297 POSTER

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

U. Langsenlehner, W. Weitzer, G. Hofmann, T. Eder, H. Samonigg, P. Krippl. *Division of Oncology, Internal Medicine, Medical University Graz, Graz, Austria*

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.

298 POSTER

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

D. Lüftner[1], P. Henschke[1], H. Dilk[1], R. Geppert[1], M. Dietel[2], H. Stein[3], K. Wernecke[4], K. Possinger[1]. [1]*Medizinische Klinik mit Schwerpunkt Onkologie und, Charité Campus Mitte, Berlin, Germany;* [2]*Institut für Pathologie, Charité Campus Mitte, Berlin, Germany;* [3]*Institut für Pathologie, Charité Campus Benjamin Franklin, Berlin, Germany;* [4]*Institut für Medizinische Biometrie, Charité Campus Mitte, Berlin, Germany*

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.

299 POSTER

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

I.H. Benoy[1], H. Elst[1], I. Van der Auwera[1], S. Van Laere[1], G. Van den Eynden[1], P. Van Dam[1], E. Van Marck[1], S. Scharpé[2], P.B. Vermeulen[1], L.Y. Dirix[1]. [1]*Translational Cancer Research Group (Lab Pathology), University of Antwerp/University Hospital Antwerp Oncology Centre, Wilrijk, Belgium;* [2]*University of Antwerp, Medical Biochemistry, Wilrijk, Belgium*

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.

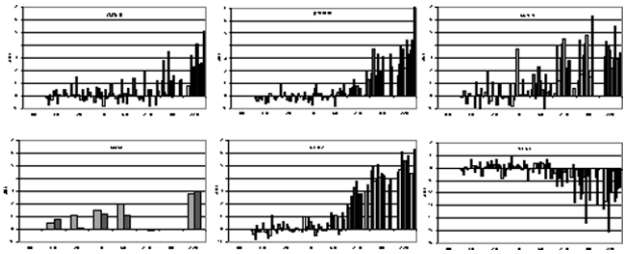

Fig. 1: Effect of pre-analytical time on the Ct values of GAPDH, β-actin, CK-19, MAM, HER2 and VEGF. The graph shows for 13 metastatic breast cancer patients and 2 healthy volunteers the ΔCt values ($Ct_{\text{time x}} - Ct_{\text{time zero}}$) versus the delay (h) of starting the RNA extraction after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

processed immediately after collection to avoid interference of the *in vivo* gene expression signature with ex vivo stress responses. An alternative is the use of an integrated system for collection, stabilization, and purification of intracellular RNA from whole blood like PAXgene (Qiagen) or Tempus Blood RNA tubes (Applied Biosystems). RNA profiles will be stabilised for up to 5 days at room temperature.

300 POSTER

Importance of XRCC1 Arg399Gln polymorphism in the development of breast carcinoma in women with and without breast cancer family history

S. Costa[1], D. Pinto[2], D. Pereira[3], H. Rodrigues[3], S. Carvalho[4], C. Portela[3], R. Medeiros[2,5], F. Schmitt[1,4,6]. [1]*Life and Health Sciences Research Institute, Health Sciences School, Minho University, Braga, Portugal;* [2]*Molecular Oncology/Department of Pathology, Instituto Português de Oncologia, Porto, Portugal;* [3]*Department of Medical Oncology, Instituto Português de Oncologia, Porto, Portugal;* [4]*IPATIMUP – Institute of Molecular Pathology and Immunology of University of Porto, Porto, Portugal;* [5]*ICBAS, Abel Salazar Institute for the Biomedical Sciences, Porto, Portugal;* [6]*Medical Faculty, Department of Pathology, Porto University, Porto, Portugal*

