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Estrogen receptor negative breast cancers express estrogen receptor mRNA

A. Arnaout[1], C. McMahon[1], A. Seth[1], J. Slingerland[2]. [1]*Sunnybrook Hospital, University of Toronto, Medical Biophysics, Halifax, Canada;* [2]*University of Miami, Breast Cancer Research, Miami, USA*

Background: Approximately one third of primary human breast cancers do not express the estrogen receptor gene alpha (α) and are classified as ER-negative. Compared to ER-positive breast cancers, these cancers have a worse prognosis and limited treatment options. The ER-negative status of breast cancers is ascertained by ER protein assays. Very few studies have assayed ERα gene expression in this aggressive form of breast cancer. In this study, we have assayed ER mRNA levels in a large cohort of archival primary human breast carcinomas to further elucidate the mechanisms leading to the ER-negative phenotype.

Materials and Methods: We examined the relationship between ERα mRNA expression using quantitative real time PCR and ER protein status as obtained by cytosolic assays in 250 archival primary human breast tumors. High quality RNA was extracted from 250 primary tumors that have been cryopreserved for up to nine years. RNA quality was verified by gel electrophoresis and visualization of ribosomal bands, by OD260/280 ratios, and by amplification of housekeeping genes. Quantitative real time PCR using the Light Cycler system was used to determine ER mRNA concentrations for all tumors.

Results: All of the 200 ER-negative tumors expressed ERα mRNA at levels that significantly overlapped those of the 50 ER-positive tumors. The mean ER mRNA concentrations for the ER-positive and ER-negative tumors were 1.14×10^3 fmol/μg RNA and 1.27×10^3 μg RNA respectively. The lowest and highest ER mRNA concentrations were similar and the mean ER mRNA values did not differ significantly between the two breast cancer groups ($p>0.50$). Quantitative PCR of housekeeping gene h-PBGD in the ER-positive and ER-negative tumors showed similar starting RNA quantities and qualities in the two groups. This was further demonstrated on agarose gel electrophoresis.

Conclusions: Thus, the lack of ERα protein expression in ER-negative breast cancers is not due to a lack of ERα gene expression but is due to post-transcriptional mechanisms. Increasing evidence links ligand-activated ERα dependent gene transcription with ER proteolysis. The presence of ERα gene expression in ER-negative breast cancers may explain why some of these cancers respond to tamoxifen. Our data raise the possibility that ER-negative breast cancers are not estrogen independent for growth.

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Mammography screening pathology, a new challenge for the histopathologist – a two year review on the first mammography screening project in Germany

G.E. Gohla. *Central Hospital Bremen-North, Pathology, Bremen, Germany*

Purpose: The aim of this investigation was to critically review the role of the histopathologist in a mammography screening project.

Methods and Materials: The first model mammography screening project in Germany has been running in Bremen for the last two years. All women between 50 and 70 years of age have been offered mammography and any suspect findings were investigated by core biopsy. These cases were reviewed in the Pathology Dept. of the University Muenster and discussed at a weekly multidisciplinary meeting. Lesions were classified using the 5 point B classification scale as recommended by the European Union.

Results: In the first two years of the screening project, a total of 22,000 women had mammography. Suspicious areas were detected and core biopsies were obtained from 401 of these women.

46% were classified B5 (malignant) 46% of these cases were classified B1,2 (regular breast tissue, benign) and 8% of these cases were classified B3 and B4 (atypia probably benign, suspicious of malignancy). In 90% of all cases there was agreement amongst the reviewing histopathologists, but in 10% there was no interobserver consensus. These disagreements centered on flat epithelial atypia, atypically hyperplasia and papillary lesions.

Conclusions: The consensus amongst experienced breast histopathologists is satisfactory in clearcut cases, but it should be improved in less well classified lesions. These lesions are more likely to be seen in a screening situation and in core biopsies. In this area it is certainly advisable to cooperate closely with the clinician and radiologist.

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The complex between urokinase plasminogen activator (uPA) and its type-1 inhibitor (PAI-1) denotes poor disease outcome in 576 node-negative breast cancer patients

P. Manders[1], L. Beex[1], V. Tjan-Heijnen[1], P. Span[2], N. Grebenchtchikow[2], J. Geurts-Moespot[2], T. van Tienhoven[2], C. Sweep[2]. [1]*UMC Nijmegen, medical oncology 550, Nijmegen, The Netherlands;* [2]*UMC Nijmegen, chemical endocrinology, Nijmegen, The Netherlands*

Background: Members of the plasminogen activation system, e.g. urokinase-type plasminogen activator (uPA) and its main inhibitor (PAI-1), are involved in tumor growth and dissemination. Both uPA and PAI-1 are established prognostic factors in breast cancer. In this study we investigated whether the complex of uPA with PAI-1 is also associated with the natural course of the disease.

