used for this purpose. The objective of this study was to test the ability of intraoperative touch imprint cytology (IC) to predict metastatic disease on SLN.

Design: SLN were received fresh and examined grossly, when less than 0.5 cm in size were bisected and when more than 0.5 cm in size were serially sectioned at 2 mm intervals along the long axis. Each surface of the sections were touched on the glass slide, stained by H&E. Results of IC were compared with results of section permanents, wich were analyzed with 10 serial levels stained with H&E and one level stained with cytokeratin AE1/AE3. Sensitivity (Se), specificity (Sp), positive and negative predictive value (PPV & NPV) and accuracy were calculated for all metastases (macro & micrometastases), micrometastases, macrometastases. False negatives were rescreened.

Results: We analyzed 179 SLN from 110 patients. The comparison between IC and definitive results of the SLN (including macro & micrometastases) showed 139 (77.65%) true negative imprints, 28 (15.64%) true positive imprints. There were not false positive imprints, there were 12 (6.70%) false negative imprints. False negative imprints were 6 macrometastases (mean size metastases 5 mm, range 3–7 mm), 3 micrometastases (mean size metastases 1.6 mm, range 2–1.5 mm) and 3 isolated tumour cells. Rescreening of the false negative imprints showed 10 negative imprints, one imprint with two diagnostic groups of cells and one imprint with multiple diagnostic groups of cells. Se, Sp, PPV, NPV, Acc for all metastases, micrometastases, macrometastases are shown in the table.

	Se	NPV	Sp	PPV	Acc
All metastases	70%	92.05%	100%	100%	93.29%
Micrometastases	73.60%	93.37%	100%	100%	94.41%
Macrometastases	82.35%	96.02%	100%	100%	96.64%

Conclusions: The majority of macrometastases can be detected by IC however IC fails to detect most micrometastases. False negative imprints for macrometastases are mainly due to sampling error. The high Sp, PPV and preservation of the arquitecture of the lymph node for histopathologic examination are the major advantages of IC for intraoperative evaluation of SLN.

Thursday, 23 March 2006 16:00–16:45

POSTER SESSION

Tumour biology and immunology

267 Poster

Bex2 identifies a novel subtype of breast cancer associated with estrogen-response and NGF/NF-KB pathway

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Background: Heterogeneity of breast cancer is a significant challenge in diagnosis and therapy of the disease. Despite advancements in the molecular profiling of breast cancer, there are still only three known molecular subtypes which can be used as classifiers; ER+, ER−, and ERBB2+. Better molecular classification of breast cancer can improve our understanding of the disease and potentially lead to the discovery of novel therapies.

Methods: In this study we performed microarray expression analysis of 135 breast tumors to identify novel classifiers in breast cancer. In addition, we evaluated clinical and biological relevance of our findings.

Results: We identified Bex1 and 2 genes as novel classifiers of breast cancer. Overexpression of these genes was present in 15% of samples and was associated with estrogen-response and apoptotic function. We showed Bex2 expression is necessary and sufficient for NGF anti-apoptotic activity. Moreover, Bex2 induction is mediated through p75NTR and located upstream of NF-κB. Furthermore, estrogen induces Bex2 in a time and dose dependent fashion and Bex2 is necessary for estrogen mediated anti-apoptotic activity. We also found cases with Bex2 overexpression responded better to tamoxifen therapy and proved the interaction between Bex2 and tamoxifen activity in breast cancer cells.

Conclusion: Although the importance of NGF/Bex3/NF-κB pathway is well known in neural tissues, NGF has recently been implicated in pathogenesis of breast cancer as well. Importantly, the function of Bex1 and 2 remains virtually unknown to date. Here, we show Bex1 and 2 classify a novel subtype of ER positive breast tumors which respond better to tamoxifen therapy. We demonstrate Bex2 is part of estrogen response and NGF/NF-κB pathways with anti-apoptotic function in breast cancer. NF-κB activity has recently gained much attention in the development of hormone refractory breast cancer and Bex2 can potentially be applied as an activity marker or therapeutic target within this pathway. The findings reported here show Bex1 and 2 are novel breast cancer-related genes and significantly advance our understanding of NGF/NF-κB pathway with potential clinical implications.

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The prognostic significance of inflammation in invasive carcinoma of the breast

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The prognostic significance of inflammation in invasive carcinoma of the breast is controversial with previous studies producing conflicting results. The predominant pattern of inflammation is a diffuse infiltrate of T cells and macrophages in the stroma between carcinoma cells. Perivascular and perilobular clusters of B and T cells are less prominent. The cells necessary for a cell-mediated immune response are often present, but there is evidence that their function is impaired. Inflammatory cells may also stimulate tumour growth by release of proleolytic enzymes or angiogenic factors. 1599 patients aged less than 71 years with operable invasive carcinomas, diagnosed from 1974 to 1988, with median follow up 9.4 years were studied. No patient received adjuvant systemic treatment. An overall assessment of the intensity of lympho-histiocytic inflammation was made on haematoxylin and eosin sections by one observer. Inflammation was associated with higher grade, ductal and medullary histological types, tumour size and inversely with patient age. On univariate analysis patients with tumours with marked or moderate inflammation had a better survival than patients with tumours with absent or mild inflammation ($P = 0.04$). On multivariate analysis survival was associated with inflammation (relative risk 0.61 (95% confidence intervals 0.47 to 0.79), $P = 0.0002$) in addition to lymph node stage, histological grade, tumour size, vascular invasion and tumour type; survival was not related to patient age or oestrogen receptor status. This study suggests that the anti-tumour effects of inflammation predominate over the pro-tumour effects. Critical review of previous large studies with assessment of histological grade and multivariate analysis shows that the majority find prominent inflammation is associated with a better prognosis, consistent with the present study. These results support further studies trying to harness the immune response in the treatment of breast cancer.

