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.

165 POSTER

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.

166 POSTER

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

of serum versus tumour levels of IGFBP-3. It is possible that future characterisation of breast tumours may include fibronectin and IGFBP-3 production, so that clinical response to agents targeting the EGF pathway may be predicted, resulting in a more targeted use of such therapies.

Acknowledgement: Funding from the Association of International Cancer Research.

167 POSTER

HER2 polymorphism and the risk of breast and ovarian cancer

D. Pinto[1], A. Vasconcelos[1], S. Costa[1], D. Pereira[2], H. Rodrigues[2], C. Lopes[1], R. Medeiros[1]. [1]*Portuguese Institute of oncology, Molecular Oncology, Porto, Portugal;* [2]*Portuguese Institute of oncology, Medical Oncoloy, Porto, Portugal*

Introduction: Breast cancer is a major public health problem around the world, and its carcinogenesis is not yet well understood. The Human Epidermal growth factor Receptor-2 (HER2) seems to play an important role in the development of this neoplasia, and genetic alterations in this gene, such as point mutations and polymorphisms have been detected in breast cancer patients, as well as ovarian cancer patients. The aim of our study was to analyze the frequency of a single nucleotide polymorphism in the HER2 gene in a southern European population.

Materials and Methods: The study included 152 patients with breast cancer, 139 ovarian cancer patients and a control group of 146 healthy donors. DNA extracted from peripheral blood was submitted to PCR-RFLP, in order to identify the possible HER2 genotypes; Ile/Ile, Ile/Val and Val/Val. The restriction fragments were analyzed in a 3% agarose gel, stained with ethidium bromide.

Results: A twofold increase in risk of breast cancer was found among women who are carriers of a Val allele genotype – Ile/Val and Val/Val genotypes (OR = 2.00; 95% CI: 1.23–3.25; p=0.005). As for the ovarian patients, we also found an increased risk in ovarian cancer, with an OR of 1.59 (95% CI: 0.96–2.63).

Discussion: Our results indicate an association between the presence of the Val allele in the HER2 polymorphism and the risk of breast and ovarian cancer. Further studies are needed to evaluate the role of this polymorphism in the behavior of breast and ovarian cancer.

168 POSTER

DNA diagnosis of hereditary breast and ovarian cancer in Latvia

O. Sinicka[1], A. Stengrevics[2], D. Smite[1], J. Eglitis[2], L. Tihomirova[1]. [1]*Biomedical Research and Study Centre of University, Riga, Latvia;* [2]*Latvian Oncology Center, Riga, Latvia*

Background: Breast cancer is the leading cancer site in Latvian women and ranks as the first highest cause of cancer-related death. Since the discovery of BRCA1 and BRCA2 genes, the mutation analysis of these genes is widely used for the identification of women with a high risk of breast and ovarian cancer and developing management strategies. The BRCA1 and BRCA2 mutation spectrum and frequencies vary significantly in different populations and geographic regions. Therefore the criteria for BRCA1/2 genetic testing should be optimised for each population.

The objective of this study was to develop effective BRCA1 gene mutation detection strategy in Latvia based on characterisation of mutation profile in breast and ovarian cancer patients.

Material and methods: Mutation analysis of entire BRCA1 gene was performed in DNA from 75 breast cancer patients and 30 ovarian cancer patients from Latvian Oncology Center selected by early onset of disease or family history of breast/ovarian cancer. Most of patients tested have insignificant cancer history in family. The analysis was performed by SSCP/HDA in polyacrylamide gels and automatic direct sequencing (ABI PRISM 310) of variants detected. The screening for recurrent mutations was performed as well in early onset breast/ovarian cancer patients unselected for family history.

Results: 5 different deleterious mutations have been detected by the analysis of entire BRCA1 gene. Three of mutations detected were recurrent. Missense-mutations, registered in BIC database as unclassified variants and common polymorphisms, have been found in this study as well. High proportion of mutation carriers were found in this study regardless insignificant cancer histories in families of patients tested. Altogether 20 mutation carriers were detected by the analysis entire BRCA1 gene and 28 by the screening for recurrent mutations.

