for changes from HER-2/neu negative primaries to HER-2/neu positive metastases (17.9% of pairs) as compared to reverse changes from HER-2/neu positive primaries to HER-2/neu negative metastases (6.4% of pairs) showed a p-value of p=0.063 in favour of preferential changes from negative primaries to positive metastases, however just missing statistical significance.

Conclusions: Clonal changes between primary breast carcinomas and their distant metastases are more frequent than generally assumed, but do not question the current practice of selecting patients for anti-HER-2/neu targeted therapies. As demonstrated in a previous study on the correlation of the HER-2/neu tissue status and the serum HER-2/neu level at stage IV disease, a simple option to reassure the current HER-2/neu status would be serum testing for HER-2/neu with a level >50 ng/ml (normal < 15 ng/ml) indicating HER-2/neu positive metastatic spread.

164 POSTER HIGHLIGHT

Role of CYP17 and SULT1A1 gene polymorphisms in breast cancer

B. Rajan[1], P. Chacko[2], M. Pillai[2]. [1]*Regional Cancer Centre, Radiation Oncology, Trivandrum, India;* [2]*Regional Cancer Centre, Laboratory Medicine&Tumour Biology, Trivandrum, India*

The common risk factors for developing breast cancer such as early age at menarche, late first full-term pregnancy, nulliparity, late menopause, family history of breast cancer and socioeconomic status are all a result of cumulative life time exposure to estrogen. Many of the enzymes involved in estrogen metabolism/biosynthesis are polymorphically distributed within human populations. Investigating the distribution of these functionally relevant genetic polymorphisms that alter the bioavailability of steroid hormone among individuals may provide a more direct evidence for estrogen and estrogen metabolites as modifiers of breast cancer susceptibility. The CYP17 gene encode for a CYP P450 C17 alpha enzyme which functions at key branch point in human steroidogenesis. The polymorphic variant to this gene (A2 allele) shows enhanced transcriptional activity due to creation of an Sp-1 promoter motif and may therefore influence breast cancer risk by increasing estrogen hormone level. The SULT1A1 gene encodes for the sulfotransferase ensyme that plays an important role in the inactivation of endogenous estrogens and biotransformation of environmental mammary carcinogens. Sulfotransferases are also found to regulate the metabolism of tamoxifen, a potent antiestrogen and a chemo preventice against breast cancer. Large interindividual variation observed both in the enzyme levels and activity of the sulfotransfereases are mainly due to the genetic polymorphisms of the SULT gene which may ultimately influence the individual susceptibility of breast cancer. The current study evaluated the role of genetic polymorphisms of these estrogen related genes-SULT1A1 and CYP17 in breast cancer susceptibility by a case-control study. In addition the relationship between the estrogen biosynthesizing CYP17 gene polymorphisim and serum estradiol levels were also analyzed. The genotype assay was done by PCR-RFLP assay and serum estradiol levels were measured by ELISA. Our data showed a significant positive association between the CYP17 (OR=2.16; 95%CI=1.23–3.79; p=0.007) and SULT1A1 (OR=1.78, 95%CI=1.09–2.89, p=0.02) gene polymorphisms and breast cancer. Our data also showed evidence for the genetic regulation of serum estradiol levels among premenopausal women with a significant 2.57 fold increase in the serum 17 beta estradiol level for the CYP17 homozygous polymorphic variant genotypes. The results from our study suggests that analysis of functionally relevant polymorphisms in these low penetrance genes would exhibit additive effects on individual susceptibility to breast cancer by influencing lifetime levels and metabolisms of estrogen. We are currently investigating the role of these genes in individual response to tamoxifen. Such a genotype analysis study holds considerable promise for individualizing diagnosis, screening and therapeutic intervention.

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The correlation between serum and normal breast tissue Insulin-like Growth Factor (IGF-I) system components

A. Vrieling[1], D.W. Voskuil[1,2], J.M.G.M. Bonfrer[3], C.M. Korse[3], A.J. Bosma[1], L.J. van 't Veer[4]. [1]*Netherlands Cancer Institute, Division of Experimental Therapy, Amsterdam, The Netherlands;* [2]*Netherlands Cancer Institute, Division of Psychosocial Research and Epidemiology, Amsterdam, The Netherlands;* [3]*Netherlands Cancer Institute, Department of Clinical Chemistry, Amsterdam, The Netherlands;* [4]*Netherlands Cancer Institute, Department of Pathology, Amsterdam, The Netherlands*

IGF-I is an important growth factor and has been associated with increased breast cancer risk in both prospective epidemiological (serum) and experimental studies (tissue). It is suggested that the association between serum IGF-I and breast cancer risk is especially seen in premenopausal women, and in women with a strong family history of breast cancer. In serum, most IGF-I is bound to IGF Binding Protein-3 (IGFBP-3). Both are mainly produced in the liver after stimulation by growth hormone. IGF system components are also locally produced in tissues (e.g. breast tissue), where IGF-I can exert its tumor promoting effect by binding to the IGF type 1 receptor (IGF-IR). In a previous study, we observed a large variation in mRNA expression of IGF-I, IGF-II, IGF-IR, and IGF-IIR in normal breast tissue. We also observed a higher expression of some IGF system components in breast tissue from women with a positive family history than in women without such a history. However, little is known about the relation between serum concentrations and normal breast tissue expression of IGF system components. Therefore, the objective of the present study was to investigate whether the serum concentration of IGF-I is correlated with mRNA and protein expression of different IGF system components in normal breast tissue.

