1303 POSTER

Effect of neoadjuvant chemotherapy on oestrogen receptor, progesterone receptor and HER 2 receptor expression in breast cancer

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Background: Neoadjuvant chemotherapy (NC) is used in treating locally advanced operable breast cancer. After surgery, further adjuvant treatment is offered based on the estrogen receptor (ER), progesterone receptor (PR) and HER2 status. Treatment post operatively can be based on the ER/PR/HER2 status of the core biopsy taken preoperatively. It is not a usual practice in the United Kingdom to repeat these markers on the surgical specimen. However a change in ER/PR or HER2 status following NC could have a profound effect on adjuvant treatment with the real possibility of appropriate therapy being unknowingly withheld. The aim of our study was to determine the percentage of patients whose ER/PR, HER2 receptor expression change with NC and if these changes lead to change in their adjuvant treatment.
Materials and Methods: This is a retrospective study of 32 patients with locally advanced breast cancer who had NC followed by breast conservation surgery or mastectomy. Quick score (Q score) for ER/PR and the HER2 expression was measured both from the preoperative core biopsy and from the excision specimen following NC.
Results: After NC, 5 patients had complete pathological response and 2 patients had residual ductal carcinoma in situ. 25 (78%) patients had residual invasive malignancy. Quantitative change in Q scores for ER and PR was seen in 6 patients (24%) and 10 patients (40%) respectively. ER status changed from positive to negative in 1 patient (4%). PR status changed from positive to negative in 4 patients (16%) and from negative to positive in one patient (4%). One patient (4%) changed from HER2 negative to HER2 positive after NC.
Conclusions: Change in 1 patient (4%) from HER2 negative to HER2 positive lead to change in adjuvant treatment who would have otherwise not received transzutumab. Though Q scores changed in 24% and 40% for ER and PR respectively no change was observed with regards to hormonal adjuvant treatment. A study with a bigger cohort might address this issue. We suggest that ER/PR/HER2 status should routinely be checked in both core biopsy sample and also resection specimen.

1304 POSTER

Immunocytochemical analysis of mitochondrial protein UCP4 and its correlation with apoptotic and predictive markers in breast cancer

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Introduction: In recent years, mitochondria have been recognized as regulators of cell death via apoptosis and necrosis, as well as modifier events via mitochondrial DNA (mtDNA) alterations providing a possible proliferative advantage to the tumor cells, in addition to their essential role for cell survival. The crucial role of mitochondria in apoptosis is reinforced by the observation that mitochondria contribute to program cell death signaling via the production of reactive oxygen species (ROS). Uncoupling proteins (UCPs) are a family of mitochondrial transporter proteins that are implicated to the maintenance of the basal metabolic rate. UCP4, a member of UCPs family, participates in apoptosis because of its early phylogenetic occurrence. Activation of the mitochondrial pathway of apoptosis is regulated by members of bcl family of proteins.
Purpose: The objective of the present study was to detective the correlation of UCP4 with other apoptotic and prognostic markers in patients with breast carcinoma.
Material and Methods: Ninety (90) imprint smears from surgically resected breast cancers were studied immunocytochemically with the use of monoclonal antibodies to estrogen receptors ER, to apoptotic markers bcl-2, and P53, to proliferative marker ki67, as well with the use of polyclonal antibody to UCP4. Immunocytochemical staining was performed by using Alkaline Phosphatase Anti Alkaline Phosphatase (APAAP) method.
Results: UCP4 positive cytoplasmic expression was detected in 41 (45.5%) of breast cancer smears, bcl2 in 36 (40%) and positive nuclear expression of P53 in 52 (57.7%), ER in 19 (21.1%) and ki67 in 59 (65.5%) of cancer breast cases. Increased UCP4 expression was significantly associated with P53, ki67 overexpression, with low expression of ER and downregulation of bcl2 in histologically poor tumor differentiation and presence of lymph node metastasis.
Conclusions: Dysregulation of normal cell death mechanisms plays an important role in the pathogenesis, progression of breast cancer and in responses of tumors to therapeutic intervention. Taken together the results of our study suggest that UCP4 as an uncoupling protein of mitochondrial membrane is implicated in the apoptosis pathway and associated with high grade breast tumors, indicating oxidative mechanism at the mitochondrial level in the tumor cells.

1305 POSTER

PSMB7 is associated with anthracycline resistance and is a prognostic biomarker in breast cancer

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Background: Chemotherapy is the most important systemic therapy of breast cancer in receptor negative patients. However, to date individual markers failed to correctly predict resistance against anticancer agents. We used gene expression patterns attributable to chemotherapy-resistant cells to detect potential new biomarkers related to anthracycline resistance. One of the genes, PSMB7 was selected for further functional studies and clinical validation.
Materials and Methods: We contrasted the expression profiles of four pairs of different human tumor cell lines of gastric, pancreatic, colon and breast origin and of their counterparts resistant to the topoisomerase inhibitors daunorubicin or doxorubicin. Top 5 genes related to chemoresistance identified by Prediction Analysis of Microarrays (PAM) were also validated by immunhistochemistry. RNA interference was used to silence one of the top genes, PSMB7, which had an elevated expression in the resistant cell lines. After silencing, doxorubicin treatment was performed, and cell vitality was measured using a Casy automated cell counter system. Finally, microarray gene expression of GEO raw microarray samples with available progression free survival data was downloaded. The arrays were MAS5 normalized, and the expression of the PSMB7 gene was used to group the samples in two group (below or above of the average expression). In these, Kaplan-Meier survival plot was generated using Winstat.
Results: Immunhistochemistry verified the differential expression of the top discriminating genes (ABCB1, TOP2A, TOP2B, MKI67 and PSMB7). We achieved a silencing of 86% of PSMB7 using an RNAi oligo at the 548. nucleotide in the 5th exon. After doxorubicine treatment 30.60%$\pm$31.2% of the resistant cell lines survived. The silencing of PSMB7 in the resistant cell lines decreased survival to 5.87%$\pm$10.2%, which had a statistical significance of $p = 0.0346$. We downloaded 1079 microarray samples with available clinical follow-up from GEO. In these, 442 patients had a PSMB7 expression higher than the average (2800). The comparison of the survival of the two groups consisting of patients having high and low PSMB7 expression resulted in high significance ($p = 0.006$).
Conclusion: Our findings suggest a role of the proteosome in the development of anthracycline resistance. High PSMB7 expression is an unfavorable prognostic marker in breast cancer.

1306 POSTER

Increased systemic microRNAs in breast cancer correlate with tumour microRNA profile and clinicopathologic characteristics

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Background: Mic(ro)RNAs have come to the fore of translational research due to their ability to control gene expression and protein translation. Dysregulated miRNA expression in breast cancer is well documented: oncogenic miRNAs have been shown to differentiate breast tumour from normal tissue (*miR-21*, *miR-10b*, *miR-155*) and other more specific miRNAs have been found to correlate with clinicopathologic parameters such as hormone receptor status. Given their small size, miRNAs are remarkably stable, rendering their potential detection in the circulation of breast cancer patients diagnostically and prognostically informative.
This study aimed to determine whether miRNAs were detectable in the circulation of breast cancer patients, to assess the relationship between breast tumour and circulating miRNA profiles, and to evaluate their potential as biomarkers for disease detection and monitoring.
Materials & Methods: Refined RNA extraction techniques were optimized for blood, serum and plasma samples. MiRNA was extracted from blood and a panel of 7 oncogenic miRNAs was quantified in 130 reverse