295 PUBLICATION

Fatty acid synthase expression in breast carcinoma patients. Correlation with hormone receptors

I. Nakamura[1], I. Kimijima[1], G.J. Zhang[1], H. Onogi[1], Y. Endo[1], S. Suzuki[1], A. Tuchiya[1], S. Takenoshita[1]. [1]*Fukushima Medical University, surgery II, Fukushima, Japan*

Purpose: Recent studies have demonstrated that high expression of fatty acid synthase (FAS) occurs in a variety of cancers including the breast cancer. The aim of this study was to compare FAS expression with the clinicopathologic and clinical outcome in breast cancer.

Methods: We evaluated 243 primary breast cancer in the period between 1989 and 1994. Immunohistochemical staining for FAS was performed on formaline-fixed, paraffin-embedded sections. FAS staining was graded for intensity as low or high.

Results: The expression of FAS was high in 145 (60%), low in 98 cases (40%). High FAS was associated with positive estrogen receptor (ER) ($p = 0.0022$) and positive progesterone receptor (PgR) ($p = 0.0085$) status. Furthermore, these finding were significantly shown in premenopausal cases than postmenopausal ones. The survival analysis showed that FAS staining intensity was not significantly associated with a high risk of recurrence in overall cases. However, we found that high FAS expression significantly related to shorter disease-free survival in ER positive cases ($p = 0.018$) and also in PgR positive cases with a borderline significance ($p = 0.057$). There was no significant association with FAS expression in both receptor negative cases ($n = 89$), while the low FAS was associated with longer disease-free survival in remaining 154 cases ($p = 0.012$).

Conclusions: This study showed close correlations between FAS expression and ER and PgR. FAS expression can be a useful tool for assessing hormonal responsiveness. It might be a possible predictor of response to endocrine therapy, especially in steroid hormone receptor positive breast cancers.

296 PUBLICATION

Altered expression of E-cadherin in breast cancer: Patterns, mechanisms and clinical significance

K.S. Ásgeirsson[1], J.G. Jónasson[2], L. Tryggvadóttir[3], K. Ólafsdóttir[2], S. Ingvarsson[2], H.M. Ögmundsdóttir[1]. [1]*Molecular and Cell Biology Laboratory, Icelandic Cancer Society;* [2]*Department of Pathology, National University Hospital, Reykjavík;* [3]*Icelandic Cancer Registry, Icelandic Cancer Society, Iceland*

Altered E-cadherin expression has been associated with increased invasiveness, metastatis formation and poor prognosis in various malignancies. In the present study, the primary tumours from 120 breast cancer patients were evaluated by immunohistochemistry using the monoclonal antibody 5H9. Possible mechanisms of altered expression were looked at by PCR. Evaluation for LOH was possible on 90 samples using two markers close to the E-cadherin gene. Finally, the clinical outcome was ascertained with a mean follow-up of 49.7 months for 109 patients. Our results show that 18 out of 97 (18.5%) of infiltrating ductal carcinomas showed complete loss of E-cadherin expression compared with with 9 out of 14 (64.3%) infiltrating lobular carcinomas. LOH was detected in 21 out of 50 (42%) infiltrating ductal carcinomas and 6 out of 8 (75%) infiltrating lobular carcinomas. LOH was associated with complete loss of E-cadherin expression in the infiltrating lobular carcinomas but not in the infiltrating ductal carcinomas. In the infiltrating lobular carcinomas, complete loss of surface E-cadherin expression and LOH was accompanied by cytoplasmic expression for E-cadherin. Univariate and multivariate analysis showed that loss of E-cadherin expression was associated with a shorter disease-free survival in node-negative breast cancer patients ($p = 0.05$ and 0.035). We conclude that different mechanisms are involved in the altered E-cadherin expression seen in different subtypes of breast carcinomas. The results implicate E-cadherin as a possible independent prognostic marker for disease recurrence in node-negative breast cancer patients, irrespective of histological type.

297 PUBLICATION

Plasminogen activator inhibitor type 2: Expression and prognostic value in primary breast cancer

N. Fersis[1], A. Ruja[1], Y.-L. Kuo[2], H. Friese[2], S. Thron[2], G. Bastert[2], D. Wallwiener[1]. [1]*Gynecology and Obstetrics University of Tuebingen;* [2]*Heidelberg, Germany*

Objective: The aim of this study was to assess the association of plasminogen activator inhibitor 2 with etablished prognostic factors and survival in tumor extracts of primary breast cancer.

