invasive tumors, 77.2% were classified as Grade II and above. 23.4% of these patients had nodal involvement and the most common stages of presentation were Stage 1 (32.9%) and Stage 2 (35.7%). Majority of the tumors were oestrogen (95.5%) and progesterone (81%) receptor positive and 27.9% and 18.6% were cerbB2 score 3+ and 2+ respectively. Where treatment data was available, 90.2% patients with ER positive tumors received tamoxifen. 18.2% received adjuvant chemotherapy and 40.3% received radiation therapy. The mean follow up time was 55.4 (2–178) months. The 2-year vs. 5-year disease free and overall survival were 93.2% and 85.9% vs. 73.8% and 74.2% respectively. Interestingly 15 patients also had a second primary cancer not of breast origin. These patients had a significantly worse overall survival ($p = 0.07$).

Conclusions: Male breast cancer in Chinese men is rare and present at an old age but at an early stage (Stage 1 and 2). Although a majority of our cohort did not have family history of breast cancer, there was a high incidence of second primary cancer not of breast origin. Further investigation with genetic study in this group of patients is likely to be of relevance.

103 Poster

Body weight and the risk of breast cancer in BRCA1/2 families – the GEO-HEBON study

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In the general population, an inverse association has been observed between both body weight and body mass index (BMI) and the risk of premenopausal breast cancer, whereas body weight and BMI increase the risk of postmenopausal breast cancer. So far, association between body weight and BMI and breast cancer risk in BRCA1/2 families is unknown.

We assessed the association between body weight and breast cancer in a large series of 485 BRCA1/2 families, consisting of 918 BRCA1/2 carriers and 142 obligate carriers from the GEO-HEBON study, a retrospective nationwide cohort study. Information on hormonal/lifestyle factors was obtained from a self-administered questionnaire. Information on breast and ovarian cancer and on preventive surgical measures was verified via the PALGA database (Pathological Anatomy National Automated Archive) and the Netherlands Cancer Registry until August 2007.

Participants were asked to report current body weight and height and body weight from age 18 years onwards in 10-year age groups (ages ranging from 20−29, 30−39, 40−49, 50−59 and 60−69 years), excluding pregnancy in these periods. Current body weight and height were used to compute current BMI in kilograms per squared meters. Analyses will be preformed to examine the effect of body weight and BMI at age 18 years and censoring, changes in body weight (20 years of age till censoring), and height on breast cancer risk in BRCA1/2 families. All analyses will be stratified according to menopausal status. Hazard ratios will be estimated using a Cox-regression approach stratified for gene and birth cohort. Results will be presented.

Wednesday, 16 April 2008 12:30–14:30

POSTER SESSION

Molecular biology, markers

104 Poster

Proteomics in mammary cancer research

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Background: This study combines a summary of proteomics techniques and protein analysis (in mammary cancer) with a report of expressed and up-regulated proteins in benign and malignant mammary tissues.

Till now, only few reports of proteomic research for mammary cancer could be found, including the techniques of two-dimensional gel electrophoresis (2-DE), MALDI/ESI/TOF and ESI/MS, browsing the PubMed database. Human carcinoma cell lines, mouse material or human serum were the most exercised materials for these studies. Only a few of them used native tissues from patients with mammary carcinomas.

The aim of this study was to search for more, not well-established (up-regulated) proteins in mammary cancer in the mean and low molecular weight (MW) range, to figure out the role of post-translational modification in biologic processes and to recognize newer pathways.

Material and Methods: Tissue samples ($n = 26$), originated from 10 healthy female donors (benign mammary tissue; K01–K10; control group) and 16 donors who had developed mammary carcinomas of a ductal type (P01–P16), were snap-frozen in liquid nitrogen and stored at −70ºC till analysis. High resolution 2-DE was performed, according to the literature, using a pH gradient from 2−11. The most abundant spots representing the selected variant spot groups were manually picked in a clean bench to provide sufficient material for MALDI-MS, as well as nano-liquid chromatography-electro spray ionization-mass spectrometry (nano-LC-ESI-MS) based protein identification.

Results: Beside hypothetical proteins a number of transcription factors, such as zinc finger proteins (ZNFs), and not well-investigated (high significant) proteins, e.g., elongation factor 1-alpha 1 (eEF1A1), were identified by mass spectrometry (MS). eEF1A1, expressed in the membrane of mammary carcinomas, is involved in gene expression/translational elongation and has a GTPase activity and an oncogenic potency.

Another protein, for example, calgizzarin (S100A11; S10AB human), which is involved in carcinoma invasion and tumor metastases, could be determined as over-expressed in the cytosol of mammary cancer tissues. It is also concerned with the regulation of numerous cellular processes such as cell cycle progression and differentiation.

Conclusion: With this study, we want to demonstrate that proteome analyses provide a powerful tool for detecting potential and new biomarkers, which could be validated for diagnostic and clinical features of mammary carcinomas.

