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

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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.

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Deregulated histone deacetylase 2 alters estrogen receptor expression in breast cancer

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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.

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

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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.

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

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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