23 POSTER

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

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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.

24 POSTER

Tumor circulating cells in low-risk breast cancer patients

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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.

25 POSTER

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

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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.

26 POSTER

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

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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.

27 POSTER

Low-dose tamoxifen trial in healthy women

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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

preliminary data suggest that lowering TAM dose by 1/2 or even by 1/4 still allows maintenance of the full effect on total cholesterol.

28 POSTER

ALL-*trans*-Retinoic (ATRA) acid induces cell cycle perturbations and apoptosis in human breast cancer

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Purpose: The antiproliferative effect of ATRA on MCF-7 human breast cancer cells was correlated with the specific effects on the cell cycle progression and apoptosis.

Methods: These effects of ATRA were investigated using DNA content evaluation and dual parameter flow cytometry (FCM) of bromodeoxyuridine incorporation and of the expression of cell cycle-related proteins (Ki-67 as proliferation marker and Statin as quiescence marker) vs DNA content. Apoptosis was studied by FCM of both DNA content and labelling of phosphatidilserine residues by Annexin V.

Results: After 4-days of ATRA treatment, the % of S-phase cells decreased significantly and cells accumulated in the G0/G1 range of DNA content. FCM analysis showed a decrease in the % of Ki-67+ve cells with a simultaneous increase of the 57% of Statin +ve cells. From 5 days of treatments onwards, apoptosis was found to occur.

Conclusions: ATRA-induced inhibition of MCF-7 cell growth is related with the block of cell proliferation mostly in a pre-DNA synthetic phase and the induction of apoptosis. This should be taken into account in the attempt to associate ATRA with other antiproliferative drugs.

29 PUBLICATION

Presence of germline BRCA2 mutations in sporadic breast cancer: Clinical correlation

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Purpose: Breast cancer is the most common malignancy in women. It occurs in hereditary and sporadic forms. At present, the sporadic breast cancer accounts for 90% of all breast cancers. Germline BRCA2 gene mutations have been identified in families prone to breast cancer. We designed the present study, now under way, to detect the presence of germline mutations at BRCA2 gene in sporadic breast cancer.

Methods: Our series consists of 93 patients diagnosed of breast cancer, without family history of breast and ovarian cancer. The mean age was 55 years, and the median age 53. The following clinical parameters were analyzing: Birth and diagnosis date, family history, menopausal status, histology of tumors, pathological stage, hormonal receptors, survival and vital status. The mutational study was performed by PCR-SSCP in peripheral blood lymphocytes DNA of the patients.

Results: We observed in 4 patients (4.3%) presence of aberrant fragment migration (now under sequencing process), 3 of them located at the same fragment (exon 11.19). The comparison of the 9 clinical parameters, between the two subgroups of patients, with and without mutation, did not show any significant difference.

Conclusion: The prevalence rate of mutations in sporadic breast cancer, considering their age, is higher than expected. It is possible that we detected a founder mutation. No implication of germline BRCA2 mutations as prognostic factor.

30 PUBLICATION

Analysis of bcl-2 and p53 genes in patients with breast cancer

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Methods: 46 primary nonmetastatic breast carcinomas. Immunohistochemical expression of bcl-2 and p53 proteins was analyzed in formalin-fixed paraffin-embedded sections. Exons 5 to 9 of the p53 gene were analyzed by PCR-SSCP in 40 of the 46 tumors. Genomic organization of bcl-2 was analyzed by southern blot from tumor tissues and from the peripheral blood of 11 patients (pt).

Results: There was a significant direct correlation between Estrogen and Progesterone receptor expression and bcl-2 protein expression ($p = 0.01$ and $p < 0.0001$). Significant correlation with tumor size was obtained ($p < 0.0001$) but not with lymph node invasion. We did not detect any molecular abnormality in the genomic organization of bcl-2; None of the 11 pt in whom peripheral blood was analyzed showed loss of heterozygosity at bcl-2 locus. The immunohistochemical expression of bcl-2 was inversely related to p53 protein expression, ($p = 0.05$), however the analysis of the correlation between p53 gene mutations and bcl-2 protein expression did not show significant correlation ($p = 0.79$). p53 gene mutations were present in 12 tumors (30% of the cases). (We detected p53 protein expression in 8 of the 12 tumors carrying p53 mutation. p53 protein staining was also detected in 14 cases without mutations at exons 5 to 9).

Conclusion: We have not found any correlation between bcl-2 immunohistochemical expression and p53 gene mutations. Bcl-2 is expressed in tumors with wild-type p53 as well as in those carrying a p53 mutation. This lack of correlation may reflect that regulation of bcl-2 expression is independent of p53.

Cancer genetics

31 ORAL

DNA mismatch repair deficient tumors exhibit length variability of repetitive DNA sequences in diverse promoter regions

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Hereditary Non-Polyposis Colorectal Cancer (HNPCC) is a cancer predisposing trait characterized by germline mutations in DNA mismatch repair (MMR) genes. Loss of MMR function in tumors of HNPCC patients results in length variability (LV) of DNA repeats within microsatellites, of coding and non-coding sequences. DNA repeats are also present within promoter regions, but have not been analyzed in HNPCC-associated cancer so far. Therefore, we analyzed HNPCC tumors for genetic instability of DNA repeats present in different human promoter regions.

Promoter repeats of the human Interleukin-10 (IL10, $[CA]_{21}$), Retinoblastoma (Rb, $[A]_{20}$), WAF1/CIP/p2l $[A_{12}]$, CyclinB1 ($[A]_{11}$), PhospholipaseA_2 (PLA2, $[CA]_{13}$), Glucokinase (GLK, $[GT]_{15}$), and Collagenase type IV (COL4, $[CA]_{21}$) genes were amplified and analyzed on an A.L.F. DNA sequencer.

11/14 HNPCC tumors showed LV within the IL10, 8/12 in the Rb, 8/14 in the CyclinB1, 9/21 in the WAF1, and 3/14 in the COL4 promoter repeat. In contrast, LV of the PLA2 and GLK promoter repeats was absent in 11 and 3 HNPCC tumors, respectively.

Our data reveal a high frequency of LV of DNA repeats within different promoter regions in tumors with DNA MMR deficiency. Case alterations might affect regulatory properties of the promoter elements and subsequently modify the gene expression profile. This new type of 'promoter instability' (PIN) might represent a common mechanism contributing to the pathogenesis of HNPCC.

32 ORAL

Genetic analysis of familal adenomatous polyposis (FAP) families: Lessons and Implications

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Aim: Germline mutations in the APC gene cause FAP. Correlations between the site of the APC mutation and the manifestation of the disease have been established. The detection of a germline mutation in the APC gene in an affected family member permits the identification of carriers and non-carriers of the mutant APC allele among relatives at risk. Non-carriers may be excluded from further endoscopic screening.

Methods: Clinical parameters were considered before mutation analysis was initiated. The APC coding region was screened for germline mutation using a non-radioactive protein truncation test (PTT). Direct sequence