Background: Breast cancer is the neoplasia with higher incidence and mortality in women all over the world. Many environmental factors have been associated with risk of breast cancer development, such as radiation, diet and endogenous and exogenous estrogens. Several studies have reported that the genes involved in DNA repair and maintenance of genome integrity are implicated in protecting against mutations that lead to cancer. Epidemiologic evidence has shown that inheritance of genetic variants (polymorphism) at one or more loci results in reduced DNA repair capacity and increased cancer risk. Base excision repair (BER) is a crucial pathway in the maintenance of genome stability. Variants of several DNA repair genes, including *XRCC1* gene, have been described, but the influence of these genetic variants in repair phenotype and cancer risk remains unclarified.
Aim: The purpose of this study was to evaluate the role of *XRCC1 Arg399Gln* polymorphism as genetic susceptibility markers to familiar and sporadic breast cancer.
Materials and methods: We have used a case-control study. We analysed 630 DNA samples from Portuguese individuals: 71 breast cancer patients with family history (FH) of breast cancer, 219 patients without FH and 340 control subjects, for *XRCC1 Arg399Gln* polymorphism using PCR-RFLP.
Results: We found *Arg/Arg* genotype in 33.8% breast cancer patients with FH, in 43.8% of patients without FH and 34.3% of healthy women. We observed statistically significant differences in *Arg/Arg* genotype of *XRCC1 Arg399Gln* polymorphism between breast cancer patients without FH and control group ($p = 0.025$; OR = 1.49, 95%CI: 1.03–2.14). Furthermore, we found that *Arg/Arg* genotype is more frequent in breast cancer patients without FH (43.8%) than in patients with FH (34.3%).
Conclusions: These preliminary results, in the Portuguese population, show a higher frequency of the *Arg/Arg* genotype of *XRCC1 Arg399Gln* polymorphism in patients without FH of breast cancer than in patients with FH and control groups, suggesting this genotype in women with no FH of breast carcinoma as a susceptibility factor to the breast carcinoma development.

301 POSTER

Very high frequency of BRCA1 5382insC founder mutation in Russian "hereditary-like" breast cancers

E. Imyanitov[1], A. Sokolenko[1], A. Iyevleva[1], E. Chekmariova[1], K. Buslov[1], E. Suspitsin[1], A. Togo[1], E. Bit-Sava[1], V. Semiglazov[1], C. Cornelisse[2]. [1]*N.N. Petrov Institute of Oncology, St.-Petersburg, Russian Federation;* [2]*Leiden University Medical Center, Leiden, The Netherlands*

Background: BRCA1 5382insC is a rare founder mutation described in Ashkenazi Jews. Some studies indicate that it may play a certain role in breast cancer (BC) incidence in Eastern Europe.
Material and methods: We analyzed the impact of BRCA1 5382insC founder mutation in BC predisposition in St.-Petersburg, Russia. In order to enhance the design of the study, we recruited a significant number of patients with "extreme" level of BC susceptibility (bilateral breast cancer (biBC) patients and/or cases with affected first-degree relative(s) and/or young BC cases) as well as an additional "cancer tolerant" control group consisting of elderly tumor-free women. BRCA1 5382insC allele was detected by allele-specific PCR.
Results: The BRCA1 5382insC carriers constituted as many as 16/184 (9%) familial and/or early-onset ($\leqslant 40$ years) BC cases and 15/144 (10%) biBC patients. The remaining BC cases, i.e. those selected against the early onset, bilaterality, and history of the disease in first-degree relative(s), showed the 5382insC mutation in 18/709 patients (2.5%). Strikingly, the 5382insC variant was not observed in any of 478 middle-aged healthy female donors or 350 elderly ($\geqslant 75$ years) non-affected women.
Conclusions: When taken together with the literature data, several aspects of this study deserve a critical discussion. 1) Unexpectedly for such a numerous nation as Russians, BRCA1 5382insC founder mutation constitute an indeed significant proportion of "hereditary-like" BC cases; 2) Since high BRCA1 5382insC occurrence was also repeatedly observed in BC-affected subjects from other Slavic countries, one may suspect that an initial allocation of this variant to the Jewish ancestry could have been wrong; 3) If the estimates found in this study are coupled together with our results on the frequency of CHEK2 1100delC (6%) and NBS1 657del5 (1%) variants in "hereditary-like" BC, 2 conclusions can be made: a) 3 simple PCR tests may reveal the genetic cause in 1 out of 6 familial and/or bilateral and/or early-onset BC in Russia; b) "comparison of extremes" approach provides a straightforward tool for the disease association analysis of rare genetic variants.

