775 POSTER DISCUSSION

Fascin, an actin-bundling protein associated with cell motility, is upregulated in hormone receptor negative breast cancer

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Loss of hormone receptor (HR) status in breast carcinomas is associated with increased tumor cell motility, invasiveness and poor prognosis. However, molecular mechanisms leading to this more aggressive tumor cell phenotype are still unknown. In an immunohistological study of 58 primary breast cancers, estrogen (ER) and progesterone (PR) receptor levels were inversely correlated with the expression of fascin, an actin-bundling protein associated with cell motility ($p < 0.0001$ and $p = 0.0019$, respectively). In addition, flow cytometry analysis revealed that fascin negative tumors were more likely diploid (26 of 43 (60.5%)) than non-diploid (17 of 43 (39.5%)), a difference that reached statistical significance ($p = 0.03$). No significant correlation was found between histological tumor grading and ploidy ($p = 0.12$). Immunohistochemically, fascin positivity appeared as cytoplasmic staining with a marked enhancement in areas of tumor–host interaction in most samples. In summary, the upregulation of fascin in HR-negative breast cancers may contribute to their more aggressive behavior.

776 POSTER DISCUSSION

Overexpression of the keratin 18 gene results in reduced malignancy of human breast cancer cells

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Purpose: In a retrospective study we could show that a high expression of the cytoskeletal protein keratin 18 (K18) in the tumor is correlated with a favorable prognosis for the patients. Experiments with cultured breast cancer cells revealed similar results. To prove the principle we now transfected the K18 gene into a metastatic and invasive cell line that shows only weak K18 expression.

Methods: The complete human K18 gene (pGC 1853, R. Oshima) was transfected (liposomal transfer with pFx-2, Invitrogene) into MCF7-LCC2 cells. Several permanently overexpressing clones were established.

Results: (i) The anchorless growth of the transfected cells in soft agar was dramatically reduced. (ii) This effect was "dose dependent": the higher the K18 expression, the lower the proliferation rate. (iii) The expression of plakoglobin (=γ-catenin) a key protein of epithelial adhesion structures was enhanced in the transfected clones.

Conclusion: The intermediate filaments in epithelial cells are formed by keratins and K18 is a marker of well differentiated luminal cells in the breast epithelial tissue. The loss of K18 seems to be part of a general loss of differentiation along with the metastatic event. A reversal of the K18 depletion by gene transfer may reverse this process in part and, thus, result in reduced aggressiveness of the cancer cells.

777 POSTER

Dihydropyrimidine dehydrogenase and thymidylate synthase in relation to 5-fluorouracil sensitivity of breast cancer

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Thymidylate synthase (TS) and dihydropyrimidine dehydrogenase (DPD) has been investigated to understand 5-FU sensitivity and to develop a biochemical modulator. It is reported that both TS and DPD activity were significantly correlated to 5-FU effectiveness in several cell lines. It is also reported that tumoral/normal DPD activity ratio was significantly lower in complete responder to 5-FU chemotherapy than in partial or non responding head and neck cancer patients. In this study, we measured TS level, DPD activity and in vitro sensitivity to 5-FU using breast cancer as a sample to assess a correlation between the enzymes and 5-FU sensitivity of a breast cancer.

Methods: This study was conducted on 23 female patients undergoing surgery for breast cancer. Immediately after resection, portions of viable tumor and adjacent normal tissue were removed and properly stored for subsequent analysis. In both normal and tumor tissues, TS level and DPD activity was measured. In vitro sensitivity of breast cancer to various concentrations of 5-FU was measured with the use of a collagen gel droplet embedded culture drug sensitivity test.

Results: TS level was significantly higher in tumor than in normal tissue which was 12.6 ± 12.5 pmol/g-tissue and 2.5 ± 1.1 pmol/g-tissue, respectively ($p < 0.05$). DPD activity was significantly higher in tumor than in normal tissue which was 75.5 ± 25.5 pmol/min/mg-protein and 39.5 ± 16.9 pmol/min/mg-protein, respectively ($p < 0.0001$). No significant correlation was observed among TS level, DPD activity and in vitro sensitivity of tumor to various concentrations of 5-FU.

Conclusion: This study provides the first analysis of DPD activity in breast cancer. Contrary to previous study using cell lines, TS level and DPD activity were not correlated to 5-FU sensitivity. However, elevated TS level and DPD activity in tumor encourage a use of biomodulator in the treatment of breast cancer, such as DPD inhibitor and 5-formyl-tetrahydrofolate.