We identified 153 women with a strong family history of breast cancer, who underwent a prophylactic mastectomy at the Netherlands Cancer Institute/Antoni van Leeuwenhoek hospital from 1990 to March 2001. For 29 premenopausal women, suitable snap-frozen normal breast tissue and a serum sample taken within one year before/after mastectomy were available for analysis. Tissue mRNA expression of IGF-I, IGF-II, and IGF-IR was measured by quantitative real-time RT-PCR. Serum IGF-I concentrations were measured by a radioimmunoassay.

In this preliminary series of 29 normal breast tissue samples, no significant correlation between serum IGF-I concentrations and tissue mRNA expression of IGF-I, IGF-II, and IGF-IR was observed [Spearman correlation coefficients (r): IGF-I mRNA: r = 0.02 (p=0.93), IGF-II mRNA: r = −0.01 (p=0.96), IGF-IR mRNA: r = −0.04 (p=0.84)]. As both serum and tissue concentrations of IGF system components within an individual may vary over time (due to e.g. dietary habits, menstrual cycle), we restricted these analyses to 7 women with a serum and tissue sample taken at the same day. In this subset of samples, somewhat stronger correlations were observed with serum IGF-I concentrations (IGF-I mRNA: r = 0.39 (p=0.38), IGF-II mRNA: r = −0.43 (p=0.34), IGF-IR mRNA: r = −0.54 (p=0.22)).

In conclusion, in this preliminary series no correlation between serum IGF-I and normal breast tissue IGF-I, IGF-II, and IGF-IR was observed. This series will be prospectively expanded with both pre- and postmenopausal women with serum and tissue taken at the same day. Immunohistochemistry will be performed to assess tissue protein expression of IGF system components. Additionally, serum and tissue IGF binding proteins will be measured. Results will provide more insight in the relation between serum and tissue IGF system components, and help explain the role of the IGF-system in tumor development and cancer prevention research.

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Insulin-like Growth Factor Binding Protein 3 (IGFBP-3) modifies Epidermal Growth Factor (EGF)-related breast cancer growth depending upon the extracellular matrix (ECM)

J. McIntosh, C.M. Perks, J.M.P. Holly, Z.E. Winters. *University of Bristol, Department of Surgery, Bristol, UK*

Introduction: IGFBP-3 is the most abundant IGFBP within serum and can modulate cell proliferation. Increasingly, the IGF axis is being implicated in Tamoxifen resistance as well as agents targeting the EGF pathway. Elevated serum IGFBP-3 has been associated with reduced breast cancer risk. Conversely, tumours with high local IGFBP-3 levels have been associated with a poor prognosis, as has tumour production of fibronectin. We have examined the effects of IGFBP-3 on EGF-mediated proliferation in breast epithelial cells, both in the presence and absence of fibronectin.

Methods: Normal breast and breast cancer cell lines were chosen with previously characterised responses to EGF. Cells were dosed with EGF (1 ng/ml and 25 ng/ml), IGFBP-3 (100 ng/ml) and combinations of each together. This was repeated in plastic wells that had been coated with fibronectin (0.25 μg/ml).

Results: In the normal MCF10A cells, EGF and IGFBP-3 each increased cell proliferation on their own (1.7 and 1.4 fold increase, respectively), but together synergistically enhanced cell growth relative to control (3.3 fold increase). When repeated on fibronectin, EGF increased proliferation (2.3 fold), but IGFBP-3 alone reduced proliferation (to 0.78 fold) and blocked the proliferative response to EGF (from 3.3 to 1.38 fold).

In HS578T breast cancer cells, EGF caused an increase in cell proliferation (1.5 fold), IGFBP-3 alone had no effect, but in combination with EGF, markedly inhibited EGF-mediated cell proliferation (from 1.5 to 1.1 fold). This is currently being repeated in HS578T cells on fibronectin-coated plastic. Whilst these cells still proliferate in response to EGF on fibronectin, IGFBP-3's effects change markedly, and it is seen to act as a mitogen in this environment.

Conclusions: IGFBP-3 has differential effects on EGF-mediated proliferation in normal and breast cancer epithelial cells. The results also suggest that the actions of IGFBP-3 may reverse with remodelling of the ECM, potentially explaining the conflicting data regarding the activity