302 POSTER

Chromosome 22 array-CGH profiling of breast cancer reveals tumor heterogeneity and 340 kb shared region of loss with ovarian cancer

M. Benetkiewicz[1], A. Piotrowski[1], M. Jankowski[2], D. Bala[2], J. Hoffman[2], W. Zegarski[2], J. Dumanski[1]. [1]*Uppsala University, Department of Genetics and Pathology, Uppsala, Sweden;* [2]*Oncology Centre, Bydgoszcz, Poland*

Breast cancer is the leading cause of death among women in developed countries. Loss of chromosome 22q has been previously established as a common event in breast malignancy, with a frequency of up to 66%. However, despite the number of studies (LOH, metaphase CGH), the identity of putative gene(s) on 22q involved in initiation and/or progression of this tumor remains unknown.
To address this issue, we have performed gene copy number profiling in a set of various stage breast cancers, corresponding surrounding healthy tissue and peripheral blood lymphocytes using a tiling-path chromosome 22 genomic microarray, with an average resolution of 75 kb. The major aberration observed was heterozygous interstitial deletions of various sizes in the telomeric part of 22q. The extent of these deletions varied from 340 kb to 12 Mb. Interestingly, the smallest 340 kb segment is shared with the region of allelic loss previously identified in ovarian carcinoma.
This finding suggests the existence of a common region of 22q, involved in the pathogenesis of these female cancers. The second prevailing type of finding was a complex pattern of low-copy-number amplifications/gains within the proximal half of 22q which were always accompanied by a loss of genetic material in the telomeric part of the chromosome. Our analysis also revealed small deletions in the centromeric region that have been previously reported as normal polymorphisms. Another aim of our project was to identify genetic heterogeneity within the studied tumors. The most remarkable finding was the presence of distinct aberrations in the two samples derived from different locations of the same large tumor.
This clearly demonstrates the co-existence of separate cell populations within the tumor mass and may reflect evolving steps of tumor progression.

303 POSTER

Bilateral breast cancer – clinical features and BRCA1, BRCA2, CHEK2 mutations

A. Niwinska[1], S. Jaczewska[1], R. Sienkiewicz-Kozlowska[1], E. Skasko[2], A. Balabas[2], A. Kluska[2], M. Piatkowska[2], T. Pienkowski[2]. [1]*M. Sklodowska-Curie Memorial Cancer Center, Breast Cancer Clinic, Warsaw, Poland;* [2]*M. Sklodowska-Curie Memorial Cancer center, Endocrinology Department, Warsaw, Poland*

Purpose: The aim of the study was to analyze clinical features of patients with bilateral breast cancer and to determine the contribution of BRCA1, BRCA2 and CHEK2 mutations in analyzed group.
Material and methods: Five hundred and nineteen case history of bilateral breast cancer patients (pts) treated at Cancer Center, Warsaw, Poland were analyzed and genetic mutations were evaluated in 120 of them. The time of observation was 1–56 years, median 24 years. There were 193 (34%) of synchronous (SBC) and 326 (66%) of metachronous (MBC) breast cancers. Mean age of diagnosis of SBC and MBC breast cancer was respectively 57 and 48 years ($p < 0.001$). In pts with MBC median time between detection of cancers were 5 years (range 1–54 years). Family history of breast or/ and ovarian cancer was verified in 37.5% of pts. Kaplan-Meier survival analysis was performed. Kohen-kappa homogeneity test was used regarding histological type and grade of cancers in both breasts of each patient.
Results: The probability of 5-year, 10-year and 20-year overall survival in MBC was 93%, 85% and 64% and in SBC– 82%, 71% and 46%