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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 analyzed 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 gen involved in the pathogenesis of breast cancer.

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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 tway. 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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Anti-idiotypes for immunotherapy of breast cancer – Tumor control in an in-vivo model

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Purpose: The idiotypic network concept offers an elegant method to transform epitope structures into idiotypic 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 his capacity for inhibition of invasion and metastasis.

Methods: 6 day old Sprague-Dawley rats (n = 3 × 12) received tumor cells (2 × 10^6) 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-idiotype treated animals 10 of 12 anials 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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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 (G_1) phase of the cell cycle, enter the resting (G_0) 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 (IFNγ-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 apoptic 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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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.