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Integrin alpha-2 and beta-3 gene polymorphisms and breast cancer risk

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Background: Integrins are cell surface receptors which mediate cell-to-cell and cell-to-extracellular matrix adhesion. Some of them, e.g. $\alpha_V\beta_3$, $\alpha_{IIb}\beta_3$ and $\alpha_2\beta_1$, have been suggested as key players for cancer development and tumor metastasis. Two polymorphisms in the gene for the α_2 component, ITGA2 807C>T and 1648G>A, have been associated with the cell-surface density of integrin $\alpha_2\beta_1$. The 176T>C polymorphism in the ITGB3 gene, encoding the β_3 subunit of integrins $\alpha_{IIb}\beta_3$ and $\alpha_V\beta_3$, modifies a variety of traits of β_3 expressing cells.
Patients and methods: To analyze the role of ITGA2 and ITGB3 polymorphisms for breast cancer risk and prognosis, we performed a case-control study including 500 female breast cancer patients and 500 healthy female age-matched control subjects. All study participants were of Caucasian origin (Austria, Middle-Europe). Genotypes were determined by 5'-nuclease assays (Applera, Austria). Primer and probe sets were designed and manufactured using Applera's 'Assay-by-Design' custom service. The PCR reaction was performed in a Primus 96 plus thermal cycler (MWG Biotech AG, Germany) using a total volume of 5 μl containing 2.5 μl SuperHot-Master-Mix (Bioron GmbH, Germany)
Results: The ITGA2 1648_AA genotype was significantly associated with breast cancer (odds ratio 3.12; 95% confidence interval 1.11–8.77). Carriers of the most common ITGA2 haplotype (807C_1648G, "wildtype") were at decreased risk for breast cancer (odds ratio 0.72; 95% confidence interval 0.53–0.98). A histological grade of 3 or 4 was found more often in ITGA2 807TT subjects ($p = 0.039$ compared to CC+CT genotypes) and carriers of an ITGA2 1648A allele ($p = 0.017$ compared to GG genotype). Carriers of the ITGA2 807C_1648G haplotype were less likely to have a histological grade 3 or 4 compared to non-carriers ($p = 0.003$). The ITGB3 176T>C polymorphisms was not associated with breast cancer susceptibility. In a Cox-regression analysis, carriers of the homozygous ITGB3 176-CC genotype had a higher risk for metastasis (relative risk 2.2; 95%CI: 1.2–4.0; $p = 0.015$).
Conclusion: We conclude that functional polymorphisms in integrin genes ITGA2 and ITGB3 influence the development and progression of breast cancer, respectively. The precise mechanism remains to be determined, but likely involves dysregulated signaling pathways.

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Moderate concordance of the HER-2/neu expression of primary breast tumours and their metachronous distant metastases: evaluation by conventional and automated immunohistochemistry

