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Immunocytochemical study of p-53 and HER-2 expression in FNA specimens from breast cancer patients

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Background: A prospective study for the prognostic value of the immunocytochemical identification of p-53 and HER-2 at the FNA cytology of the primary tumors of patients withbreast cancer. An attempt to evaluate the correlation of the p-53 and HER-2 expression with the infiltration of the ipsillateral axillary lymph nodes (LN).

Methods: We applied immunocytochemistry at the FNA specimens of the primary tumor (tumor size until 3cm) of 224 breast cancer patients and clinically negative axillary LN for the identification of p-53 and HER-2. These patients consequently underwent modified radical mastectomy or breast-conserving surgery and standard axillary dissection of level I and II at the Metaxa Memorial Anticancer Hospital. Then we examined histologically the presence or not of ipsillateral LN metastasis in every case.

Results: The median age of these patients was 52.8 years (32–82 years old). 97 patients had free (negative for metastatic infiltration) LN, whereas 127 had at least 1 positive LN. Among the patients with positive HER-2 at the FNA (88 patients) the 65 (73.86%) presented an infiltration of at least 1 LN, whereas only the 45.58% of the 136 patients with negative HER-2 (p-value<0.001). As far as the p-53 was concerned 31 (67.39%) of the 46 patients with positive p-53 and 53.93% of the 178 patients with negative p-53 had LN invasion (non significant). 30 of our patients (13.39%) presented a simultaneous positiveness for p-53 and HER-2 and 26 of them (86.66%) had infiltrated LN (p-value < 0.001).

Conclusions: Tumors with positive p-53 and HER-2 express an aggressive behaviour. The preoperative evaluation of these two biological markers at the FNAs of patients with breast cancer could assist for the better therapeutic plan and prognosis of the disease.

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The role of tumor markers in breast cancer recurrence

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The aim of the present study was to evaluate the clinical performance of tumor markers in breast cancer recurrence. One hundred and seventy-four breast cancer cases were entered into the study and followed up for 132 months (mean=34.0, SD=28.0). The mean age of the patients at diagnosis was 47.1 (SD=12.3) ranging from 23 to 81 years. Most patients (51%) presented with stage II breast cancer. 81% of the patients underwent modified radical mastectomy and the remaining 19% had breast preservation surgery. Recurrence occurred in 22% of patients during the follow up time. The association between clinical recurrence with age, tumor size, nodal involvement, stage, type of surgery and pathology, serum CA15.3, CEA, P53, ER, PR, and HER-2 status were examined by using the forward conditional logistic regression analysis. The results indicated that recurrence was significantly predicted by the existence of CA15.3 (odds ratio = 6.1, 95% CI = 1.62–23.1, P=0.007). The findings showed that independent of age and other know prognostic factors; CA15.3 is a significant predicting factor for recurrence in breast cancer patients.

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Expressions of O (6)-methylguanine-DNA methyl transferase MGMT and p53 in breast cancer

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Background: MGMT is one of the DNA repair protein. It is reported that MGMT is a strong predictor of survival in brain tumors and that mutant p53 protein might be associated with down regulation of MGMT expression in brain tumor.

Purpose: This study investigated the expression s of both MGMT and p53 in breast cancer to clarify the correlation between MGMT and p53 expression.

Methods: Using immunohistochemical staining with an anti-MGMT and anti-p53 antibody, the MGMT and p53 expressions in tissues from 48 consecutive cases of primary breast cancer patients was examined. From the correlation between MGMT and p53 expression and the clinicopathological findings, the prognosis for survival were analyzed.

Results: The expressions of both MGMT and p53 were classified as negative or positive on the basis of staining. The specimen which had negative MGMT expression showed significantly higher expression of p53 (p=0.026, Chi-squared test). No relationship between MGMT expression and each of clinicopathological findings was identified. However, a relationship of negative staining of p53 and estrogen receptor expression was observed. The prognosis of the patients with negative staining of MGMT was worse than that of other patients. The prognosis of the patients with positive staining of p53 was worse than that of other patients.

Conclusions: The p53 protein might be associated with regulation of MGMT expression in breast cancers. MGMT immuno-negativity and p53 immuno-positivity migit be strong predictors of survival in breast cancers.