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Identification of cell-of-origin subtypes and a wound healing response signature in inflammatory breast cancer

S. Van Laere[1], G. Van den Eynden[1], I. Van der Auwera[1], V. Huygelen[1], H. Elst[1], C. Colpaert[1], P. van Dam[1], E. Van Marck[1], P. Vermeulen[1], L. Dirix[1]. [1]*Translational Cancer Research Group, (Lab Pathology University of Antwerp and Oncology Center, GH Sint-Augustinus), Wilrijk, Belgium*

Introduction: Recently, gene expression studies demonstrated the significance of different biological breast cancer subtypes with regard to prognosis and treatment. In this study we tested to what extent these subtypes contribute to the specific inflammatory breast cancer phenotype (IBC).

Materials and Methods: The presence of different cell-of-origin subtypes (Perou et al.) and of a wound healing signature (Chang et al.) was analyzed in gene expression data sets from 16 IBC and 18 nIBC specimens. A set was compiled of genes being part of respectively the intrinsic gene set (Perou et al.) and the wound healing response signature (Chang et al.) which were also present on the cDNA microarrays used to compare IBC and nIBC specimens (Van Laere et al). 144 and 98 genes were selected from both gene lists. These gene lists were then tested for performance in the original data sets. Next, centroids for each cell-of-origin subtype and for the quiescent and activated fibroblast signature were calculated. These centroids were than used to classify our specimens. For the cell-of-origin subtype classification, the robustness of the taxonomy was confirmed using an alternative data set of 141 genes related to the cell-of-origin subtypes. Contribution of each of the cell-of-origin subtypes to the IBC phenotype was tested by principle component analysis (PCA).

Results: The performance of the selected data sets was 84% and 100%, respectively. 8/16 IBC specimens belonged to the combined Basal-like and ErbB2-overexpressing cluster, compared to only 3/18 nIBC specimens

(p = 0.037). The classification was in good agreement with IHC data for ER and CK5/6. PCA revealed that only 35% of the differences between IBC and nIBC can be explained by the presence of the cell-of-origin subtypes. 12/16 IBC specimens correlated with the quiescent fibroblast signature centroid compared with 5/18 nIBC specimens (p = 0.006). When only significant correlations were taken into account, 6/6 IBC and 3/8 nIBC specimens correlated with the quiescent fibroblast signature centroid (p = 0.03). These data are currently confirmed using real-time qRT-PCR.

Discussion: These data sustain our previous findings that IBC and nIBC are two distinct biological entitities. Different cell-of-origin subtypes in IBC were identified, but cannot fully explain the specific phenotype. Other processes must determine the biology of IBC, as shown by the differential expression of the wound healing response signature.

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Effect of tibolone on breast cancer cell proliferation in postmenopausal ER+ patients: results from a double-blind, placebo-controlled, randomized clinical trial (STEM)

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Experimental design: Postmenopausal women with stage I or II, ER+ primary breast cancer, were randomly assigned to 14 days of placebo or 2.5 mg/day tibolone. Core biopsies of the primary tumor were obtained before therapy, and a representative sample of the excised tumor was obtained from the operative specimen after treatment. For each patient, Ki67 and, apoptosis index were analyzed in both baseline and the corresponding post-treatment specimen.

Results: Of 102 enrolled patients, 95 had evaluable data. Baseline characteristics were comparable between both treatment groups. Most breast cancer cases were invasive (99%), stage I or II (42% and 50% respectively) and ER+ (99%). Median intratumoral Ki67 expression at baseline was 13.0% in the tibolone group and 17.8% in the placebo group, and decreased to 12.0% after 14 days of tibolone while increasing non-significantly to 19.0% in the placebo group. Similarly, no significant differences were observed between the treatment groups when the median baseline apoptosis index (1.4% in both groups) was compared to the corresponding post-therapeutic indices of 1.6% (tibolone) and 1.7% (placebo). No differences between tibolone and placebo were observed with respect to the incidence of adverse effects.

Conclusion: 2.5 mg/day tibolone given for 14 days has no significant effect on tumor cell proliferation and apoptosis in ER+ tumors.