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Introduction: The determination of HER-2/neu overexpression, mostly tested in the tissue of primary breast cancer, is required for the selection for trastuzumab therapy in metastatic breast cancer patients. Clonal changes in the course of the disease may compromise patient selection.
Patients and methods: In a 10-year retrospective study from 1994–2004, we searched for paraffin-embedded breast carcinomas in two university institutes of pathology. A total of 136 slides of breast carcinoma tissues obtained from 68 patients (primary tumour and one metachronous metastatic lesion each) were stained for HER-2/neu expression. The HER-2/neu tissue results of the primary tumours were correlated to the tissue results of the corresponding metastases. We used 2 immunohistochemistry (IHC) evaluation techniques: the conventional IHC method by the DAKO HercepTest® and a computerized automated IHC of the same slide using the ChromaVision ACIS® system. The concordances of those HER-2/neu results were determined using the concordance index kappa (κ), the McNemar test and the intraclass correlation coefficient (ICC).
Results: Tumour characteristics were distributed as follows: 71% invasive ductal, T1/2-tumours 41/34%, N0/1 staging 41/38%, G1/2 grading 10/49%, ER/PR positivity 34/35% in the primary tumours. Metastatic lesions (78% soft tissues, 7% visceral organs, 12% bone, 3% others) were biopsied from 24–918 weeks after initial surgery. Metastases were ER/PR-positive in 62/53%. A total of 50% of the patients was HER-2/neu-positve in the primary tumour using the DAKO test (+2/3 positive), whereas only 34% were HER-2/neu-positive with the ACIS test ($\geqslant 2.0$). In the metastatic lesion, 59% of the patients were DAKO-positive and 34% ACIS-positive. The concordance between the HER-2/neu expression in the primary tumour and the metastatic tissue was moderate with $\kappa = 0.53$ for the DAKO test and even decreased to $\kappa = 0.28$ for the ACIS test. Altogether, 77 (i.e. 68%) of the patients had the same HER-2/neu status in the primary tumour and the metastatic tissue. The comparison of the metric results of the ACIS test for the primary tumour and the metastases revealed a weak correlation ($ICC = 0.514$, $p < 0.001$). The McNemar test for a change from HER-2/neu negative primary tumours to HER-2/neu positive metastases as compared to a change from HER-2/neu positive primary tumours to HER-2/neu negative metastases revealed no statistically significant differences ($p = 0.210/1.000$ for DAKO/ACIS, respectively). The comparison of the two IHC techniques DAKO and ACIS showed a moderate concordance with $\kappa = 0.49$. In 73% of the measurements, the HER-2/neu status was concordant. Of note, the McNemar test demonstrated a highly significant difference ($p < 0.001$) in the evaluation of the HER-2/neu expression by the two tests. In 24% of the cases, DAKO-positive slides were stained negative with the ACIS test, whereas only 3% were negative with the DAKO test and positive with the ACIS test.
Conclusions: A clonal change of the HER-2/neu-expression, tested by immunohistochemistry, can be noticed more often than generally assumed. Clinical selection of metastatic breast cancer patients for trastuzumab therapy requires an individual weighing of the evaluation method of the HER-2/neu status. HER-2/neu testing in metastatic tissue could possibly improve the probability of therapeutic effects. In a further study, we will verify our data by testing the tissue slides for HER-2/neu-amplification using the fluorescence-in situ-hybridisation (FISH) technique. Moreover, we will include serum HER-2/neu results at the time of metastatic disease.

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Detection of disseminated epithelial cells by quantitative real-time RT-PCR: effect of pre-analytical time

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Introduction: We and others have recently explored the use of quantitative real-time RT-PCR analysis for the detection of circulating tumour cells in blood of patients with breast cancer (BC). A major problem in such experiments is the instability of the cellular RNA *in vitro*. Copy number of mRNA can change during storage or transport at room temperature. Accurate quantitative measurements of specific transcripts may be critically when working with small numbers of target mRNA.
Methods: Peripheral blood samples were obtained from 2 healthy volunteers and 13 patients with BC. Blood was stored at room temperature for 0, 1, 2, 4, 6, 24, 48 and 72 hours. The potential alteration of gene expression for 6 target genes was investigated by quantitative real-time RT-PCR.
Results: For β-actin, GAPDH, cytokeratin-19 and HER2 a significant decrease in expression level occurs after 6 hours (CK-19 and HER2), 24 hours (β-actin) or 48 hours for GAPDH. Mammaglobin expression was only measurable in two samples and seems to be stable for at least 6 hours. For VEGF, a statistically significant increase in expression level is observed in samples processed 24 hours after collection. Fig 1 shows the effect of pre-analytical time expressed as difference in CT value (ΔCt) at the different time points versus time zero.

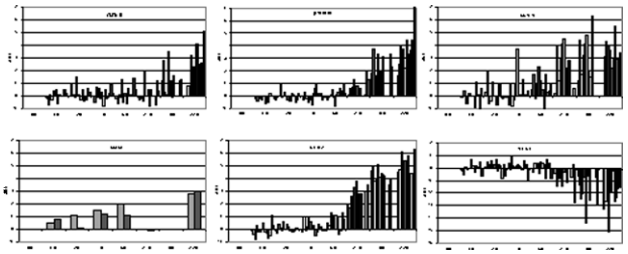

Fig. 1: Effect of pre-analytical time on the Ct values of GAPDH, β-actin, CK-19, MAM, HER2 and VEGF. The graph shows for 13 metastatic breast cancer patients and 2 healthy volunteers the ΔCt values ($Ct_{\text{time x}} - Ct_{\text{time zero}}$) versus the delay (h) of starting the RNA extraction after venipuncture.

Conclusion: Most transcripts are reduced in samples that where stored overnight at room temperature, compared with fresh samples, but also up regulation of transcripts as an active response to cellular stress may happen when blood is removed from its *in vivo* environment and stored at ambient temperature. Optimally, blood samples and RNA should be