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Immunohistochemical analysis of bilateral breast carcinomas: tendency to concordance of ER, PgR and erbB2/HER2 status

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Background: Bilateral breast cancer (biBC) is a common disease however its molecular features have not been systematically studied. Although being clonally independent, biBC tumor pairs do share essential host and environmental factors. Here we addressed the question whether this similarity of the natural history of the disease results in a concordance of selected immunohistochemical (IHC) characteristics of the bilateral neoplasms.
Materials and methods: Expression of estrogen receptor (ER), progesterone receptor (PgR) as well as erbB2/HER2 and p53 proteins was evaluated in 51 patients (102 tumors) using IHC staining of tissue arrays.
Results: Expression of ER, PgR, erbB2 and p53 was detected in 71%, 75%, 70%, and 31% of tumors, respectively. Based on the above frequencies, the expected concordance of the IHC status was calculated to be 58%, 62%, 59%, and 58% for ER, PgR, erbB2, and p53, respectively. Actual concordance tended to be higher than the expected one for ER (76%), PgR (78%), and erbB2 (71%) but not for p53 (59%). The difference between actual vs. expected concordance was especially prominent in synchronous biBC (ER: 83% vs. 56%; PgR: 87% vs. 56%; erbB2: 87% vs. 56%).
Conclusions: biBC demonstrate tendency to concordance of IHC status for ER, PgR, erbB2/HER2 but not p53. The concordance of expression profiles is particularly evident in the synchronous form of biBC.

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Prognostic significance of IFN-gamma receptor (IFNGR1) in breast carcinomas

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Introduction: IFN-γ and STAT-1 (Signal transducers and activators of transcription) knockout mice are more susceptible to both chemically induced and spontaneous tumours implicating this cytokine and its signalling in immune-surveillance. Tumours may therefore escape immune recognition by down regulation of the IFN-γ receptor or abnormal signal transduction. STAT-1 has a very short half life and can not be visualised by immunohistochemistry in normal tissues. However mutation or hyper-activation may increase its half life and allow detection in tumours.
Material and methods: Samples from 668 patients with primary operable breast cancer diagnosed between 1987 and 1992 (median follow-up of 86 months) were analyzed in tissue microarray format. Immunohistochemical analysis of expression of IFNGR1 and STAT1 was performed and the results correlated with different prognostic factors and patient outcome. All tumours expressed IFNGR1 but only 43% expressed STAT-1.
Results: Univariate analysis showed a positive relationship between intensity of expression of IFNGR1 and lymph node involvement ($p < 0.001$). In lymph node positive patients a significant association was noted between higher expression of STAT1 and increased tumour grade ($p = 0.039$), development of distal metastasis ($p = 0.012$) and vascular invasion ($p = 0.043$). Survival analysis demonstrated that patients with tumours with low expression of STAT1/IFNGR1 had an improved survival compared with those with high expression (log rank=0.045).
Conclusion: It is concluded that breast cancer patients with a STAT1(+)/IFNGR1(+) phenotype demonstrate poor survival times. Abnormal expression of STAT-1 may make tumours resistant to immune control by IFN-γ and thus develope a more aggressive phenotype.

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Inactivation of the *BRCA1*-, *BRCA2*- and *p53*-genes in sporadic breast carcinomas

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Background: Breast cancer is the most frequently diagnosed malignancy affecting women in Europe. Mutations in the *BRCA1* and *BRCA2* genes, the two major susceptibility genes in hereditary breast cancer, are responsible for approximately 80–90% of all inherited breast tumors. However, the large majority of breast cancers belongs to a sporadic form. The role of the *BRCA1/2* genes in noninherited tumors is not exactly defined. The *p53* gene is the most frequently mutated gene in human cancers. In breast carcinomas, the *p53* gene is inactivated in about 20–30% of the tumors.
Material and methods: In this study, we analysed loss of heterozygosity (LOH) of the microsatellite markers intragenic or flanking to the *BRCA1, BRCA2* and *p53* genes in 40 unselected breast carcinomas. In the samples with allelic deletions, we screened for mutations in the respective the genes. The analysis included entire coding regions of the genes and was performed by protein truncation test (PTT) for the *BRCA1/2* and by sequencing for the *p53*. Automated sequencing of the appropriate genomic DNA fragments was used to confirm and characterize the mutations.
Results: LOH was identified in 8 of 38 (21%), 13 of 39 (33%) and 16 of 35 (46%) informative tumor samples in the *BRCA1, BRCA2* and *p53* genes, respectively. Allelic losses in *BRCA1* and *BRCA2* were linked to losses in *p53* in 100% and 77% of cases. On the other hand, losses in *p53* occurred often individually. We identified six somatic missense mutations in the *p53* gene, two somatic truncating mutations in the *BRCA1* gene and no mutation in the *BRCA2* gene.
Conclusion: Our results prove the role of the *p53* gene in the breast cancer development. Furthermore, inactivation of the *BRCA1/2* genes is involved in tumorigenesis of at least a minor subset of sporadic breast tumors and is often associated with inactivation of the *p53* gene. Somatic inactivation of *p53* thus seems to precede further alterations in the *BRCA1/2* genes.
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E-cadherin and Snail expression in epithelial cells isolated from primary tumor and peritumoral tissue from breast cancer patients and their relation to epithelial circulating cells