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Association between pS2 and cathepsin-D in breast carcinoma: biological and clinical aspects

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From the biological point of view our aim was to define estrogen-regulated versus nonestrogen-regulated pS2 and cath-D expression and, then, to examine the association between estrogen-regulated pS2 and cath-D in relation to pT and pN status of carcinomas. From the clinical point of view, our aim was to define high risk subgroups of breast cancer in relation to cath-D expression. This study included 152 patients with histologically verified breast carcinoma. ER and PR were assessed in accordance with recommendation of EORTC. pS2 and cath-D were determined using immunoradiometric assay. The results were analysed using non-parametric statistical methods. Estrogen-regulated cut-off value for pS2 was defined on the basis of the ER-status- and histological grade-, as well as menopausal-related pS2 quantitative values. No overlapping of pS2 protein values was obtained between ER-positive and ER-negative carcinomas within postmenopausal subgroup with histological grade II and within pre- and postmenopausal subgroups with histological grade III. The highest pS2 protein level observed in ER-negative subgroups was considered as the cut-off value (15 ng/mg). Estrogen-regulated cut-off value for cath-D was defined on the basis of the ER–PR status-, auxiliary lymph node- and tumor size-related cath-D quantitative values. No overlapping of cath-D protein values was obtained neither between pN0 and pN+ nor pT1 and pT2/3 carcinomas within ER, PR-negative subgroup. The highest cath-D protein level observed in ER, PR-negative TN-stage favorable subgroups was considered as the cut-off value (28 pmol/mg). Our further analysis aimed at examination of pT- and pN-related association between estrogen-regulated pS2 and cath-D expression. A statistically significant association was only obtained in pT1 carcinomas. Evaluation of disease free interval in the first three years, among patients bearing pT1 carcinomas, showed a statistically significant difference between estrogen-regulated and nonestrogen-regulated cath-D expression. An unfavorable course of disease was observed in patients with carcinomas expressing estrogen-regulated cath-D. From the biological point of view it is important to point out that the positive association of estrogen-regulated pS2 and cath-D expressions is an early biological event occurring in pT1 carcinomas. Moreover, the estrogen-regulated cath-D defines high-risk subgroup within generally accepted low-risk pT1 breast carcinomas group.

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Expression of multidrug resistance associated genes MRP1, MRP2 and MRP3 in primary and anthracycline exposed breast cancer

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Introduction: Multidrug resistance (MDR) associated proteins MRP1, MRP2 and MRP3 confer in vitro resistance to a wide range of drugs. We investigated their possible role in clinical breast cancer resistance to anthracycline-based chemotherapy.

Methods: Using real-time reverse transcriptase polymerase chain reaction (RT-PCR) and immunohistochemistry (IHC) the expression of MRP1–3 was quantified in nine breast cancer cell lines and 30 breast carcinoma samples.

Results: Detectable levels of MRP1–3 mRNA were present in all breast cancer cell lines and tumor samples. No increase of expression was detected between primary untreated carcinoma samples and samples taken after neoadjuvant anthracycline treatment. IHC was not suitable for

detection of the proteins at these expression levels. MRP1–3 expression was not associated with tumor response to treatment or with impaired outcome.

Conclusions: MRP1–3 are expressed in breast cancer cells, but the immunohistochemical detection failed. We have found no evidence linking these proteins to clinical drug resistance.

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S-HER2 by chemiluminescence versus ELISA

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The purpose was to establish corresponding discriminatory values and if possible create an algorithm for conversion of values from one to the other.

Sera from 80 cancer patients and 120 healthy controls randomly selected from the county population database were run on the Bayer Centauer HER2 chemiluminescence kit and compared to the DAKO HER2 ELISA kit.

The correlation was ELISA = 0.6508 Centauer + 1.66 ng/ml and the R2 = 0.9174.

With a discriminatory value of 15 ng/ml on the Centauer, the corresponding value was 11.8 ng/ml on Elisa.

We conclude that the results can be converted between the two, and the precision on both is sufficient for monitoring the same patient using both methods.

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The influence of oral contraceptives on breast cancer's mitotic activity

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Background: To correlate the administration of oral contraceptives with the mitotic activity of the breast in breast cancer patients.

Material and Methods: The correlation of previously administered oral contraceptives with the tumor's mitotic activity was investigated in 58 breast cancer patients. The PCNA (Proliferating Cell Nuclear Antigen) expression was immunohistochemically evaluated in histologic specimens of the tumor. According to the PCNA expression the tumors were divided in those of low (<20%) or high (>20%) mitotic activity.

Results: 67% of the patients were oral contraceptive users in the past and 38% of these had been using the pill for a long time (>48 months). Increased PCNA expression was ascertained in the group of patients who had been using oral contraceptives in the past ($p<0.05$). No statistically significant difference was noticed in the PCNA expression among different groups of patients according to the time period of oral contraceptive use.

Conclusions: The administration of oral contraceptives in the past might be correlated with the mitotic activity of the tumor in breast cancer patients.

