy to the breast/chest wall and axilla under similar regime. Immunohistochemical analysis was performed on primary tumour, axillary node samples for p53, anti-apoptotic markers (bcl-2,bcl-xl,mcl-1) and pro-apoptotic markers (bad,bak,bax). Scoring was done by 2 independent observers.

Results: In 5 out of the 10 cases, the primary tumour was positive for p53 as opposed to none in the controls, the difference being statistically significant (p= 0.016,Fisher's exact test). A statistically significant difference was also found in the expression of bcl-xl between the axillary samples of cases and controls (p=0.035, Fisher's exact test). None of the other markers were significantly different between the 2 groups.

Conclusion: p53 mutation in the primary tumour is a significant risk factor for axillary recurrence following axillary radiotherapy. Over-expression of bcl-xl in the axillary sample may also contibute to radioresistance by failing to initiate an appropriate apoptotic response in cells with radiation induced damage.

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p53 and p21WAF1/CIP1 proteins and cells proliferation in ovarian carcinomas

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Now time the selection of therapeutic approach of patients with ovarian carcinoma predominantly is based mainly on morphological characteristics. However the molecular profile of ovarian neoplasm is necessary also for correct prognosis.

Aim: To determine the proliferative activity and the overexpression of supressor genes p53, p21WAF1/CIP1 as possible prognostic factors in ovarian tumors.

Materials and methods: Archives materials from 50 patients (age 17-74) were used. Fourty patients were with serous ovarian carcinomas and 10 patients – with normal ovary obtained from women with hysteromyoma (control group). Tissue sections were immunohistochemically stained using a monoclonal antibodies: for Ki-67 clone MIB, for p53 clone DO-7 and for p21WAF1/CIP1 clone SX118 ("Dako"). The presentage of immunopositive cells was calculated as labeling index (LI).

Results: The low proliferation activity (Ki-67 LIs = 1,0) and negative expression of p53 and p21WAF1/CIP1 were found in normal ovary. There were heterogeneity in Ki-67, p53 and p21WAF1/CIP1 expression in ovary tumors. Ki-67 was detected in 92,5% ovary tumors. The most tumors (90,0%) were highly proliferating (Ki-67 LI > 10,0). Nuclei p53 staining was found in 95,0% tumors (LI = 40,3±0,3; 6,7 œ 72,5) and p21 - 87,5% (LI = 6,8±0,3; 1,5 œ 31,3). p53 and p21WAF1/CIP1 overexpression was found respectively in 72,5% tumors (LI > 30,0) and 12,5% (LI > 15,0). It was observed dependence between Ki-67, p53 and p21 expression and the level of ovarian carcinomas differentiation. Ki-67 and p53 increase from well to poorly differantiated and were equal to 14,0±0,4 and 37,1±0,4, respectively and Ki-67 - 34,9±0,7 and 45,8±0,5, respectively for p53. However, p21 was changed in oposite direction from 9,4±0,8 and 3,4±0,3. It was determined that Ki-67 level was higher in tumors of patients with survival time 5-10 years, than more 10-20 years, p53 expression was nearly similar.

Conclusion: The levels of Ki-67, p53 and p21WAF1/CIP1 expression objectively reflect ovarian tumor cells biological particularities and can be used for prescription of adequate treatment and as independent factors for prognosis.

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Prognostic value of Ki-67, p53, p21(WAF1) and p16 expression in glandular and atypical hyperplasia of endometrium

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Aim: Determination of tissue proliferation activity, expression of tumour supressor genes P53, P21, P16 in patients with glandular, atypical hyperplasia in comparison with high differentiated adenocarcinoma.

Materials and methods: Formalin-fixed scrapes and operative materials were obtained from 67 patients (age 31-69) with glandular and atypical hyperplasia. Tissue sections were immunohistochemically stained for Ki-67 using a monoclonal anti-Ki-67 antibody (clone MIB), p53 antibody (clone DO-7), p21 (clone SX118), p16 (clone DCS-50), DAKO Cytomation.

Results: Analysis of proliferative potential showed significant variation of expression Ki-67 in hyperplastic endometrium (individual variations 1-37,1%), and high differentiated carcinoma (5-45%). Hight proliferation (>10% cells staining) was observed in 50% cases of hyperplastic endometrium and 83,3% cases of carcinoma. Reliable increasing of proliferative activity in comparison with normal endometrium was determined in

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

Conclusion: The moust of cases of hyperplastic endometrium were characterized hight 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 $AgNO_3$ 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 determinated progressive increasing of expression of NOR's in high-, middle- and low differentiated carcinomas (LI= 2,91±0,27%; 4,63±0,41%; 8,57±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±0,3% in high- in comparison with 47,6±0,4% in low differentiated carcinomas) and p53 (LI= 35,9±0,8% in high- compared with 57,4±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.

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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 cy 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