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Background: To invade, breast cancer cells reduce their intercellular cohesion, enhance their motility and proteolytic activity, and acquire mesenchimal cell characteristics, in a process similar to epithelial-mesenchimal transition, that takes place during embriogenesis. E-cadherin is the main molecule of cell-cell adhesion and defects on its fuction was described in carcinomas. E-cadherin expression is modulated by some transcriptional factors, among them, Snail, which may repress genes mainly expressed in epithelial tissues, as well as induce the expression of certain mesenchimal markers. Our aim was to study isolated epithelial cells, obtained from the tumor itself or its adjacent tissue, to determine whether variations in E-cadherin, Snail (related to the epithelial-mesenchimal transition) expression, between transformed and non-transformed cells, might occur. We have also evaluated whether the expression ot these genes might be correlated to the presence of epithelial circulating cells, as detected by the expression of cytokeratin 19.
Patients and methods: We have studied 48 samples from breast cancer patients, whose median age was 49 years (33–88 y). Most of them presented invasive ductal carcinoma (79.2%) and 52%, histopathologically involved lymph nodes. Early breast cancer (clinical stages I/II) was detected in 33 patients. Primary tumor and peritumoral samples, as well as peripheral blood, were collected and epithelial cells were isolated, by an immunomagnetic method. RNA was extracted from each individual sample and gene expression was evaluated by real-time PCR.
Results: No variations in the expression of E-cadherin and Snail were observed between cancerous and normal samples. Cytokeratin 19 (CK19) expression in mononuclear cells, obtained from peripheral blood, was positive in six and negative in 23 patients. No relation was observed between CK19 expression in the peripheral blood and lymph node involvement. However, there was a trend towards lower expression of E-cadherin, but not of Snail, in tumor specimens from patients presenting epithelial circulating cells.
Conclusions: In breast cancer, expression of E-cadherin and Snail, in epithelial cells obtained from tumor and peritumoral tissues seems similar. There was a trend towards a lower expression of E-cadherin in tumors from

patients with epithelial circulating cells, which might indicate a capacity of these malignant cells to invade blood vessels.
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Development of a measles virus vector targeting breast cancer cells by expression of single chain antibody against HER-2/neu

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Background: HER-2/neu is overexpressed in 25% of breast cancers, and is associated with poor prognosis. In order to develop effective therapies for breast cancer, we used a novel virus vector targeting the neu protein. We previously established a reverse genetics system for measles virus (MV) using the MV-HL strain. By using this system, we have previously constructed a recombinant MV (rMV) that expresses a single chain antibody (ScFv) against human alpha-fetoprotein (AFP). This recombinant virus inhibited colony formation of AFP-positive human hepatoma cells. In the present study, we constructed rMV expressing ScFv against activated rat HER-2/neu protein to be tested *in vivo* in a transgenic mouse model of spontaneous breast cancer.
Materials and Methods: We constructed a cDNA in which the ScFv against rat HER-2/neu is fused with the transmembrane domain (TMD) of vesicular stomatitis virus (VSV)-glyco (G) protein, and inserted it as an additional transcription unit between the N and P genes of the MV genome. We then rescued the virus (rMV-aneu), and investigated the ability to replicate in breast cancer cells and the potential as an antitumor agent.
Results: We succeeded in rescuing the rMV-aneu from the construct using the reverse genetics system. The rMV-aneu replicated as well as the parent MV in B95a cells, derived from marmoset B lymphoma. The rMV-aneu grew in N2C cells, a mammary carcinoma cell line established from a spontaneous BALBneuT tumor expressing rat HER-2/neu on their surface, whereas the parental MV did not show any infectivity. The protein produced by the inserted gene within the recombinant MV was properly expressed in the infected N2C cells. The rMV-aneu significantly reduced cell viability as measured by the metabolic activity of the infected cells. In contrast, the parental MV and mock infection did not cause any change in the activity. The effects of the rMV-aneu on the rat neu transfected human cells and on the transformed cells *in vivo* are currently under investigation.
Conclusions: As an approach to develop tumor-targeted, replication-competent viruses useful in breast cancer treatment, we constructed an rMV that express ScFv recognizing activated HER-2/neu. The insertion of the ScFv gene fused with G-TMD into the rMV genome induced its infectivity. The rMV-aneu significantly inhibited the HER-2/neu+ cell activity *in vitro*. These results suggest the possibility that the rMV-aneu may be useful in breast cancer therapy.