778 POSTER

Somatic mutations in bilateral breast carcinomas

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Purpose: The molecular pathogenesis of various categories of breast cancer (BC) has been well described, but surprisingly few reports have appeared on analysis of somatic mutations in bilateral BC. This study analysed common genetic lesions in paired bilateral carcinomas, with an especial emphasis on 2 questions: 1) is there a concordance or discordance between two tumours within the same patients? 2) does bilateral BC show distinct features as compared to regular, monolateral BC?

Methods: PCR-driven analysis of common losses of heterozygosity (LOH) was performed for 23 cases (46 tumours) from patients diagnosed with bilateral BC.

Results: LOH was observed in 15/46 (33%) informative cases for chromosome 1p, 7/40 (18%) for 5q, 12/44 (27%) for 11q, 15/40 (38%) for 13q, and 7/32 (22%) for 17p. These values are within the range of interlaboratory variations reported for monolateral BC. There was no strong evidence for concordance of LOH within the same patient for any chromosomal loci tested. Atypical for breast carcinomas, 7/46 (15%) tumours accumulated a high frequency (ranging from 11% to 29%) of shortened dinucleotide CA repeats, implying microsatellite instability (MI). Further analysis with the highly informative BAT-26 marker allowed for the classification of 2 of these cases as replication error positive (RER+) phenotype, whereas the remaining 5 tumours harboured so-called border-line MI.

Conclusion: An involvement of both RER+ and border-line microsatellite instability appears to be a distinct feature of bilateral breast carcinomas as compared to monolateral lesions.

779 POSTER

The influence of c-myc gene expression on disease-free interval in breast cancer

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Purpose: The current studies show the correlation between the amplification of c-myc oncogene and a shorter patient survival as well as a shorter disease-free interval. This especially concerns patients with the axillary lymph nodes clear from metastases, but with positive estrogene receptor and negative progesterone receptor. The aim of our study was to examine this correlation, however, in patients with the metastatic axillary lymph nodes.

Methods: We qualified 26 patients with the same degree of clinical advancement T1-2N1M0 (the number of metastatic lymph nodes ≤ 3), with positive estrogene receptor, aged 54–68, (average age 58) for the study. All the patients had been treated with hormonotherapy until the first symptoms of the disease recurrence appeared. The presence of c-myc oncogene expression was examined by means of the 'In situ' hybridization method, and its intensity was read in the microscope and evaluated in the four-degree scale.

Results: The considerable correlation was shown between c-myc oncogene expression and disease-free interval. 16 patients (61.53%) with short disease-free intervals (21.6 months on average) were found to have very intensive expression (III and IV degree), however, in women with long disease-free intervals (over 5 years) c-myc oncogene transcription was weak, and was evaluated to reach I and II degree of hybridization signal intensity.

Conclusion: The expression of c-myc oncogene in breast cancer cells implies high malignancy and a short disease-free interval.

780 POSTER

Comparison of clinical, biological and pathological characteristics in symptomatic vs asymptomatic breast cancers (BC): Study of 1969 patients (pts)

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Purpose: The aim of the present study was to compare the biological, pathological and clinical characteristics of BC in clinically palpable vs occult tumors.

Methods: A series of 1969 consecutive pts who underwent surgery for stage I and II infiltrating BC at Verona hospitals between 1.1.1992 and 30.3.1998, and had positive ultrasound and/or mammography results before surgery, were divided into two groups on the basis of the reason why they decided to do the imaging exam: group A refers to the 1320 pts with a palpable lump, group B to the 649 asymptomatic pts.

Results: As expected, group A pts had larger tumors and a higher percentage of positive nodes than group B ($p < 0.001$); they also had significantly higher grade tumors, higher Ki-67 levels and a higher percentage of ER negative tumors: all the p values were highly significant ($p < 0.001$). The differences in PgR and c-erb B2 were also statistically significant, although to a lesser degree ($p = 0.02$ and 0.03 respectively), with more PgR negative and c-erb B2 positive tumors in group A.

Conclusion: Our results suggest that smaller asymptomatic tumors are biologically different from their clinically presenting counterparts, thus confirming the hypothesis that progression towards greater malignancy can occur during the natural history of breast cancer.

781 POSTER

Detection of genomic instability in fibroadenomas using a simple method

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Purpose: Genomic instability (GI), which regroups microsatellite instability (MIN) and loss of heterozygosity (LOH), may play a role in the ethiology of breast cancer. Therefore, 26 benign breast lesions were examined for GI.

Methods: 9 microsatellite loci were amplified using the Polymerase Chain Reaction (PCR), followed by separation on non-denaturing polyacrylamide mini-gels and visualization with ethidium bromide staining. In order to validate the technique, we used two cancereous cell lines known to harbor the MIN phenotype, which were confirmed using a genetic analyser (ABI PRISM 310). Afterwards, a population of 26 paired benign breast lesions (basically composed of fibroadenomas) and normal tissue, were screened for GI.