105 Poster

Approaching molecular classification of breast cancer by using a panel of molecular tumor markers

P. Sinn[1], S. Aulmann[1], P. Schirmacher[1]. [1]*University of Heidelberg, Gynecopathology, Heidelberg, Germany*

Background: Gene expression profiling has resulted in the definition of distinct and robust clusters of distinct types of breast cancer (luminal A and B, erbB2-like, basal-like, normal breast-like). However, the question how to transform this classification into clinical practice is quite uncertain.

Materials and Methods: We have used a panel of 6 immunohistological markers on primary invasive breast cancers and collected data from 3733 tumors. The panel includes ER (1D5), PgR (PgR636), HER2 (A0485), Ki-67 (MIB1), bcl-2 (124), and p53 (DO7, all antibodies by Dako). Data were clustered with the aim of creating groups similar to those obtained by gene expression classification.

Results: 75% of all breast cancers could be clustered using this panel of 6 immunohistological tumor markers. 30.2% were clusted as Luminal A, 18.0% as Luminal B, 11.3% as erbB2-like, 5.4% as basal-like, and 10.1% as normal-breast like.

Conclusions: While phenotypic clustering is very well possible for hormone receptor positive tumor into Luminal A and B subgroups, the markers are not sufficient to predict basal phenotype. With the addition of basal cytokeratins to this panel, a good immunohistological classification should be possible.

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Basal- and non-basal phenotypes in triple-negative breast cancers

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Background: Triple negative breast cancers are characterized by lack of expression of oestrogen, and progesterone hormone receptors, and lack of HER2 overexpression. Frequently they are associated with a basal phenotype, but there is a distinct subgroup of “quadruple-negative” cancers which not basal-like, and are yet ill defined yet. Therefore, in the present study, we have analysed the expression of basal and other markers the triple negative breast cancers.

Materials and Methods: 158 triple-negative invasive breast carcinoma were selected from the archives and used for construction of tissue microarrays. A panel of immunohistochemical markers, which included ER, PgR, HER2, CK5/6, CK14, CK18, EGFR, p53, c-KIT, MKI-67, bcl-2, and p16 was used to further characterize these tumours.

Results: 102 tumours (66%) showed a basal phenotype by being positive for either CK5/6 or CK14 using a cutoff value of 10%. The non-basal triple-negative cancers differed from the carcinomas with a basal phenotype by having a lower proliferative rate ($p = 0.04$), and were less frequently CD117 positive (14% vs. 32%, $p = 0.01$) and less frequently overexpressed p16 (31% vs. 52%, $p = 0.01$). No differences were seen for bcl-2 (9% vs. 10%) and p53 overexpression (37% vs. 36%).

Conclusions: Non basal-like triple-negative breast cancers differ from basal-like triple-negative breast cancers in several aspects, and have a lower malignant phenotype. Therefore, this subgroup of triple-negative breast cancers is important to distinguish from the basal-like triple-negative breast cancers.

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VEGF-C in association with VEGFR-3 promotes nodal metastases but does not stimulate peritumoral lymph vessel growth in breast cancer with extensive intraductal component

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One of the assumed mechanisms of lymphatic dissemination in breast cancer is lymphangiogenesis. Although the underlying molecular mechanisms in animal in vitro and in vivo studies have been clarified, controversies in clinical breast cancer studies do exist. By performing immunohistochemistry stainings on breast cancer with extensive intraductal component (N = 46), we try to clarify existing controversies. We studied breast cancer with extensive intraductal component (EIC) because it is a representative of early disease, also the assumed mechanism of intratumoral lymph vessel collapse due to high pressure does not take place.

We found a significant correlation between vascular endothelial growth factor receptor-3 (VEGFR-3) positive vessel density and lymph node metastases (P = 0.007). Lymph node status also correlated with CD34 positive blood vessels (P = 0.027). Only vascular endothelial growth factor-C (VEGF-C) cell expression in the invasive component correlated with lymph node dissemination (P = 0.047). However combining expression of VEGF-C or D with strong VEGFR-3 vessel density, which is in fact the assumed lymphangiogenesis pathway, VEGF-C both in the in situ and invasive as well VEGF-D in the invasive component correlated with lymph vessel metastases. All LYVE-1 positive lymph vessels were located peritumorally and LYVE-1 vessel density did not correlate with lymph node metastasis neither with any clinicopathological factor or growth factor.

In this study with a rather limited number of patients with EIC we could not prove that lymphangiogenesis is a mechanism of tumor spread in early disease. We did not see intratumoral lymph vessels, not even in the in situ component but we stained with the LYVE-1 antibody only. While VEGF-C and VEGFR-3 and also VEGF-D in combination with its receptor VEGFR-3 correlated with lymph node metastatic spread, peritumoral lymph vessel density did not correlate with lymph node metastases. Therefore we conclude that lymphangiogenesis based on the VEGF-C/D VEGFR-3 pathway is not solely responsible for lymph node dissemination.