Material and Methods: The study was performed on patients ($n = 252$) with operable breast cancer. Tissue from benign breast lesions ($n = 20$) served as control.

Results: The PAI-2 median value (2.43 ng/mg protein) was significantly higher ($p < 0.001$) in the cancer samples than in the extracts of the control group (0.19 ng/mg protein). Tissue extracts from invasive ductal breast cancer showed higher levels of PAI-2 (2.64 ng/mg) than invasive lobular breast cancer ($p = 0.037$). Patients without lymph-node involvement had significant higher levels of PAI-2 (2.82 ng/mg) than patients with lymph-node metastasis (2.30 ng/mg) at the time of the surgery ($p = 0.015$). After a follow-up of 44 months (range 11–57 months) we found that patients with low levels of PAI-2 had a shorter overall survival ($p = 0.140$).

Conclussion: Measurment of PAI-2 is prognosticator for breast cancer and high levels of PAI-2 antigen exercise a protective function in tumor metastasis.

298 PUBLICATION

Quantitative analysis of circulating tumour cells in breast cancer patients using reverse transcriptase polymerase chain reaction

I.H.N. Wong, W. Yeo, A.T. Chan, P.J. Johnson. *Chinese University of Hong Kong, Prince of Wales Hospital, Department of Anatomical and Cellular Pathology, Shatin, NT; Chinese University of Hong Kong, Prince of Wales Hospital, Department of Clinical Oncology, Shatin, NT, China*

Purpose: The major cause of morbidity and mortality in cancer patients is metastatic spread of the tumour to secondary sites. To monitor the dissemination of tumour cells, we study the feasibility of detecting tumour cells in the peripheral blood of breast cancer patients by reverse transcriptase polymerase chain reaction (RT-PCR) using cytokeratin 19 (CK19) and carcinoembryonic antigen (CEA) mRNAs as molecular markers. We aim to develop a semiquantitative method for distinguishing levels of the mRNA markers between breast cancer patients and healthy subjects.

Methods: We have analyzed peripheral blood samples from 36 female breast cancer patients and 29 healthy subjects. The levels of CK19 and CEA mRNAs were quantified by semiquantitative RT-PCR with reference to a breast cancer cell line.

Results: We detected variable CK19 mRNA levels in the peripheral blood from 6 of 29 healthy subjects and 11 of 36 breast cancer patients. The normal upper reference range was found exceeded by 7 breast cancer patients. Different CEA mRNA levels were also detected in 28 of 29 healthy subjects and 33 of 36 breast cancer patients and The normal upper reference range for CEA mRNA was found exceeded by 3 breast cancer patients.

Conclusion: We have developed semi-quantitative RT-PCR for distinguishing the CK19 and CEA mRNA levels in the peripheral blood between breast cancer patients and healthy subjects. Much higher CK19 and CEA mRNA levels concomitantly detected in the seven breast cancer patients appeared to originate from circulating tumour cells. Low CK19 and CEA mRNA levels detected in healthy subjects were possibly attributed to skin contamination in the peripheral blood caused by needle aspiration and/or "illegitimate" transcription. This suggests the importance of our quantitative approach in monitoring the levels of mRNA markers for risk assessment and prognostic indication.

Acknowledgement: This research project is supported by the Direct Grant for Research from the Chinese University of Hong Kong.

299 PUBLICATION

Breast cancer: Does tumour location influence survival and cosmesis? Experience from 644 patients (1984–1995)

J. Hammer, C. Track, D.H. Seewald, J.P. Zoidl, W. Labeck, E. Putz, B. Gruy. *Barmherzige Schwestern Hospital, Department of Radiotherapy, Linz, Austria*

Purpose: The authors demonstrate the unfavourable survival rate in patients with medially and centrally located breast tumours (m/c) compared to patients with cancer in the lateral quadrants (lat).

Methods: From 1984 to 1995 644 patients with 649 T1–2 tumours were treated. 429 presented with lat and 220 with m/c. Treatment method included breast conserving surgery and radiotherapy (45 to 50 Gy) and one interstitial 10 Gy boost. All axillary nodal positive patients underwent systemic therapy (6 × CMF or tamoxifen). Mean follow up of survivors: 77 months (25 to 158). From the first 216 patients the cosmetic results were evaluated using a 4 grade scoring system.