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Association between serum estrogen and androgen concentrations and tumour receptor status in postmenopausal breast cancer

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Elevated levels of endogenous sexual hormones; estrogen (E2), estrone (E1), testosterone (TE) and their precursors; androstenedione (AD), dehydroepiandrosterone (DHEA) and DHEA sulphate (DHEA-S), E1 sulphate (E1-S), have been associated with the risk of breast cancer in postmenopausal women. In this study we investigate the correlation between serum hormone concentrations and tumour receptor status. Besides the levels of sexual hormones and precursors, sex hormone-binding globulin (SHBG), insulin-like growth factor-1 (IGF-1) and IGF binding protein-3 (IGFBP-3) were also measured by fully automatized equipment using RIA and IRMA methods. The estrogen (ER) and progesterone receptors (PR) expression in tumour tissues were determined by ICH, and MedCalc Software was used for statistical analysis.
Our study involved 444 postmenopausal patients with primary breast cancer of Stage I, II prior to surgical intervention and 250 healthy controls [average age in both groups was 64 years]. 358 of cancer patients were diagnosed for invasive ductal carcinoma (DC), 55 for invasive lobular carcinoma (LC), 29 for DC in situ (DCIS), and 2 for LCIS. 297 were ER and PR-positive [ER+/PR+], while 78 were ER and PR-negative [ER−/PR−]. Significant increase of E1, E1-S, AD, TE, DHEA, DHEA-S levels and significant decreases of SHBG level in cancerous cases were found by Mann-Whitney statistical analysis.
The median value of serum E1, AD, E1-S, IGF-1, E2 and TE were higher in patients with [ER+/PR+] receptor status than in patients with [ER−/PR−] receptor status. In E1 ($p < 0.0003$), AD ($p < 0.0009$) and E1-S ($p < 0.0096$) levels the difference was highly significant. Patients were rank ordered according to the increasing serum E1, E1-S and AD concentrations. In the highest quintile of each series 74–80% of patients were [ER+/PR+], while 9–11% of them were [ER−/PR−]. Using logistic regression analysis, the probability of tumour receptor positivity can be predicted based on the knowledge of serum hormone (E1, E1-S, AD) levels. In addition, E1-S and TE levels tend to be markedly associated with invasive DC (DC vs. LC, $p < 0.0109$ for E1-S; $p < 0.019$ for TE).
Our study supports the hypothesis that the circulating sex steroid hormone levels are strongly correlated with risk of [ER+/PR+] breast tumours.
Some kits for IGFBP-3, E1-S, AD and MedCalc software were gratefully donated by Laborexpert Ltd., Budapest, Hungary.

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The prognostic influence of Plasminogen Activator Inhibitor-1 in early breast cancer is not related to estimates of angiogenesis

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Introduction: Plasminogen Activator Inhibitor type-1 (PAI-1) is one of the key proteases involved in tumour invasion and microenvironment remodelling. Indeed, high levels of PAI-1 have been associated with poor prognosis in several tumour types. In several experimental studies PAI-1 has been shown to play a role in angiogenic processes, and since estimates of tumour angiogenesis have been demonstrated as predictors of poor prognosis this study investigates the relationship between estimates of tumour angiogenesis and protein levels of PAI-1 in breast cancer.
Materials and methods: Tumour specimens from 438 patients diagnosed with primary unilateral non-metastatic breast cancer were used. Median follow-up was 9.5 years, and 168 patients (38%) had died from cancer. Angiogenesis scores were performed on paraffin-embedded tissue slides stained with anti-CD34, and vessels were counted using a Chalkley grid in hot spots. Protein levels of PAI-1 were measured in supernatants from frozen tumour tissue using a sandwich ELISA kit with monoclonal catching and detecting antibodies.
Results: Median Chalkley count was 5.00 (range, 2.67–12.00), and median PAI-1 level was 0.70 ng/mg protein (range, 0–90 ng/mg protein). Chalkley counts were not correlated with PAI-1. Both high Chalkley counts and high PAI-1 were significantly correlated with high malignancy grade and lack of estrogen receptor, and high Chalkley counts furthermore correlated with large T size. High Chalkley counts and PAI-1 in tertiles were both correlated with poor disease specific survival (DSS) ($P = 0.002$ and $P = 0.05$, respectively). Combining low/low versus high/high tertiles of Chalkley counts and PAI-1 showed actuarial survival rates of 75% versus 52%, respectively ($P = 0.0008$). In multivariate analysis high N-stage ($P' < 0.0001$), grade ($P < 0.0001$) and increasing levels of PAI-1 ($P = 0.004$) were identified as independent markers of cancer-death.
Conclusions: In univariate analysis both Chalkley counts and PAI-1 levels were associated with poor DSS. Combining lowest versus highest tertiles of both factors separated the patients into groups with significantly different survival. This study suggests that the prognostic impact of PAI-1 is independent of its supposed involvement in angiogenic processes.

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Gene expression associated to response to doxorubicin based primary chemotherapy in breast cancer

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Background: This study was undertaken to identify genes that could predict response to doxorubicin based primary chemotherapy in breast cancer patients.
Patients and methods: Patients (pt) with confirmed invasive breast cancer on samples obtained by core or incisional biopsy, clinical stages (CS) II or