23 POSTER

Frequency and prognostic significance of genetic instability at tumour suppressor gene loci in mamary carcinomas

J. Ryś[1], R. Schneider-Stock[2], A. Kruczak[1], A. Stelmach[1], A. Sokolowski[1], A. Gruchala[1], W. Szklarski[1], D. Markiewicz[1], A. Roessner[2], A. Niezabitowski[1]. [1]Cracow Center of Oncology, Poland; [2]Institute of Pathology, University of Magdeburg, Germany

The evolution of tumour is accompanied by a number of serial genetic events which lead to the malignant phenotype. The inactivation of tumour suppressor genes plays a critical role in multistage carcinogenesis. At present analysis of LOH using polymorphic microsatellite markers is the most common methodology employed for the localisation of sites in the genome with high probability for the presence of candidate tumor suppressor genes. LOH has been described in human breast carcinomas at several chromosomal arms with frequently lost regions being at 3p, 6q, 7p, 16q, 17p. The role of LOH at p16 gene (localized to 9p) and Rb gene (localized to 13q) in human breast carcinomas is still controversial. That is why we investigated the role and possible interactions of three suppressor genes: p16, Rb and p53 in mammary carcinomas.

Material and Methods: 70 sporadic breast cancer patients were screened for LOH with microsatellite markers on 5 different loci: C5.1 and D9S162 for p16, intron20 and D13S263 for Rb and intron 1 for p53. DNA fragments were amplified by PCR from tumor (fresh material) and reference tissue (from paraffin blocks). Simultaneously the same tumours were investigated for the presence of p53 mutation basing on analysis of exons 4–8 by PCR-SSCP technique. The results of molecular studies were compared to the datas obtained through the immunohistochemical analyses of p16, Rb and p53 proteins, and they were correlated to others clinico-morphological parameters like histological staging, proliferation activity measured by expression of Ki67 (MIB) antigen, ploidy, estrogen and progesterone receptor status, node status and survival of the patients.

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Tumor circulating cells in low-risk breast cancer patients

F. Ferrara[1], E. Pezzica[2], M. Cremonesi[3], D. Corti[2], E. Makovec[1], R. Ciotti[3]. [1]Servizio Integrato Medicina di Laboratorio, HSR Istituto Scientifico San Raffaele Milano; [2]Servizio Anatomia Patologica; [3]Servizio Oncologia Medica; Ospedale Azienda U.S.S.L 13 Treviglio BG, Italia

PCR or immunocytochemistry performed in breast cancer pts detect tumor cells on the apheresis during ABMT or in peripheral blood immediately after the surgical manipulations. (J Clin Oncol 1996 14, 6: 1868–76 Br. J. Cancer 1996, 73: 79ndash;82)

Methods: The peripheral blood samples from 41 pts before and after the surgery were labeled either with anti-cytokeratins (CK) MoAb or propidium iodide for nuclear stain. The samples were then analyzed for a simultaneous double fluorescence detection. 32 pts had T < 2 cm and 27 had nodal involvement <6. Chemotherapy (CMF or Epirubicin-CMF) was carried out in 31 pts, Radiotherapy in 18 pts for conservative surgery.

Results: 10/41 samples (25%) contained CK positive events (CK+). We observed that the CK+ samples were positive too for aneuploid DNA content (aDNA+): No samples were only CK+ or aDNA+. The peripheral blood aneuploidy was also confirmed in frozen specimens from primary tumors. After 3 months seven samples aDNA+/CK+ shifted to aDNA –/CK – but in 3/10 cases we again observed aDNA+/CK+ without disease evidence.

Conclusions: We cannot confirm that the chemotherapy, the G-CSF or the surgery release tumor cells. 1) No correlation were found with a) nodal status b) p53 c) Ki67 d) ormonal pattern. 2) The circulating cells were only associated with lymphatic and blood vessel invasion found at breast cancer specimens evaluation. 3) Efforts should be addressed in order to understand their clinical significance and/or their metastatic potentials.

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PCR-SSCP technique for the detection of mutation in the exons 5 and 6 of the p53 gene in breast cancer. Higher sensitivity than immunohistochemical technique

A. Dueñas[1,2], J.J. Cruz[2], M.M. Abad[3], R. González-Sarmiento[1], C.A. Rodríguez[2], E. Fonseca[2], A. Gómez[2], G. Martín[2], P. Sánchez, R. Salazar[1,2]. [1]Dept. Genetica Molecular, [2]Dept. Oncologic, [3]Dept. A. Patológica. University Hospital. Salamanca, Spain

Purpose: p53 mutation, is the most frequent genetic abnormality in breast cancer, providing prognostic information and a better understanding of tumor biology. In this study we compare the sensitivity of immunohistochemistry and mollecular genetics methods for the detection of p53 mutations.