Results: Comparing m/c and lat the survival parameters were highly significant in favour of the lateral tumours (p-values: OS 0.0011, DSS 0.009, DFS 0.0001, LC 0.051). Cosmetic results after surgery were 1.65 in lat and 2.15 in m/c ($p < 0.005$). These values hadn't changed 5 years after RT with 1.69 and 2.13 respectively ($p < 0.025$).

Conclusion: The medial and central tumour location in the breast is associated with lower survival rates and unfavourable cosmetic results. The reason may lie in the fact that the pathological stage of the internal mammary chain is unknown, while in lateral tumours all patients with positive axillary nodes underwent systemic therapy and (in part) supraclavicular irradiation. The difference in the cosmetic outcome may lie in the fact that the medial part presents with a smaller tissue volume than the lateral half.

300 PUBLICATION

Relationship between insulin-like growth factor 1 (IGF-1), prolactin (PRL), human growth hormone (hGH) and steroid receptors in breast cancer patients

L. Delgado[1], O. Alonso[2], I. Alonso[1], N. Artagaveytia[3], B. Alvarez[2], G. Sabini[1], R. Roca[3], I.M. Musé[1]. [1]*Clinical Hospital of the University of Uruguay, Department of Clinical Oncology, Montevideo;* [2]*Clinical Hospital of the University of Uruguay, Department of Nuclear Medecine, Montevideo;* [3]*Clinical Hospital of the University of Uruguay, Department of Medicine, Montevideo, Uruguay*

IGF-1, PRL and hGH, seem to play an important role in the growth regulation of breast cancer.

The aim of this study was to evaluate if estrogen and progesterone receptors contents (ER, PR) of breast carcinomas were related to serum levels of PRL, hGH, and IGF-1. We studied 90 patients with primary breast cancer. Preoperative serum measurements of PRL, hGH, and IGF-1 were performed by RIA. ER and PR tumor levels were determined by binding assay using radioactive ligands (DCC method). Statistical association was assessed by the Spearman test. We found a significant negative correlation between PRL and ER levels ($p = 0.011$). Furthermore, a significant negative correlation was also observed between IGF-1 and ER levels ($p = 0.002$), and with PR levels ($p = 0.018$). This findings are in favour of the importance of PRL in ER regulation and suggest a possible role for IGF-1 in the regulation of both receptors in breast cancer patients. Therefore, both PRL and IGF-1 serum levels may be factors to be considered when evaluating hormone sensitivity in breast cancer patients.

301 PUBLICATION

Expression of cyclin dependent kinase inhibitor protein p27kip in localized invasive ductal carcinoma of the breast

H. Kourea[1], A. Koutras[1], V. Zolota[1], M. Marangos[1], E. Tzorakoeleutherakis[1], D. Koukouras[1], H. Kalofonos[1]. [1]*University of Patras, Medical School, University Hospital, Patras, Greece*

Purpose: Expression of p27KIP cyclin-dependent kinase inhibitor, a negative cell cycle regulator, was studied in a series of localized invasive ductal breast carcinoma and correlated with clinicopathological parameters and outcome.

Methods: 103 invasive ductal breast carcinomas, T1 and T2, N0, M0 were reviewed. Formalin-fixed, paraffin-embedded normal (n = 87) and neoplastic (n = 103) tissue samples were studied by immunohistochemistry for p27KIP. Samples were considered positive if >/= of 50% of tumor nuclei showed immunoreactivity. The findings were correlated to standard clinicopathological parameters and outcome.

Results: 43% of cases were T1 and 57% were T2. Disease free survival (DFS) and overall survival (OS) were both 5–144 months (mean 47 and 49 months, respectively), p27KIP expression in tumor and normal tissue was noted in 35% and 62% of cases respectively ($P = 0.045$). P27KIP expression was positively correlated with estrogen (ER) and progesterone (PR) receptor status ($P = 0.0004$ and $P = 0.05$, respectively). Loss of p27KIP was associated with higher tumor grade ($p = 0.02$). No association with tumor size, DFS and OS was observed.

Conclusions: Localized, early stage invasive ductal carcinomas tend to lose p27KIP expression compared to benign breast epithelium. Although loss of p27KIP is correlated with higher tumor grade and negative ER and PR status in these tumors, loss of p27KIP does not appear to adversely affect their prognosis. This finding is contrary to previously reported series of invasive breast carcinoma of all stages, emphasizing the biological differences among the various tumor stages.