Material and Methods: The levels of uPA, PAI-1 and uPA:PAI-1 complex were measured by our in house ELISAs in tumor tissue of 576 patients (median follow up = 61 months) with node-negative invasive breast cancer. Patients did not receive adjuvant systemic therapy. Univariate and multivariate survival analyses with well-known clinicopathological factors were performed.

Results: uPA:PAI-1 complex levels were associated with adverse histological grade and inversely associated with estrogen and progesterone receptor status. In univariate survival analyses, increasing levels of uPA:PAI-1 complex were associated with a reduced relapse-free survival (RFS) and overall survival (OS, P<0.001 for both). In multivariate analyses, uPA:PAI-1 complex level was an independent prognostic factor for OS (P=0.039), but not for RFS (P=0.240). However, when uPA and PAI-1 were not included in this analysis, uPA:PAI-1 complex reached statistical significance for both RFS and OS (P=0.029 and P=0.007, respectively).

Conclusions: uPA:PAI-1 complex levels have a strong prognostic value in univariate analysis of RFS and OS in breast cancer. This prognostic value persisted in multivariate analysis with established clinicopathological factors. When uPA and PAI-1 are included in the multivariate analysis, uPA:PAI-1 complex has still independent prognostic value for OS, suggesting an interaction of the complex with progression of metastasis or with therapy.

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Predictive value of activated tyrosine kinase (Tyr1248) in patients with HER2-overexpressing metastatic breast cancer (MBC) treated with trastuzumab + chemotherapy regimens

R. Giuliani[1], M. Paesmans[1], V. Durbecq[1], A. Di Leo[1], D. Larsimont[1], V. Cocquyt[2], F. Majois[3], C. Sotiriou[1], M.J. Piccart[1], F. Cardoso[1]. [1]*Jules Bordet Institute, Translational Research Unit, Brussels, Belgium;* [2]*University Hospital, Medical Oncology, Ghent, Belgium;* [3]*Jolimont Hospital, Medical Oncology, Haine-St-Paule, Belgium*

Background: The activation of HER2 receptor through the autophosphorylation of its tyrosine residues, mainly the Tyr1248, has been regarded as one of the possible key points at which *de novo* resistance to single agent trastuzumab (H) could occur. Results from our previous experience with H given as single agent to pts with MBC did not show any statistically significant imbalance in the clinical outcome according to the phosphorylated status of HER2 (pHER2). The aim of this study was to investigate the role of pHER2 when H is given in combination with chemotherapy (CT).

Patients and Methods: Fifty eight pts received H with paclitaxel (38 pts), docetaxel (10 pts), vinorelbine (8 pts), cisplatin (1 pt) and capecitabine (1 pt) within a compassionate use program of H in Belgium. Their medical files were reviewed and the archival samples were centralised in order to assess the status of HER2 by FISH (Path-Vysion probe, Vysis, Illinois, USA), or by IHC (moAb CB-11, Novocastra, Newcastle, UK), and HER2 phosphorylation in the Tyr1248 (clone PN2A, Neomarker, CA, USA).

Results: Median age of the study population was 51 years (29–76), ECOG PS 1 (0–3). Twenty two of 58 pts (40%) had received adjuvant hormonotherapy (HT) and 38 (65.5%) adjuvant CT. Median number of metastatic sites was 2 (range 1–4) and 40 pts (69%) had visceral involvement. Median number of lines for advanced disease was 1 for HT and 2 for CT. Median duration of H-CT treatment was 8.1 months (0.5–35.2 months). Ten pts had a complete response (17.2%), and 20 a partial response (34.5%), for an overall response rate of 51.7%. Stable disease was documented in 17 pts (29.3%), progressive disease in 10 pts (17.2%), while in one patient response to treatment was not assessable. Actuarial median time to progression was 6.1 months, actuarial median survival time was not reached, with a survival rate of 75% of pts at 13 months. Fifty four pts fulfilled the criteria to enter the analysis (evaluable for response, amplification/overexpression of HER2, available pHER2). A statistically significant difference (p=0.03) in response rate between pts with pHER2+