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.

183 POSTER

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.

184 POSTER

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.

186 POSTER

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.

187 POSTER

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.

Materials and methods: Fresh tissue samples were obtained from 55 breast cancer patients undergoing mastectomy or breast conserving surgery. Total RNAs were isolated from 55 surgical specimens of breast cancer tissue and 16 non-cancer breast tissue. The relative mRNA abundance of CxCR4 and CCR7 was measured by real time reverse transcription-PCR analysis based on TaqMan method, and the results were standardized with b-globin mRNA expressions. Statistical analyses were performed using Mann-Whitney test and Kruskal-Wallis test, and the statistical significance was defined as $p<0.05$.

Results: CxCR4 mRNA expression was significantly enhanced in breast cancer tissues compared to non-cancer tissues ($p<0.01$). CCR7 mRNA expression was also significantly enhanced in breast cancer tissues compared to non-cancer tissues ($p<0.01$). However, neither mRNA expression of CxCR4 or CCR7 correlated with any clinicopathological fact ors such as lymph node status, lymphatic invasion, venous invasion, hormone status, distant metastasis or tumor stage.

Conclusions: These results suggest that both CxCR4 and CCR7 mRNA expressions, significantly up-regulated in tumor specimens comparing to non-cancer breast tissue, might have an association with carcinogenesis in breast cancer. However, because the metastasis will be formed by not only chemokine receptor but also its ligand at site, it seemed to be difficult to predict cancer metastasis only by measuring the mRNA expression level of CxCR4 or CCR7.

188 POSTER

The rates of growth of breast cancers

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The diagnostic rate at prevalent screening, the excess of cancers diagnosed at screening over expected presentation, the natural rate of symptomatic presentation and the diameter of screen detected and symptomatic tumours have been used to calculate the diameter doubling time during the observable phase of primary breast cancers (10 mm to 30 mm). These are for grade I 5 years, grade II 2 years and grade III around 3 months. The relative rates match the ratios of mitotic counts for each grade. If growth was by volume doubling, from inception to presentation this would take 150 years in grade I tumours! We have previously (Connor, 1988) shown that mitoses are concentrated in the outer 2 mm shell. We suggest that growth initially is by volume doubling but from 4 mm is by doubling of the outer shell only. Cell doubling rate remains constant. Screen detected tumours average 12 and symptomatic 22 mm diameter. This represents 15 cell doublings in the outer shell and cell doubling rates may be calculated. From the cell doubling rate the time from inception to presentation may be calculated (25 doublings).

Grade	15 cell doublings (months)	Cell doubling time (months)	Mean time from inception to 12 mm (months)
I	62	4.1	102
II	24	2.6	40
III	6	0.4	10

The length of time from inception to presentation in many tumours casts doubt on the role of tamoxifen in 'prevention', more easily explained by inducing responses in undiagnosed cancers. The observation also explains the predominance of grade III tumours at presentation in young women, allowing inception at the same time in good grades but longer to diagnosis.

References

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189 POSTER

Overexpression of eukaryotic elongation factor-1 subunits in breast carcinoma

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Background: Wide evidence suggests the involvement of translation elongation factors (EFs) at the onset of oncogenesis. To investigate the potential role of the EF-1 subunits (alpha, beta, and gamma) in formation and progression of breast cancer, we compared their expression in breast cancers with that in non-cancerous tissues.

Materials and Methods: Total RNA was isolated from fifty eight frozen specimens including 20 primary tumors, 9 fibroadenomas, and matched normal adjacent tissue. The expression of the EF-1 subunits (alpha, beta, and gamma) mRNA in breast cancer tissues was determined using RT-PCR. The mRNA expression was also examined in three breast cancer cell lines and one normal breast cell line using northen blot analysis.

Results: EF-1 alpha, beta and gamma mRNA expression was significantly higher in cancerous over normal tissues ($p<0.05$). However, there was no significant difference in the expression of the three EF-1 subunits between grades I, II, and III tumors. A 2–3 fold increase was observed in mRNA expression in breast cancer cell lines (MCF-7, T47D, and MDA-231) when compared to a normal cell line (MCF-10A).

Conclusion: Overexpression of the three EF-1 subunits was observed in malignant but not in normal breast tissues. Similar results were obtained in cell lines of breast tissue. The elevated levels of these translation factors are indicative of a possible role in the pathogenesis of breast cancer.

190 POSTER

Tartrate-resistant acid phosphatase is expressed by breast cancer cell lines

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Tartrate-resistant acid phosphatase (TRAP, EC 3.1.3.2) is a histochemical marker of osteoclasts, however its biological function is not fully understood. TRAP is also expressed by macrophages and dendritic cells and occurs in a wide variety of tissues including spleen, lung, thymus, skin, the linings of the gastrointestinal tract, tissues in the nervous system, as well as the skeleton. Studies using mice lacking TRAP as a result of targeted gene disruption have demonstrated that TRAP is essential for the normal mineralisation of cartilage in developing bones and the maintenance of the adult skeleton. Macrophages and dendritic cells lacking TRAP displayed an abnormal immunomodulatory response and cytokine profile. It has been suggested that TRAP may be involved in cell recruitment in bone and the immune system.

Osteopontin, identical to the T-cell cytokine Eta-1, is a substrate for TRAP. It is a highly phosphorylated protein with a wide tissue distribution like TRAP. A variety of functions are associated with it some of which are known to be phosphorylation dependent. This cytokine contributes substantially to metastasis formation by various cancers. Breast cancer, one of the principal neoplasms that metastasise to bone causing extensive destruction by osteoclasts, is associated with an abundant secretion of osteopontin. However the mechanism by which cancer cells interact with osteoclasts is not fully understood. Serum TRAP is a marker of metastatic bone disease in breast cancer patients and can be used to monitor its response to treatment.

Our aim in this study was to investigate TRAP in breast cancer, to determine if TRAP is expressed by breast cancer cells. Breast cancer cell lines MCF-7, T47-D, MDA-MB-435 were used for experiments. Cell line Hb4a derived from normal human mammary epithelial cells was used as a control. Cells were cultured and lysates assayed for TRAP activity using p-nitrophenyl phosphate as the substrate. The MDA-MB-435 cell line had an activity of 114 nmoles/mg/min, which was 2 fold higher than the other cell lines. Immunohistochemistry using an antibody that specifically recognises TRAP showed positive staining in all cell lines compared with non-immune controls. We conclude that breast cancer cells do express TRAP and initial studies show that activity is increased in cells that are more tumourigenic.

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191 POSTER

Analysis of cell growth inhibitory effects of antineoplaston through MAPK in human breast cancer cell line SKBR-3

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We have investigated the cell growth inhibitory effects of antineoplaston that are naturally occurring peptides and amino acid derivatives on the human breast cancer cell line SKBR-3, and the mechanism of its action, with emphasis on the cell cycle and mitogen-activated protein kinases (MAPK). A significant dosage-dependently growth inhibition was observed after treatment with antineoplaston. At 48 hours after the