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ORAL

Frequent inactivation of the CDKN2 gene by methylation and deletion in breast carcinomas

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Purpose: Allele loss on 9p are common in a wide variety of human carcinomas including breast cancer. The putative tumor suppressor gene CDKN2 encoding an inhibitor of the CDK4/cyclin D-complex has been identified in this region. Homozygous deletion of CDKN2 has been detected in 60% of breast cancer cell lines. However, homozygous deletions and point mutations of CDKN2 in primary breast cancer have rarely been observed. Although another mechanism of inactivation of CDKN2, methylation of the 5' CpG island silencing of gene expression, was detected frequently in breast cancers, the involvement of this gene in carcinogenesis of primary breast tumors remains to be established.

Methods: To delineate the role of CDKN2 as a tumor suppressor gene in the genesis of breast cancer, we examined allele loss on 9p21-22 and homozygous deletions, mutations and hypermethylation of the CDKN2 gene in sporadic breast cancer by using microsatellite markers, fluorescent multiplex PCR, direct sequencing and PCR-based methylation assay.

Results: Allele loss at one or more microsatellite markers flanking CDKN2 were detected in 32 (42%) of 77 breast cancers. Homozygous deletions and methylation of the CDKN2 gene were detected in 6 and 14 cases of tumors with allele loss respectively. No other mutations were found by direct sequencing of both exons of CDKN2.

Conclusion: The results of this study suggest that homozygous deletions and methylation combined with allele loss are the predominant inactivation mechanisms of the CDKN2 gene involved in the pathogenesis of breast cancer.

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ORAL

Phosphotyping of c-ErbB-2 in human breast tumours

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Overexpression of c-ErbB-2 commonly accompanies human breast tumourigenesis, but the precise pathogenetic and prognostic significance of this phenotype remains controversial. Using antibodies which detect different c-ErbB-2 phosphorylation states, we have characterised patterns of receptor activation and transmodulation in 101 primary human breast tumours. Over 90% of tumours were transphosphorylated on either tyrosine or threonine residues, consistent with modification by the action of heterologous growth factor receptors. In contrast, less than half of all tumours exhibited significant tyrosine autophosphorylation; this tumour cohort displayed prominent c-ErbB-2 proteolysis on immunoblotting, consistent with lysosomal degradation of activated receptors. Overexpression of epidermal growth factor receptors (EGFR) was detectable only in tumours without c-ErbB-2 upregulation, and co-immunoprecipitation studies revealed oligomeric association of transphosphorylated c-ErbB-2 with EGFR. In vitro studies confirm that c-ErbB-2 overexpression prevents the downregulation of ligand-activated EGFR by redirecting it away. We conclude that c-ErbB-2 acts as a form of "molecular glue" in human breast tumours, leading to upregulation of heterologous growth factor receptors and consequent amplification of ligand-dependent receptor signalling.

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ORAL

Anti-Idiotypes for immunotherapy of breast cancer – Tumor control in an in-vivo model

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Purpose: The idiotype network concept offers an elegant method to transform epitope structures into idiotype determinants, which are expressed on the surface of antibodies. We generated a monoclonal anti-idiotypic antibody (IgG1), designated ACA 14C5, against the cell substrate adhesion molecule CA 14C5 on breast cancer cells and introduced the mAb ACA14C5 in an in-vivo model to prove its capacity for inhibition of invasion and metastasis.

Methods: 6 day old Sprague-Dawley rats (n = 3 × 12) received tumor cells (2 × 10⁶) subcutaneously. After one week series Ab2 received mAb

ACA14C5 intraperitoneally at a dosage of 100 µg weekly (n = 12). 2 control groups received polyvalent mouse IgG at the same dosage intraperitoneally weekly (n = 12) and a negative control received only tumor cells (n = 12). The tumor growth was evaluated over a period of 60 days. 8 applications were administered in total.

Results: The results showed a highly significant difference in the tumor growth as the ACA 14C5 treated group developed a mean tumor size of 6.5 ± 12.7 mm and the IgG control showed a mean diameter 37.2 ± 14.9 mm (p < 0.005) and the tumor control group showed a diameter 15.3 ± 16.3 (p < 0.05). In the anti-idiotypic treated animals 10 of 12 animals were cured from their tumor burden and a T-cell response (lysis of HH16cl. 1/2) could be evaluated, which was not present in the controls.

Conclusion: In summary, this is the first report of an inhibition of tumor growth in-vivo caused by an anti-idiotypic antibody (ACA 14C5) reacting with a human cancer antigen, which is a cell substrate adhesion molecule of 90 kd and is expressed on different invasive tumors, especially on invasive breast cancer.

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ORAL

Antibody-guided cytokine therapy (AGCT) chemoresistant human breast cancer

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Breast cancer constitutes one of the most lethal malignancies due to limited effects of chemotherapy, radiotherapy and immunotherapy. A high metastatic chemoresistant human breast cell line has exhibited overexpression of MDR-1 and MRP mRNA by both RT-PCR. Furthermore, this cell line exhibited by the same method low expression Fas mRNA and overexpression of bcl-2 oncogene. Our aim is to inhibit or reduce the synthesis of RNA and proteins which are essential for the initiation of DNA synthesis during the post mitotic (G₁) phase of the cell cycle, enter the resting (G₀) phase and mainly enhance expression of Fas cell surface antigen, by using IFN-γ. Our final goal is to induce apoptosis by cytotoxic Fas antibody upon binding with its antigen. Thus, we use pH sensitive immunoliposomes, in whose hydrophilic space we entrap IFN-γ-gene and on their surface we link anti-Fas (IgM) antibodies (CH-11). Then, we incubate a sample of tumour cells with empty liposomes as controls, and then we use another sample which we incubate with the loaded liposomes. After incubation, we observe morphologically and biochemically no apoptosis in control tumour cells and no bcl-2 change in expression. For the tumour cells which have been incubated with the loaded liposomes we observe by TEM binding of the immunoliposomes by the cell surface antigen, subsequent initiation of receptor-mediated endocytosis, internalization of the immunoliposomes into acidic endosomes, fusion of the pH-sensitive immunoliposomes with the endosome membrane and the release immunoliposome content (IFN-γ-gene) into the cytoplasm. This leads to apoptosis exhibited biochemically by reduced metabolic activity according to MTT analysis and reduced DNA synthesis according to BrdU analysis. Morphologically, we observe typical features of cells undergoing apoptosis such as condensation of chromatin in crescentic caps adjacent to the nuclear membrane, incomplete nuclear membranes and translucent cytoplasmic vacuoles. Furthermore, transduced cells can cause cell death in non-transduced cells by mediating a bystander effect due to phagocytosis of apoptotic vesicles. Finally, after the interaction of Fas antigen with the monoclonal Fas antibodies there is enhanced expression of Fas mRNA and downregulation of bcl-2, according to RT-PCR.

Conclusion: We have achieved to circumvent drug efflux pump genes and to induce apoptosis in resistant human tumour cells by enhancing expression of Fas and downregulating bcl-2.

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POSTER

The human 14C5 cell substrate adhesion molecule – Expression patterns and functional activity

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Purpose: Cell substrate adhesion is a prerequisite for invasion and the subsequent formation of metastases. Therefore, we designed a monoclonal antibody against an epitope on the extracellular cell membrane domain of SK-BR-3 cells. This Mab 14C5 is able to inhibit cell substrate adhesion and therefore prevents invasion.

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.

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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 untreated 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.

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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.

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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.

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POSTER

BRCA1 mRNA expression and allele 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 fluorescent PCR products on an automatic 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- 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)