Wednesday, 17 March 2004

POSTERS

Tumour cell biology

185 POSTER

Activated Akt expression in breast cancer – relationships to p53, Mdm2 and patient outcome

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Background: Activation of protein kinase-B/Akt downstream of phosphatidyl-inositol 3-kinase is mediated by oestrogen and oncogenic pathways, of which HER-2 is important. pAkt phosphorylates Mdm2 to influence its subcellular localisation and to enhance p53 cytoplasmic localisation and degradation, blocking apoptosis. This study examined expression of all activated Akt isoforms (pAkt) together with p53/Mdm2 subcellular expression in a series of invasive ductal breast cancers (IDCs), to evaluate whether *in vitro* findings were related to clinical data and the effect on outcome.

Methods: Immunohistochemical expression of pAkt, was evaluated in 103 patients with invasive ductal carcinoma and related to clinicopathology, p53 and Mdm2 subcellular expression, as well as outcome. pAkt was scored 0–3+, with 0–1+ considered negative and 2–3+ positive. A score of 3+ was considered strongly positive.

Results: pAkt was evaluable in 101 patients with a ubiquitous pattern of cytoplasmic expression in 82% of IDCs. Strong pAkt expression was evident in 24%, with 18% of breast cancers showing no activation of Akt. pAkt is more likely associated with larger tumours (P=0.02), and showed no correlation to other clinicopathologic criteria or HER-2 expression. pAkt is correlated with increasing levels of cytoplasmic p53 (P=0.01), but not nuclear p53. Activated Akt did not correlate with the subcellular localisation of Mdm2. pAkt was associated with a reduced disease-free survival (P=0.04; univariate), but was not an independent predictor in relation to the Nottingham Prognostic Index.

Conclusion: Akt has implications in breast cancer growth through mechanisms inactivating p53 that involve Mdm2. We have demonstrated that activation of Akt is associated with immunohistochemical p53 expression, which is preferentially cytoplasmic. Despite *in vitro* associations, pAkt appears to be a poor marker of HER-2 expression to suggest a greater complexity of these pathways in human cancers.

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Effect of zoledronate on persisting disseminated tumor cells (DTC) in the bone marrow (BM) of breast cancer patients

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Background: Adjuvant systemic therapy reduces the risk for recurrence in breast cancer by approximately 10% (Early Breast Cancer Trialists' Collaborative Group, Lancet 1998). Patients with persisting DTC in the BM after primary therapy show an increased risk for distant relapse and shortened survival (Janni et al., Cancer 2001). Adjuvant chemotherapy, however, seems to have only limited effect on DTC in dormant state (Braun et al., 2000).

Aim of this study was to investigate the therapeutic efficacy of zoledronate on the persistence of DTC in BM after completion of primary therapy.

Methods: Zoledronate was applied at 4 mg q4wx6mon (loading dose 8 mg) to 14 breast cancer patients with persisting DTC in the BM. Patients were to have completed surgery and adjuvant chemotherapy for at least 6 months and had no evidence for recurrence at this point of time. In a matched pair analysis, these patients were compared to 14 patients with DTC in the BM receiving no further therapy. The BM was re-examined after a median of 8 months (range 6.5–9.83) in the treatment group and 9 months (range 2.33–29.17) in the control group. DTC were detected by immunocytochemical staining using the pan-cytokeratin antibody A45-B/B3 and the APAAP technique.

Results: Primary tumor characteristics, i.e. tumor size (P=0.66), axillary nodal status (P=1.0) and histopathological grading (P=0.76), as well as primary surgery (P=0.23), adjuvant systemic therapy (P=0.10) and radiotherapy (P=0.36) were well balanced between both patient groups. While DTC were detected in all 28 patients at the time of first BM aspiration, no patient showed DTC in the BM after 6 months of zoledronate therapy. In contrast, persisting DTC were detected in 4 patients (29%) without treatment (P=0.03).

Conclusion: These preliminary results indicate potential antineoplastic effect of the cell-cycle independent agent zoledronate on persisting DTC in dormant state. In our view, these data provide a hypothesis generating basis to investigate the therapeutic efficacy of zoledronate on DTC in a secondary adjuvant setting by prospectively randomised trials.

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The enhanced expressions of CxCR4 and CCR7 mRNA in breast cancer tissue do not always correlate with cancer metastasis

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Backgrounds: Cancer metastasis is a major prognostic factor for breast cancer patients. The recent findings indicated that the chemokine receptors CxCR4 and CCR7 which found on breast cancer cells, and their ligands that highly expressed at sites have an association with breast cancer metastases.

The aim of the present study was to measure CxCR4 and/or CCR7 mRNA expression in the clinical specimens of primary breast cancer, and to explore whether CxCR4 and/or CCR7 mRNA expression in breast cancer correlate with cancer metastasis and other conventional clinicopathological parameters.