302 PUBLICATION

Circulating antibodies against a breast tumor antigen

R. Pasquinelli[1], I. Capasso[2], M. D'Aiuto[2], P. Barba[3], A.M. Anzisi[1], G. D'Aiuto[2], J. Guardiola[3]. [1]*INT – Oncologia E;* [2]*INT – Chirurgia A, Senologia;* [3]*CNR, Istituto Internazionale di Genetica e Biofisica, Napoli, Italy*

Purpose: Determination of circulating antibodies against tumor neoantigens is important for the development of preventive or therapeutic vaccines. We report here the finding of antibodies against the breast tumor antigen, GCDFP-15gp 17.

Methods: An ELISA assay in which GCDFP-15/gp17 was used as a substrate was developped. Sera from three groups of patients were then examined (Group A including 73 breast carcinoma patients; Group B including 38 patients carrying benign breast conditions and Group C including 16 controls.

Results: Patients were considered positive when the amount of anti-GCDFP-15gp17 circulating Ab present was above the average value observed for control group C + 3× standard deviation. 5.5% of patients with breast carcinoma and 2% of patients with benign diseases expressed these antibodies. The specificity of circulating Ab was determined by competition with an anti-GCDFP-15gp17 mAb and their IgG and IgM isotypes were also assessed.

Conclusions: Patients with breast carcinoma or benign lesions secreting GCDFP-15gp17 exhibit a humoral immune reaction against the tumor antigen. The presence of IgM and IgG isotypes indicates that this response is mediated by T helper cells and suggests an approach to breast tumor vaccination.

303 PUBLICATION

Correlation between proliferating cell nuclear antigen (PCNA) and p53 protein expression in breast carcinomas. Can they have a prognostic value?

L. Lazar[1], Ioana Berindan[2], O. Balacescu[2], Rodica Risca[2], E. Neagoe[1], G. Lazar[1]. [1]*Surgical Department – Oncological Institute, Cluj-Napoca;* [2]*Tumor Biology Department-Oncological Institute, Cluj-Napoca, Romania*

Purpose: The correlation between overexpression of PCNA and p53 protein in different stages of breast carcinomas together with histopathological parameters were studied, for establishing a prognostic value.

Methods: 93 patients with in situ and invasive breast carcinomas were studied for both PCNA and p53 overexpression. Correlation with tumor size, histological and malignancy grade and the lymph node status was done. The study was made on paraffin-embedded tissues; (MoAb DO-7 from Dako, for p53 and MoAb PC10 for PCNA) from Boehringen-Mannheim,. The scale of positive PCNA tumor cells was between 0–3.

Results: a number of 52/93 (55.9%) revealed in different grades positive reaction for PCNA, and 80/93 (86%) revealed overexpression of p53 protein. Positive reaction for PCNA was associated with invasive tumor size, lymph-node metastasis and high malignancy grade. 54% from invasive carcinomas were p53 positive. We had 20% from positive PCNA cases marked on scale 1 (SI: weak) 18% on scale 2 (SI: moderate) and 12% on scale 3 (SI: strong).

Conclusion: our results reveal that high PCNA immunoreactivity and overexpression of p53 can be associated with poor prognosis (PCNA and p53 distributed in invasion areas). This study demonstrate that both PCNA and p53 protein have no independent prognostic significance but if they are correlated with histological parameters, they can become of great interest in the prognosis.

304 PUBLICATION

Increased E-cadherin and keratin 18 expression is associated with better prognosis in patients with human breast cancer

Y. Glienke, I. Fuchs[1], H. Bühler, A. Lorenz, F. Opri, H. Voss[2], G. Schaller. [2]*Dep. of Gyn. and Inst. of Pathology of the Medical Center, Free University Berlin and Dep. of Gyn.;* [1]*Charité Humboldt University Berlin, Germany*

Purpose: Besides classical prognostic factors in Human Breast Cancer i.e. lymph node status, tumor size/grading and estrogen (ER) and progesterone (PE) status, the value of adhesion and cytoskeletal proteins as inhibitors of metastasis are largely underestimated. Therefore an immunohistochemical examination for E-cadherin (E-cad) and keratin 18 (K18) was performed to