permutation t-test. In a point of repeated aberrations, 78 repeated loci were detected from the whole specimen, gained in 1p36.33, 19p13.13, and lost in 14q32.33, 4q32.3, 10p15.3, 14q21.1 from. In all 4 cases of c-erbB-2 (+) group, repeated signal were gained in 17q12, 17q21.1 and lost in 14q32.33, 22q11.1.

Conclusion: 17q11.2 gain and 15q11.2 loss were statistically significant but not frequent aberrations, so was the 14q32.33 vice versa. We propose that not only the statistically marked signaling genes but also the genes that show continuously repeated abberation should be included in ongoing study design not to lose meaningful abberations that could influence on the tumor expression. (−); not stained or in <10% cytoplasmic membrane proportion of the field. (+); at least >10% proportion, the intensity classified faint/barely, weak to moderate, moderate to strong, to (1+), (2+), (3+) respectively.

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Array comparative genomic hybridization analysis and real time PCR reveal genomic alterations in tissue and blood of breast cancer patients

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Background: Genomic alterations are important events in the origin and progression of cancers. DNA copy number changes has been shown to be associated with progression and treatment response in cancer. The aim of our study is to compare differences of DNA copy number alterations in blood and tumor tissue for breast cancer.

Material and Methods: Tumor tissue and blood were derived from 30 patients with breast cancer. DNA copy number changes in blood were compared to those in tumor tissue using array-comparative genomic hybridization (array-CGH). Array CGH was performed using MACArray™-Karyo 4K BAC-chip (Macrogen, Seoul, Korea) which contains 4,030 bacterial artificial chromosome (BAC) clones on the whole human genome with a resolution of about 1Mbp. The data analyses were using a MAC Viewer Software and the relative degree of chromosomal changes was analyzed using log2 ratios. For validation, we used real time polymerase chain reaction (PCR). The relative genomic copy number was calculated using the comparative threshold cycle (CT) method.

Results: We identified 46 regions of gains present in more than 30% of the tissues and 70 regions of gains present in more than 30% of blood. The most frequently gained region is chromosome 8q24. Thirty regions of copy number gains were detected in at least 30% both primary tumors and blood. Of 30 regions, 7 regions of copy number gains were found in more than 50% both tissues and blood. 7 regions include 5p15.33, 8q24.3, 16p13.3, 17q11.2, 17q25.3, 20q13.33, and 22q13.33. This region include AHRR, EXOC3, SLC9A3, HSF1, DGAT1, SCRT1, FBXL6, GPR172A, ADCK5, MYO18A, LAMA5, RPS21, CABLES2, C20orf151, MOV10L1, PANX2, TUBGCP6, HDAC10, MAPK12, MAPK11, PLXNB2. There was one region of copy number loss more than 30% both tissues and blood. SCRT1 and MYO18A was confirmed by RT-PCR.

Conclusions: These data support the utility of array CGH for the identification of genomic alterations in breast cancer. Although there are more frequent genomic alterations in tumor tissue, the pattern of gain and loss in blood is similar to that seen in the tumor tissue. So further study will be needed to validate our results. These findings suggest that array CGH in blood could be used for identification of candidate genes for breast cancer.

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Potential clinical application of serum proteome mass spectrometry analyses in breast cancer patients diagnosis and management

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Background: Proteomics is the study of the proteome – a complete protein component of the cell. In contrast to the genome, the proteome is dynamic and its fluctuations depend on combination of numerous internal and external factors. Identifying and understanding changes in the proteome related to a disease development and therapy is a subject of clinical proteomics. Here we aimed to identify in the circulating blood a set of polypeptide biomarkers that could be useful for the early detection, diagnosis, prognosis and management of cancer and to correlate them with known pathological and clinical prognostic and predictive factors.

Methods: Analysis of the low-molecular-weight region of the blood proteome (using either serum or plasma samples) by mass spectrometry (MS) methods is one of the basic approaches of clinical proteomics. Although no single peptide is expected to be a reliable bio-marker in such analyses, multi-peptide sets of markers selected in numerical tests have been already shown in a few studies to have prognostic and predictive value in cancer diagnostics. In our study we have analyzed low-molecular-weight serum polypeptides (<10 kD) using MALDI-TOF mass spectrometry.

Results: Blood samples were collected in the group of 92 operable breast cancer patients before the start of therapy, as well as in the group of 104 healthy controls matched according to age. The clinical data and pathological characteristics are presented. Specific patterns of low-molecular-weight polypeptides (2−10 kD) were identified due to mathematical analyses and cross-correlated between experimental groups. A multi-component set of polypeptides has been selected as a classifier that differentiate control and cancer samples. Components of spectra blood serum peptides differentiating certain groups of breast cancer patients are shown.

Conclusions: Here we have presented report from the project aimed to identify a set of polypeptide biomarkers that could be used for diagnostics and management of breast cancer patients. Preliminary data showed that cancer-specific multi-component polypeptide pattern could be identified in serum of breast cancer patients. However, their importance for cancer diagnostics remains to be validated.

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Cox-2 is a target gene of Rho GDP dissociation inhibitor beta in breast cancer cells

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Background: Rho-GDIbeta, an inhibitor of Rho-GTPases, is primarily expressed by haematopoietic cells, but also found in epithelial cancer cells. Recently, we have identified Rho-GDIbeta as a target Ets1 (Oncogene, 24, 2005, 650–661), a transcription factor that is involved in controlling cellular invasiveness. Here we confirm that Ets1 regulates Rho-GDIbeta. We further analyzed the function of Rho-GDIbeta and its importance for the prognosis of breast cancer patients.

Material and Methods: MDA-MB-231 cells were transfected with siRNA against Rho-GDIbeta or Ets1. Gene expression was determined by quantitative RT-PCR, cDNA-Microarray, Western blot analysis and immunohistochemistry. Promoter assays were performed by using a luciferase-containing reporter construct. In vivo-Ets1 binding was investigated by chromatin-immunoprecipitation assays. Two cohorts (263 and 117 patients) were used to determine the effect of Rho-GDIbeta RNA and protein levesl on disease-free and overall survival.

Results: We show that, in breast cancer cells, Ets1 regulates Rho-GDIbeta expression on the RNA and protein level and binds to the upstream region of the Rho-GDIbeta gene. Furthermore, in primary breast cancer, Rho-GDIbeta is co-expressed with Ets1. Studying the function of Rho-GDIbeta in breast cancer, we found that a Rho-GDIbeta-specific siRNA increased cellular migration, but also decreased the expression of the Cox-2 oncogene. Further studies revealed that Rho-GDIbeta regulates Cox-2 gene at least partly on the transcriptional level most likely by activating NFAT-1. Vav-1, an interaction partner of Rho-GDIbeta, was also found to interfere with Cox-2 expression and NFAT-1 cellular distribution suggesting a cooperative action of Rho-GDIbeta and Vav-1 on Cox-2 expression. To explore the importance of Rho-GDIbeta for the survival of breast cancer patients, two cohorts including 263 and 117 patients were analyzed for clinical outcome in relation to Rho-GDIbeta RNA and protein levels, respectively. Expression of Rho-GDIbeta was not associated with either disease-free or overall survival in the two patient population.

Conclusions: Our data suggest that the expression of Rho-GDIbeta in breast cancer is neither beneficial nor disadvantageous to the patient. This may be the net effect of two opposing activities of Rho-GDIbeta, one that suppresses tumor progression by inhibiting migration and the other that stimulates it by enhancing Cox-2 expression.