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Expression of FOXP3 and vascular endothelial growth factor in human breast cancer: its correlation with angiogenesis and disease progression

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Background and Objective: The Forkhead/winged helix transcription factor FOXP3 is positively associated with the induction of $CD4^+CD25^+CD45RA^-$ T regulatory cells, which have a suppressive effect on the effector T-cells. To determine whether FOXP3 might be involved in the progression of breast carcinoma, we measured the expression of FOXP3 in infiltrating breast carcinoma along with their corresponding normal breast tissues and a smaller number of ductal carcinoma *in situ* (DCIS) specimens and correlated it with the expression of Vascular Endothelial Growth Factor (VEGF, an invasogenic and angiogenic growth factor) and intratumoral microvessel density (IMD, a prognostic marker for angiogenesis).

Methods: FOXP3 and VEGF mRNA expression was semiquantitated by RT-PCR assay in 20 biopsies of infiltrating breast carcinoma, 5 biopsies of DCIS along with 20 biopsies of normal breast tissues and analyzed their correlation with each other and with the IMD, determined by immunohistochemical staining using anti-CD34 antibody. We also analyzed whether FOXP3 mRNA expression correlated with other pathological variables like tumor size, histological grade and lymph node status, the prognostic indicators of breast carcinoma.

Results: Invasive cancers had nearly three times greater FOXP3 mRNA expression than did ductal carcinoma *in situ* and nearly eight times greater than normal tissue and the difference was statistically significant ($P < 0.05$; $P < 0.02$, two tailed t-test respectively). There appeared to be a trend towards increasing FOXP3 mRNA expression with increase in tumor size, with the larger tumors (≥ 2.0 cm) having approximately two-fold higher FOXP3 expression than the smaller tumors (< 2.0 cm), although the difference was statistically insignificant. FOXP3 expression was also found to be increased in higher grade tumors ($0.05 < P < 1.0$). There was a clear trend towards increasing VEGF mRNA expression with increase in FOXP3 expression, however, the statistical comparison revealed no significance. There was no significant correlation between FOXP3 mRNA expression with IMD and lymph node status.

Conclusion: These findings suggest that the expression of FOXP3 transcription factor has a direct correlation with other clinicopathological indicators of aggressive tumor behavior, consistent with the hypothesis that FOXP3 is a biological factor that may play a role in breast cancer progression.

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Real-time RT-PCR of CD146 and VE-cadherin mRNA to detect circulating endothelial cells in peripheral blood of patients with breast cancer

I. Van der Auwera, H. Elst, V. Huygelen, S. Van Laere, G. Van den Eynden, P. van Dam, E. Van Marck, P. Vermeulen, L. Dirix. *Translational Cancer Research Group, Laboratory of Pathology University of Antwerp,Oncology Centre, GH St-Augustinus, Antwerp, Belgium*

Angiogenesis is a fundamental process in tumour growth and metastatic dissemination. The number of circulating endothelial cells (CECs) in peripheral blood (PB) of patients with cancer reflects the amount of proceeding angiogenesis and can therefore be used as a surrogate marker to monitor antiangiogenic therapy. The standard quantification method of CECs is currently based on a complex four-color flow cytometry. Real-time RT-PCR technology to quantify EC-specific mRNA in PB samples has been shown to be a promising alternative approach. This study aimed to compare mRNA expression levels of EC-specific markers (CD146 and VE-cadherin) in PB of healthy volunteers and patients with breast cancer using real-time RT-PCR.

PB samples have been collected from 18 healthy volunteers and 18 metastatic breast cancer patients. RNA was subsequently isolated with the PAXgene Blood RNA isolation kit. Real-time PCR analysis was performed with primers and TaqMan probes for both CD146 and VE-cadherin mRNA. Ct values were normalised for beta-actin mRNA expression and gene expression levels were calculated relative to a reference sample (RGE).

VE-cadherin mRNA was increased in patients with breast cancer in comparison to healthy volunteers: the median VE-cadherin mRNA expression level in PB of healthy volunteers was 1.20 (range 0.50–4.18); this was 2.45 (range 0.69–25.80) for patients with breast cancer (p = 0.040). However, the difference in CD146 mRNA expression levels between healthy volunteers and patients with breast cancer did not reach statistical significance: the median CD146 mRNA expression level in PB of healthy volunteers was 0.037 (range 0.020–0.058); this was 0.058 (range 0.013–0.488) for patients with breast cancer (p = 0.077). CD146 and VE-cadherin mRNA expressions were significantly correlated (r = 0.401, p = 0.017). A cut-off value was determined as the 95^{th} percentile of the RGE values of the healthy volunteers: this value was 0.058 for CD146 and 4.184 for VE-cadherin mRNA. 9 out of 17 patients with breast cancer had a RGE of CD146 above the cut-off value;for VE-cadherin 7 out of 18 patients with breast cancer had increased RGEs.

Our preliminary results suggest that the quantitative evaluation of EC-specific mRNA by real-time RT-PCR could indeed be a promising tool to monitor the efficiency of antiangiogenic therapy in patients with breast cancer but a larger study population and a comparison with flow cytometry is necessary to confirm this. These studies are ongoing.

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Local aromatase and sulfotransferase protein expression in malignant breast tumors vs adjacent and distant breast tissue

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The suppression of local estrogens levels is of key importance in the treatment of ER positive breast cancer. Most endocrine strategies now