108 Poster

Common expression of estrogen receptor type A and its gene targets in proliferating breast epithelial cells

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Introduction: It is supported that estrogen receptor type a (ERa) exerts estrogen's mitogenic activity through induction of expression of genes that control cell proliferation, i.e. cyclinD1, c-Myc and stromal growth factor-1 (SDF-1). Failure to detect co-expression of ERα and proliferation 'markers' such as Ki67 in human mammary epithelium led to the proposal that estradiol acts indirectly in this tissue by stimulating ER-positive epithelial cells to release unknown mitogens that then trigger proliferation in neighboring ER-negative epithelium (model of paracrine breast estrogenic action).

To evaluate co-expression of ERa and proliferating marker Ki67 in normal breast epithelial cells under short-term estrogenic stimulus and increased proliferation. The simultaneous with ERα expression of estrogen-induced target proteins SDF-1, MYC and cyclin D1, that are involved in the proliferation of breast epithelial cells.

Methods: Immunohistochemistry was used on mammary tissue sections from short-term estrogen treated women to investigate co-expression of ERα and the proliferation antigen Ki67. Using the same methods, we investigated the cell localization of proteins involved in estrogen-induced proliferation, including cyclin D1, stromal cell-derived factor (SDF)-1, and MYC. To determine the percentage of double-labeled mammary epithelial cells, an observer scored 500 to 1,000 cells for each combination of antibodies (number of women used: 4).

Immunohistochemistry results:

- 23±10% of mammary epithelial cells was positive for ERa and Ki67 antigens.
- Co-expression of ERa and SDF-1 antigens was found in ~25% of cells, while co-expression of ERa and Myc was found in ~26% of cells.
- Most positive for SDF-1 cells were also positive for Myc.

Results: ERα is expressed in proliferating mammary epithelial cells together with the estrogen-induced proteins MYC, cyclin D1 and SDF-1, consistent with a direct mitogenic action by estrogen in mammary epithelium.

Conclusion: These observations obviate the need to invoke unknown paracrine mediators of estrogen action in the mammary gland.

109 Poster

SPARC in breast tumors of different histological types: defined its role in patients' outcome and nodal status

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Background: to characterize the immunohistochemical distribution of secreted protein acidic and rich in cystein (SPARC) in a series of breast tumors of different histological types, and to define its role in malignant transformation and association with patients' outcome.

Materials and Methods: A total of 268 Samples of different histopathological benign and malignant breast lesions were retrieved from National Taiwan University Hospital (NTUH) between 1994 and 2005. Up to 11 year's clinical follow-up data were available for 185 infiltration ductal carcinoma (IDC) cases. Immunohistochemistry staining with SPARC were performed in tissue microarray (TMA) or whole section. The association of expression of SPARC and cumulative overall survival of IDC patients were analyzed using Kaplan–Meier survival analysis and the Cox Proportion analysis.

Results: SPARC was expressed only in benign breast phylloides of all benign lesions, in 16% IDC, 85% Metaplastic carcinoma of the breast (MCB) and all malignant breast phylloides. SPARC was strongly expressed in mesenchymal component of MCB and different expression level in epithelial component. The correlation of positive expression of SPARC and poor long-term survival is significant (p = 0.004). The result also showed strongly statistically significant association between SPARC expression and nodal status by using Chi-Square test (p = 0.037).

Conclusions: SPARC plays a crucial role in the process of epithelial-mesenchymal transition and its expression correlates with poor overall survival in IDC.

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Insulin like binding protein 7 – evidence for a possible paracrine protective effect in human breast cancer

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Background: Insulin like growth factor (IGF) has a well recognised pro-neoplastic role in various human malignancies. This study examined paired mRNA expression of Insulin like growth factor binding protein (IGFBP) 3 and 7 genes in malignant breast tissue and associated 'adjacent non cancerous tissue' (ANCT) correlating this with various prognostic parameters.

Materials and Methods: Prospectively collected breast cancer/ANCT pairs were analysed for levels of IGFBP 3 and 7 mRNA using real time Q-PCR. mRNA levels were analysed against tumour grade, nodal status, Nottingham prognostic index (NPI) stage, size, recurrence and disease free survival (DFS). Full ethical approval was obtained.

Results: Non parametric analysis was performed throughout. The number of validated results were, BP7anct = 90, BP7tumour = 84, BP3anct = 57, BP3tumour = 58. When ANCT IGFBP7 was correlated with NPI, significantly more binding protein was expressed adjacent to favourable prognostic tumours (NPI 1) when compared with poor prognostic tumours (NPI 3), (p = 0.016). This trend repeated for tumour grade, with greater expression adjacent to low grade tumours (Grade 1) compared to high grade tumours (Grade 3) (p = 0.047) and for recurrence, with significantly greater expression adjacent to tumours who remained recurrence free (p = 0.006). Survival analysis using Kaplan–Meier curves also revealed improved DFS associated with high ANCT IGFBP7 levels, which was statistically significant (p = 0.004).

Conclusions: Our data suggests that IGFBP7 may act to limit progression of the malignant phenotype in a paracrine fashion. This needs to be evaluated further with larger studies.