111 Poster

Robust PCR-quantification of RNA from formalin-fixed paraffin-embedded breast cancer tissue slides with variable tumor cell content

G. Hennig[1], T. Acht[1], H. Euting[1], J. Overgaard[2], T. Tramm[2]. [1]*Siemens Medical Solutions Diagnostics, Molecular Research, Leverkusen, Germany;* [2]*Aarhus University Hospital, Department of Experimental Clinical Oncology, Aarhus, Denmark*

Background: It would be of substantial benefit if robust gene expression profiling for predictive or prognostic biomarkers with kinetic PCR could be performed on routinely collected, formalin-fixed paraffin-embedded (FFPE) tissue. The goal of this study was to establish a FFPE assay to assign ESR1, PGR and ERBB2 status to breast carcinoma based on mRNA. In addition we analyzed assay reproducibility comparing different tumor content and different blocks of FFPE tissue from the same tumor.

Materials and Methods: Two independent paraffin blocks from the same tumor were taken from each of 22 patients diagnosed with breast cancer in 1991−93 in Aarhus. The tissue had been routinely formalin-fixed and paraffin-embedded. From each tumor tissue block a whole slide section and a manually trimmed, tumor enriched section (discarding non-invasive background tissue) were prepared and RNA was isolated with a Siemens Diagnostics experimental, silica bead-based and fully automated isolation method for RNA from FFPE tissue slides. Tumor content defined as invasive carcinoma with intervening stroma was estimated from an HE-section. All eluates were analyzed with kinetic one-step RT-PCR for the gene expression of 1 housekeeping gene RPL37A and 3 target genes (ESR1, PGR and ERBB2). Raw data (Ct values) were normalized to RPL37A expression and gene expression levels of target genes were calculated by the Delta Ct method and compared to standard immunhistochemistry data of these genes.

Results: RNA from all archival FFPE tissue slides was successfully isolated. The gene expression for ESR1, PGR and ERBB2 were reliably quantified over a dynamic range of 3−4 logs. The correlation for ESR1, PGR and ERBB2 between different tumor contents (range 30–100%) including independent isolations from two different sections from the same specimen was very good with spearman correlations of about 0.94, 0.87 and 0.77, respectively. In addition the correlation between RNA expression and protein level was very good, so it was possible to define Delta Ct RNA cut offs for each gene distinguishing between immunhistochemistry positive and negative samples.

Conclusions: In this study we demonstrated that the isolation and quantification of total RNA from 14 to 16 years archived FFPE tissue is reproducible using the Siemens Diagnostics automated isolation technology despite the variation of clinical relevant variables like different tumor contents and intra-tumor heterogenicity. Furthermore, these data suggest that the quantitative RNA expression level is a correlating surrogate marker to the semi-quantitative protein level.

112 Poster

Deregulated histone deacetylase 2 alters estrogen receptor expression in breast cancer

M.F. Hou[1], Y.T. Yeh[2], F. Ou-Yang[1], T.H. Shieh[2], S.S. Yuan[3], F.M. Chen[1]. [1]*Kaohsiung Medical University Hospital, General Surgery, Kaohsiung, Taiwan;* [2]*Fooyin University Kaohsiung County Taiwan, Medical Technology, Kaohsiung, Taiwan;* [3]*E-DA Hospital I-Shou University Taiwan, Medical Research, Kaohsiung, Taiwan*

Background: The modulation of non-histone proteins by Histone deacetylases (HDACs) confers to protein instability and transcriptional repression. Although absent information concerning their roles in breast cancer, alternations of HDACs may contribute to breast cancer.In the present study, we thus aim to explore the potential role of HDAC2 belonged to class I HDACs in breast cancer.

Material and Methods: The expression profile of HDAC2 was determined by immunoblotting and immunohistochemical approach in 71 paired breast cancer and adjacent non-cancer tissues. The results obtained were further correlated with clinicopathological characteristics.

Results: Our results showed that HDAC2 was increased in 73.3% of breast cancer tissues as compared with the matched non-cancer tissues. The increased HDAC2 expression was only correlated with decreased estrogen receptor (ER) expression ($p = 0.015$) but not other clinicopathological characteristics and overall survival. Intriguingly, however, we found that HDAC2-specific siRNA and inhibitor, valporic acid, increased ER expression, while 17-beta-estradiol (E2) decreased ER expression but did not alter HDAC2 expression in MCF-7 cells. E2 increased the interaction between ER and HDAC2 and the ubiquitination of ER. In addition, HDAC-2 specific siRNA and valporic acid increased the sensitivity of MCF-7 cells to tamoxifen.

Conclusions: Altered HDAC2 may play some roles but not a determined role in breast cancer through an ER-dependent manner. The modulation of ER by HDAC2 may provide some treatment information in a subset of breast cancer patients.

113 Poster

D-glycuronyl C5-epimerase expression is lost in human breast fibroadenoma

T. Eshchenko[1], A. Chernakov[2], E. Zabarovsky[3], S. Sidorov[2], E. Grigorieva[1]. [1]*Institute of Molecular Biology and Biophysics, Laboratory of Molecular Mechanisms of Carcinogenesis, Novosibirsk, Russian Federation;* [2]*Municipal central hospital N1, Oncology department, Novosibirsk, Russian Federation;* [3]*Karolinska Institute, Microbiology and Tumor Biology Center, Stockholm, Sweden*

Background: Human D-glycuronyl C5-epimerase (GLCE) is one of key enzymes of glycosaminoglycan/proteoglycan biosynthesis, which expression is decreased in human breast tumors. However, nothing is known about expression in other kind of tumors. In this study, we decided to examine if there is a change in GLCE expression in human benign breast tumors and compare with malignant ones.