Conclusions: Breast cancer diagnosed before the age 48 is suggestive for DNA testing to be offered to patients in Latvia, regardless cancer history in the family. The identification of three founder mutations in Latvian population allows rapid and cost-effective detection of mutation carriers. Further study of founder mutations could be useful for understanding the role of these mutations in the incidence of breast and ovarian cancer in order to provide individual risk assessment and to design better therapeutic strategies.

169 POSTER

Are lobular carcinomas more often steroid receptor positive?

P. Neven, H.J. Huang, M. Drijkoningen, R. Paridaens, F. Amant, P. Berteloot, I. Vergote, M. Christiaens. *UZ-Leuven, Multidisciplinary Breast Centre, B-3000 Leuven, Belgium*

Background: Invasive lobular carcinomas (LC) and invasive ductal carcinomas (DC) differ with respect to their expression of a variety of molecular tumour markers including oestrogen (ER) and progesterone receptor (PR) expression. LC are generally referred to be more likely ER and PR positive compared to DC. We analysed whether tumour grade affects differences in ER/PR expression by histologic tumour types.

Patients and Methods: Charts from 1472 consecutive female patients diagnosed with primary operable invasive breast cancer (Jan. 2000–May 2003) were reviewed, excluding those who received neoadjuvant therapy. The highest tumour grade was retained for each case and each tumour was classified according to its histological type as LC or non-lobular carcinoma (non-LC). Immunohistochemical stains for ER (antibody 6F11/2) and PR (antibody 312) were categorised using the H-score as follows: <50/300 negative; ∗ 50/300 positive.

Results: 204 (13.8%) of invasive tumours were classified as LC. LCs were more frequently ER/PR positive than non-LCs ($p<0.001$ – table 1). The great majority of LCs (85.3%) were grade 2 where only 40% of non-LC were classified as grade 2. When we only classified grade 2 tumours by receptor state, there was no difference in incidence of ER/PR positivety between LC and non-LC (table 2); differences however, were significant for grade 3 lesions.

Table 1

Type	ER-positive	PR-positive
LC (n=204)	92.7%	79.5%
non-LC (n=1268)	80.0%	62.4%

Table 2

Type	ER-positive	PR-positive
Gr 2 LC (n=174)	94.3%	79.9%
Gr 2 non-LC (n=508)	94.7%	76.0%
Gr 3 LC (n=25)	76.0%	72.0%
Gr 3 non-LC (n=535)	59.1%	42.9%

Conclusion: Regarding the frequency of positive steroid receptors in invasive breast cancer, grade 2 LCs do not differ from grade 2 non-LCs. The difference for ER/PR positivety between both histologic tumour types lies in grade 3 lesions; grade 3 LCs are more often ER/PR-positive than grade 3 non-LCs.

170 POSTER

The progesterone receptor has a prognostic value in oestrogen receptor negative breast cancers

H.J. Huang, P. Neven, M. Drijkoningen, R. Paridaens, P. Berteloot, F. Amant, I. Vergote, M. Christiaens. *On behalf of all members, Multidisciplinary Breast Centre (MBC), UZ-KULeuven, Leuven, Belgium*

Background: Considering oestrogen (ER) and progesterone receptor (PR) expression in breast cancers, the ER-negative (−ve) PR-positive (+ve) phenotype is the least common variant. Some believe that non-expression of ER in the presence of PR is a laboratory error whereas others consider this as a separate category. The aim of this study is to analyse whether prognostic factors are differently expressed in ER−ve breast cancers with or without PR expression.

Patients and methods: Charts from 1358 women who underwent primary breast surgery and complete axillary clearance for invasive breast cancer between Jan 2000 and June 2003 (excluding those who had neoadjuvant therapy and those with sentinel lymph node only) were examined. We compared age, mean tumor size, histologic type, incidence of grade 3 lesions, axillary lymph node status, HER-2/neu expression [immunohistochemical (IHC) measurement] and menopausal status between ER−ve PR+ve and ER−ve PR−ve tumours. Steroid receptors were measured by IHC using the H-score and defined negative with a score of less than 50/300.