Results: Cell lines showed GI in 8 of 9 loci analysed. Of the 26 breast lesions, 2 presented GI in one locus.

Conclusions: The technique is able to detect GI and is easily adapted to a medical background because of its simplicity and rapidity. GI could be a very early event in cancer progression.

782 PUBLICATION

Cytokine activity of tumor draning lymph nodes in breast cancer

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The spontaneous TNF production by lymphocytes, isolated from non-metastatic tumor draining lymph nodes (TDLN) studied in 16 breast cancer patients with bioassay using sensitive transformed fibroblasts L-929 cell line. These data were compared with TNF activity in the conditional mediums of 24 hour primary cultures of breast cancer, peripheral blood lymphocytes (PBL) in breast cancer patients, lymphocytes isolated from lymph nodes in nonspecific lymphadenitis, in serum of breast cancer patients and donors. The lymphocytes from TDLN demonstrated the lowest level of TNF activity (0.058 ng/ml) comparing to all other supernatant sampels. TNF activity in breast cancer cells supernatants was 0.17 ± 0.03 ng/ml, in breast cancer PBL – 0.15 ± 0.02 ng/ml. TNF level in serum of breast cancer patients was 0.85 ± 0.02 ng/ml, in serum healthy volunteers – 0.088 ± 0.014 ng/ml.

Conclusion: Obtained data may be interpreted as the evidence of down regulation of TDLN immunocytes cytokine activity by fators of breast tumor microenvironment.

783 PUBLICATION

Expression of lymphocytes activation markers of peripheral blood dependent on phases of menstrual cycle of young patients with breast cancer

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Purpose: Breast cancer is the most common cancer in patients of pre-menopausal age. In the past decade the incidents of breast cancer in young patients are increase. Prognosis of young patients is still poor. The comparative analysis of the expression of the activation markers in the peripheral blood of young breast cancer patients have done in the different phases of menstrual cycle under control.

Method: The comparative analysis of the expression of activation markers (CD30, CD38, RFB-1) of mononuclear cells of peripheral blood was established in 60 young (20–35 years old) breast cancer patients in different phases of menstrual cycle using monoclonal antibodies.

Results: Unlike the control, the maximal level of lymphocytes activation markers CD30, CD38 and RFB-1 in population of peripheral blood cells of young patients was revield only in pholiculare phase of menstrual cycle. In luteinizing phase of cycle were established the narrowing levels of natural killers and cytotoxic lymphocytes both in young patients and contol group. In breast cancer patients expression of RFB-1 antigen has the minimal level only in ovulation phase of cycle compared with control.

Conclusion: The different type of expression of the activation markers in the peripheral blood of breast cancer patients compared with healthy women of young age is discovered in different phases of menstrual cycle, that may reflect an existence of basic differences in endocrine regulation of the immune system, especially activated lymphocytes, in breast cancer patients of young age.

784 PUBLICATION

Expression of the genes of adenylate cyclase (AC) G-protein subunits in breast cancer (BC) tissue: Connection with estrogen-dependency?

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Purpose: Response of breast cancer tissue to external and local estrogen stimulation needs to be thoroughly evaluated in relation to the cross-talk with different signaling systems. Accordingly expression of the genes of AC G-proteins stimulatory ($G_{s\alpha}$) and inhibitory ($G_{i2\alpha}$) subunits was compared with age of patients, ER content and aromatase gene expression in this tissue.

Methods: In 14 BC samples expression of AC G-protein subunits and aromatase (coding region and exons I.3, I.4 and II) genes was evaluated by RT-PCR according to Itoh et al., 1988 and Santner et al., 1997. ER content in the same samples was studied by dextran-charcoal radioligand assay according to Saez et al., 1980.

Results: Expression of $G_{s\alpha}$ gene correlated positively ($\rho + 0.70$) with the age/existence of menopause in patients and with ER content in tumor tissue. $G_{i2\alpha}$ gene expression demonstrated tendency ($p = 0.07$) to positive correlation with the age of patients too and negative correlation ($\rho - 0.77$) with expression of I.3 exon of aromatase gene.

Conclusion: Expression of AC G-protein subunits genes in breast tumor tissue correlates with more old age (menopause), sensitivity to estrogens and certain signs of their local production that confirms involvement of cAMP-dependent mechanisms in the latter process.

Acknowledgements: To Dr. R. Pauley (USA) for the help with RT-PCR of aromatase gene exons and to Prof. R. Santen (USA) for useful discussions.