Methods: We evaluated the tissue distribution of the 14C5 antigen by immunohistochemistry.

Results: The antigen is specifically overexpressed in 64% of invasive ductal adenocarcinomas of the breast (n = 33), in all investigated cases of invasive squamous cell carcinoma (n = 7) and in 40% of basocellular carcinomas of the skin (n = 5). The 14C5 molecule is located on the cell membrane of the carcinoma cells. However, when the tumour is characterized by a highly invasive phenotype, 65% of the cases also show an extensive stromal expression on the fibroblasts between the tumour cells (n = 71). In normal tissues as well as in the stroma surrounding *in situ* carcinomas of the breast (n = 15), no expression of the 14C5 antigen occurred. A 90 kDa protein was purified from lysates of human breast cancer cells using a 14C5 Mab sepharose column and is considered as the antigen, recognized by the Mab 14C5.

Conclusion: The antigen was considered to be an effective target for passive and active immunotherapy and is therefore been introduced in in-vivo models to prove his efficacy as an immunological approach to tumor therapy in breast cancer.

19 POSTER

Inhibition of breast cancer tissue aromatase activity and estrogen concentrations by the third generation aromatase inhibitor vorozole

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Purpose: To study the effects of the third generation nonsteroidal aromatase inhibitor vorozole (Rivizor™) on intratumoural aromatase activity and estrogen concentrations in breast cancer tissue of postmenopausal patients.

Methods: During seven days preceding mastectomy eleven postmenopausal breast cancer patients were treated with vorozole (2.5 mg/d). During surgery tumour tissue samples were obtained, in which aromatase activity and estrogen concentrations were measured and compared to results obtained in nine unpretreated postmenopausal breast cancer patients.

Results: Eight patients were evaluated. In treated patients median tissue aromatase activity was 89% lower than in controls ($p < 0.001$). Similarly, median tissue estrone and estradiol concentrations were 64% and 80% lower respectively in treated patients ($p = 0.001$ resp. $p < 0.05$).

Conclusion: Vorozole is able to significantly lower aromatase activity and estrogen concentrations in breast tumour tissue. Impairing estrogenic stimulation may be an important mechanism in the antitumour activity of aromatase inhibitors, which is further to be tested in clinical investigations.

20 POSTER

The role of desmosomal glycoproteins in the adhesion & invasion of human breast cancer cells

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Purpose: The invasion and metastasis of cancer cells is of prime importance to breast cancer patients. This study determined the role of Desmoglein 2 (Dsg2), a member of the desmosomal glycoproteins in adhesion and invasion of cancer cells and its interaction with E-cadherin (E-cad).

Methods: Three human breast cancer cell lines were used, MDA MB 231, MCF7 and BT 474. The expression of Dsg2 and E-cad was determined using Western blotting and Immunocytochemistry. Cell adhesion, invasion and migration were assessed using cell-cell aggregation, *in-vitro* invasion and colloidal gold phagokinetic tracking assays. We compared these cell functions in cancer cells alone and those treated with a monoclonal antibody (Mab) to Dsg and/or E-cad.

Results: All three cell lines expressed Dsg-2. Both MCF-7 and BT474 were E-cadherin positive but MDA MB 231 was negative. Treatment with Dsg-2 Mab resulted in a markedly reduced cell-cell aggregation in all three cells with aggregation indices at 30 minutes (median ± standard deviation, control vs mab) 0.68 ± 0.27 vs 0.0 ± 0.5, 0.72 ± 0.1 vs 0.43 ± 1.5, and 0.69 ± 0.15 vs 0.15 ± 0.32, respectively. Mab to E-cad reduced cell aggregation in BT474 and MCF7 cells, with no effect on MDA MB 231 cells. Both the invasive potential in Matrigel & the cell migration in colloidal gold were increased in pretreated cells.

Conclusion: We propose that Dsg2 is another factor in addition to E-cadherin, which may regulate the adhesion and invasion of human breast cancer cells, which may have important implications in the metastasis.

21 POSTER

Mutations in the BRCA1 gene in Italian breast and ovarian cancer patients

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To evaluate the role and the frequency of germ-line BRCA1 mutations in the pathogenesis of the heterogeneous familial forms of breast and/or ovarian cancer in Italian population we are currently analyzing by *in vitro* transcription-translation the coding sequence of the BRCA1 gene in 150 unrelated breast and/or ovarian cancer patients, including: 86 cases of breast or ovarian cancer with family history positive for these forms of cancer; 15 cases of breast cancer with family history of cancers other than breast and ovary (prostate, colon, stomach, endometrium, lung, kidney); 37 cases of breast cancer with early onset (aged 40 or less at disease diagnosis); 12 cases of bilateral breast cancer.

Genetic analysis was performed first on exon 11, which includes about 61% of coding region. Thus far, mutations were detected in 2 cases. One of the two cases (1254delAG) corresponded to a 64 years old breast cancer patient, whose daughter was diagnosed to have breast cancer at age 24, after two pregnancies and 14 years before the onset of the disease in the mother and died of breast cancer at age 26. The other case (1623del5bp) corresponded to a 45 years old ovarian cancer patient, whose mother died of ovarian cancer at age 57. The same BRCA1 mutations were also detected in 4 unaffected relatives of the two positive cases. In addition to the two protein-truncating mutations, a missense mutation at nucleotide 3232 (A/G), was detected in a 32-year-old breast cancer patient. In conclusion, exon 11 truncating germline mutations occurred in only 2 out of the total group of 150 cases, the 2 mutations occurred in the subset of 25 kindreds characterized by disease in mother and daughter (8%). The characteristics of the mutations and their clinico-pathologic correlations are of some interest. Patients that resulted negative for exon 11 mutations are presently being analyzed for the entire coding sequence of the gene and other patients are being recruited in the study.

22 POSTER

BRCA1 mRNA expression and allel loss at chromosome 17q21 in sporadic breast and ovarian carcinoma

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Objective: Germline BRCA1 mutations predispose to breast and ovarian cancer, but the role of BRCA1 alterations in sporadic breast and ovarian cancers is still unclear. Allele loss at the BRCA1 locus is a frequent genetic alteration, but point mutations of BRCA1 in sporadic carcinoma are rare.

Methods: In an ongoing prospective study we determine allele loss and BRCA1 mRNA expression in sporadic breast and ovarian cancers. Loss of heterozygosity (LOH) at the BRCA1 locus was analyzed in 121 invasive breast and in 40 ovarian carcinoma with at least two intragenic microsatellite markers (D17S1322, D17S855). In 32 breast and 24 ovarian carcinoma BRCA1 expression was determined using a fluorescent quantitative reverse transcription (RT)-PCR technique followed by fragment analysis of flourescent PCR products on an automatique DNA sequencer.

Results: LOH could be detected in 37.5% of the breast, in 27% of the ovarian tumors; LOH of BRCA1 correlated with higher tumor grade and -in breast cancers- with positive PgR expression. Reduced BRCA1 expression levels (<50%) were found in 62.5% of the breast and in 38% of the ovarian tumors. These values were compared to BRCA1 expression in normal tissues. In breast tissues reduced BRCA1 expression was found in all tumors with BRCA1 allele loss as well as in 44% of the tumors without LOH.

Conclusions: Reduction of BRCA1 expression due to loss of one gene copy may be one mechanism of genetic BRCA1 alteration, however also cis- or trans- regulator mechanisms are possible. Inhibition of BRCA1 mRNA expression may be one mechanism of BRCA1 gene inactivation in sporadic breast and ovarian carcinoma. (supported by Grant DFG Be 1215/6-2)