Methods: Forty tumors obtained from breast cancer patients submitted to a modified radical mastectomy were analyzed for p53 mutations at exons 5–6 through PCR-single stranted conformational polymorphism (SSCP)-sequencing. Moreover, the expression of p53 protein was analyzed by immunohistochemistry.

Results: Six of the 40 tumors (15%) amplified by PCR and analyzed by SSCP, showed an altered electrophoretic mobility. In these cases a mutation was confirmed by gene sequencing. Only two of these six tumors were positive in the immunohistochemical analysis. Immunchistochemical analysis of all tumors were positive in 15 cases (37%), which is explained by the presence of mollecular abnormalities in other region of the gene.

Conclusion: These results confirm the utility of the PCR-SSCP technique for detection of p53 mutations and suggest to be more sensitive than immunohistochemistry.

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Tumor lymphocytic infiltration, hormonal-metabolic status and aromatase gene expression in breast cancer

L. Berstein, T. Poroshina, E. Tsyrtina, V. Gamajunova, O. Chernitsa, A. Larionov, I. Kovalenko, T. Zimarina, A. Mikhailov, V. Semiglazov, N.N. Petrov. Research Institute of Oncology, St. Petersburg, 189646, Russia

Mononuclear inflammatory cells infiltrating tumors are believed to represent marker of the host immune response to neoplastic growth but also may be considered as hormonocytes metabolizing steroid hormones and reacting to the local and systemic hormonal signals (L. Berstein et al., 1993, 1995). Such capacities of these cells may affect both the size of tumor macrophagal-lymphocytic infiltrates (LI) and their influence on the neighbouring malignant cells. As first step to approach this problem we compared intensivity of LI with hormonal-metabolic parameters (113 pts) and aromatase gene expression (42 pts) in breast cancer. Age of patients varied from 25 to 77 yrs; 63.7% of patients were in I–IIa stages of disease. LI density (LID) was evaluated in hematoxylin-eosin stained preparations and graded into 7 groups (from ± till ++++). Age and menopause do not influence LID in breast tumors, though in smoking menopausal pts LID was higher than in non-smokers. LID correlated positively with SHBG, LH and cholesterol level in blood, progesterone receptors content in tumor and lean body mass content and negatively with thyroid hormones blood level, cortisol and norepinephrine in urine and body fat/lean body mass ratio. No any correlation was revealed in the whole group of patients between LID and tumor aromatase gene expression evaluated by dot-blot. In sample of tumors (n = 14) treated by irradiation increase in aromatase expression and positive correlation with LID was discovered. Thus, LID may depend of hormonal metabolic-status and be connected in certain conditions with estrogen production in breast tumors.

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Low-dose tamoxifen trial in healthy women

B. Bonanni[1], A. Decensi, R. Travaglini, A. Guerrieri Gonzaga, A. Tessadrelli, M.T. Sandri, G. Farante, D. Bettega, Chris Robertson, A. Costa. [1]FIRC Chemoprevention Unit at the European Institute of Oncology, Milan, Italy

Since Tamoxifen (TAM) has been associated with an increase in endometrial cancer incidence at the usual standard dose, a dose reduction could lift up its cost-benefit ratio. This study was aimed at assessing whether a short course of TAM treatment at the dose of 10 mg/d or 10 mg/qod is associated with a significant modulation of a number of estrogen-regulated target systems, including total cholesterol as the primary endpoint. A total of 69 healthy hysterectomized women have been randomized. Results have been compared with a previous study performed on the same type of population, where the effect of TAM 20 mg/d or placebo were studied (table). Other parameters of the lipid profile, clotting system, hemogram, IGF system and markers of bone metabolism are being assessed. Our

Effect on total cholesterol (mg/dl, mean ± SD)

	Baseline	1 month	2 months
Placebo (n = 37)	239 ± 34	253 ± 35	249 ± 41
Tam 10 mg qod (n = 35)	223 ± 46	197 ± 37	98 ± 35
Tam 10 mg (n = 34)	222 ± 48	188 ± 42	189 ± 39
Tam 20 mg (n = 31)	223 ± 33	201 ± 36	204 ± 43