and the disease-free patient group showed the same trend of differential expression as they did in the xenograft model. These trends could be confirmed for all of the 5 down-regulated as well as for 3 of the 6 up-regulated genes. Comparing the expression data for each gene in individual patients, distinct expression differences were observed for NNAT, IGFBP5 and HOXB13.

Conclusion: TaqMan® Low Density Arrays provide an efficient and reliable RealTime PCR method in a 2 µl reaction volume for the simultaneous analysis of multiple genes. Expression data for several genes in clinical samples reveal differences with respect to recurrence under tamoxifen therapy. We were able to define candidate genes possibly related to tamoxifen responsiveness that will be subjected to further functional evaluation.

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Gene profiling analysis of tissue-specific metastases from human breast cancer

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Breast cancers are prone to metastasise, particularly to the lymphatics, bone, liver, lungs and central nervous system. Once solid secondary tumours are established, the chances of long-term survival fall from over 90% to around 5%. The European project "MetaBre" investigates the molecular mechanisms of breast cancer organ-specific metastasis. The first step of this project has studied the gene expression profiles of tissue-specific metastases by use of microarray analysis (Affymetrix HG U133 plus 2.0, over 47,000 transcripts). To this aim, we hybridised 21 human breast cancer metastases resected from 5 different organs: lung (5), liver (6), bone (4), brain (4) and skin (2). We also analysed normal tissues from each corresponding target organ.

Statistical analyses were performed using BRB ArrayTools 3.2.2. "One class versus all" class prediction analysis was used to identify genes involved in organ-specific metastasis. 4 signatures of approximately 20 genes were established for lung, liver, bone and brain metastases. The validation of the signatures was performed by quantitative RT-PCR. These 4 signatures were able specifically to distinguish metastases from different sites from each other. Furthermore, our lung metastasis signature was able to discriminate breast cancer primary tumours relapsing to lungs from a series of tumours metastasizing to different organs. Thus, our expression profiling study allowed us to identify genes potentially involved in organ-specific metastasis.

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Gene copy numbers and expression of ErbB-1 and ErbB-2 in breast cancer

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Background: The family of ErbB oncogenes and their receptors play important role in breast cancer development, however prognostic relevance of ErbB-1 and ErbB-2 abnormalities is debatable. The aim of this study was to determine clinical impact of ErbB-1 and ErbB-2 gene copy numbers and expression in a large series of breast cancer patients.

Material and Methods: Study group included 225 consecutive stage I-III breast cancer patients treated between 1998 and 2002 in three Polish institutions. Average gene copy numbers (AGCN) of ErbB-1 and ErbB-2 were determined by double differential polymerase chain reaction (ddPCR). Expression of ErbB1 (63 patients) and ErbB-2 (171 patients) was assessed by tissue microarray immunohistochemistry (TMA-IHC) and by IHC-based HercepTest, respectively. Disease free survival (DFS) and overall survival (OS) were computed by the Kaplan-Meier method. Univariate and multivariate survival analysis was performed with log rank test and Cox proportional hazard model.

Results: ErbB-1 amplifications and deletions were found in 15% and 31% of cases, respectively, and ErbB-2 amplifications and deletions – in 26% and 3% of cases, respectively. Deletions of ErbB-1 occurred more frequently in node negative ($p = 0.03$) and in PgR negative cases ($p = 0.06$), whereas ErbB-2 AGCN was not related to major clinicopathological characteristics. Overexpression of ErbB-1 and ErbB-2 occurred in 17% and 18% of patients, respectively and both abnormalities were correlated with negative estrogen receptor status ($p = 0.007$ and $p = 0.02$, respectively). ErbB-1 was correlated with lymph node metastases ($p = 0.06$) and larger tumor size ($p = 0.027$). The correlation between expression and AGCN was strong for ErbB-2 ($p = 0.0003$) and insignificant for ErbB-1. ErbB-1 amplification was associated with shorter DFS and OS ($p = 0.03$ and 0.02, respectively) and overexpression – with shorter DFS ($p = 0.04$). ErbB-2 overexpression was associated with shorter OS ($p = 0.02$), whereas prognostic impact of ErbB-2 AGCN did not reach statistical significance. There was a strong correlation between AGCN of ErbB-1 and ErbB-2 ($p = 0.000036$). Patients with co-amplification of both genes tended to be node-positive, but small number of this subset did not allow for statistical analysis.

Conclusions: Overexpression of both ErbB-1 and ErbB-2, and amplification of ErbB-1 carry adverse prognosis in breast cancer patients. Strong correlation between ErbB-1 and ErbB-2 AGCN may indicate an important role of ErbB heterodimers in tumor progression. Clinical relevance of these findings warrant further studies.

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VEGF-D in assocation with VEGFR-3 promotes nodal metastasis in human invasive lobular breast cancer

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The aim of this study was to investigate the role of lymphangiogenesis in lymphatic dissemination in invasive lobular breast cancer by examining peri- and intratumoral lymph vessel density as well as the expression of VEGF-C, VEGF-D and VEGFR-3 in these tumors.

By performing immunohistochemistry stainings on human invasive lobular breast cancer tissue samples we assessed the expression of vascular endothelial growth factor C (VEGF-C) and vascular endothelial growth factor D (VEGF-D) in breast cancer cells and the density of lymph vessels and vascular endothelial growth factor receptor 3 (VEGFR-3) vessels in and around the tumor.

We found a significant correlation between peritumoral lymph vessel density and the presence of lymph node metastases ($P = 0.001$). Lymph vessel density also correlated with the number of metastatic lymph nodes ($P < 0.001$). Furthermore a significant correlation was detected between tumor cell VEGF-D expression and lymph node status ($P = 0.001$).VEGF-D expression also correlated with the density of LYVE-1 positive vessels ($P = 0.035$). Tumors positive for both VEGFR-3 and VEGF-D or both VEGFR-3 and VEGF-C had a significantly higher number of metatstatic lymph nodes than tumors with other staining patterns ($P < 0.001$). Finally, tumors that were neither VEGF-D nor VEGFR-3 positive had a lower density of LYVE-1 positive vessels compared with the tumors with other staining patterns ($P = 0.033$).

Our study represents the first simultaneous analysis of VEGF-C/-D expression with LYVE-1, CD34 and VEGFR-3 vessel densities in breast cancer. The results indicate that peritumoral lymph vessel density is associated with lymph node metastases in invasive lobular breast cancer. Moreover, these findings show, that invasive lobular cancer producing VEGF-D, surrounded by VEGFR-3 positive vessels, have a significantly higher peritumoral lymph vessel density as well as a higher number of metastatic lymph nodes.

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Real time RT-PCR detection of disseminated tumour cells in bone marrow has superior prognostic significance in comparison with circulating tumour cells in patients with breast cancer

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Purpose: This study assessed the feasibility of using real time RT-PCR analysis to detect disseminated epithelial cells (DEC) in peripheral blood (PB) and bone marrow (BM) of patients with breast cancer (BC). Detection of DEC in BM is an obvious choice in BC, but blood sampling is more