Materials and Methods: The study included 21 patients with diagnosis fibroadenoma and 73 patients with breast tumors. Clinical samples were matched pair for each patient – from the central part of tumor and a more distant part of the breast. Level of D-glycuronyl C5-epimerase expression in fibroadenoma and human breast tumors was studied using multiplex RT-PCR, qReal-Time PCR and Western-blot assays. All patients presented their written informed consents concerning their participation in the investigation, and experiments were performed in accordance with ethical principles of the Helsinki Declaration and standards of the Committee of Bioethics of the State Research Institution of Molecular Biology and Biophysics, Siberian Branch of the Russian Academy of Medical Sciences.

Results: It was found that the epimerase expression was changed in human breast fibroadenoma from some patients – 4 patients from 21 studied showed a total lost of expression compared with normal human breast tissue. 17 patients of 21 had a comparable D-glycuronyl C5-epimerase expression both in tumor and normal breast samples. According Western-blotting, D-glycuronyl C5-epimerase protein (Mv 68 kDa) was detected in breast fibroadenoma tissue from all 21 patients studied. Because previously we have shown that the GLCE expression is strongly decreased in human breast cancer, a loss of epimerase expression in fibroadenoma could reflect a tendency of the benign tissue to malignization.

Conclusion: Taken together, the data shows that the loss of D-glycuronyl C5-epimerase mRNA expression in human breast fibroadenoma could be a new potential marker for the malignancy and should be investigated in more detail.

114 Poster

Preliminary study of genomic DNA aberration differences on c-erb B2 overexpression in Asian breast cancer

T. Kang[1]. [1]*Maryknoll Hospital, General Surgery, Busan, South Korea*

Background: Array-CGH (comparative genomic hybridization) is one of effective techniques to detect multiple chromosomal abnormalities in genomic DNA. Compared with conventional CGH, there are many advantages for array-CGH, such as high resolution, simplified image analysis and high throughput, and its oligo-strategy allows a genome based design. We analyzed various genomic aberrations that could influence on c-erbB-2 amplification, which is an important prognostic and treatment factor in breast cancer.

Materials and Methods: 10 cases of breast cancer patients were selected to equally stratify on the c-erbB-2 immunostain status and analyzed with array-CGH in paraffin embedded tumor tissues. We emphasized several genes that shown not only a marked signals but also continuously repeated abberations though its signals were not statistically significant.

Result: There were 4 (+) and 4 (−) c-erb B2 immunostained specimen in this study as we first stratified so. By a-CGH test, all (+) cases showed genomic aberration in c-erb B2 region, and all (−) cases showed no signal amplification on the same region. Of 4 IHC(+) cases, 2 cases were (3+) and other 2 cases were (2+), and we validated interobserver reproducibility among these 2 (2+) cases by other qualified laboratory. According to 2nd test, 1 case showed IHC(3+) and another showed IHC(2+) and FISH(−) result. The unsupervised dendrogram showed no significant classifier, might be due to its limited case number in this preliminary study. By the supervised clustering on the c-erbB-2 factor, 18 statistically significant aberrations (gained in 17q12−21.1, 17q12, 17q21.1, 17q11.2 and lost in 22q11.1, 15q11.2) were found in c-erbB-2 (+) group with the

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.

115 Poster

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

J.S. Bae[1], B.J. Song[1], Y.J. Suh[2], W.C. Park[1], J.S. Kim[1], S.S. Jung[1].
[1]Kangnam St. Mary's Hospital, Surgery, Seoul, South Korea; [2]St. Vincent Hospital, Surgery, Seoul, South Korea

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.

116 Poster

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

E. Nowicka[1], M. Pietrowska[2], K. Behrendt[1], A. Walaszczyk[2], J. Polanska[3], A. Polanski[3], L. Marczak[4], M. Stobiecki[4], P. Widlak[2], R. Tarnawski[1].
[1]Center of Oncology Maria Sklodowska-Curie Memorial, Radiotherapy Department, Gliwice, Poland; [2]Center of Oncology Maria Sklodowska-Curie Memorial, Department of Experimental and Clinical Radiobiology, Gliwice, Poland; [3]Silesian University of Technology, Faculty of Automatic Control Electronics and Computer Science, Gliwice, Poland; [4]Polish Academy of Science, Institute of Bioorganic Chemistry, Poznan, Poland

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.

117 Poster

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

D. Schunke[1], P. Span[2], H. Ronneburg[1], A. Dittmer[1], M. Vetter[1], E. Kantelhardt[1], V. Mueller[3], F.C.G.J. Sweep[2], C. Thomssen[4], J. Dittmer[4].
[1]Universitaet Halle, Klinik fuer Gynaekologie, Halle/Saale, Germany; [2]Radboud University Nijmegen Medical Centre, Department of Chemical Endocrinology, Nijmegen, The Netherlands; [3]Universitaetsklinikum Hamburg-Eppendorf, Klinik für Gynaekologie, Hamburg, Germany; [4]Universitaet Halle, Klinik für Gynaekologie, Halle/Saale, Germany

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.