of lymph node metastasis. We analyzed prognostic factors in each group such as age, histologic grade, nuclear grade, lymphovascular invasion (LVI), estrogen and progesterone receptor status, HER-2/neu expression, Ki67-labelling index, bcl-2 expression, extensive ductal component (EIC), DCIS, and comedonecrosis.

Results: The node negative (T1N0) group included 157 cases and the remaining 73 cases were allocated to the node positive (T1N1−3) group. In the univariate analysis, lymphovascular invasion ($p = 0.000$), histologic grade ($p = 0.012$), HER-2/neu ($p = 0.012$) and bcl-2 ($p = 0.025$) were the statistically meaningful prognostic factors that were related to the node metastasis in T1 breast cancer. But in the multivariate analysis, LVI ($p = 0.000$), bcl-2 ($p = 0.048$), and HER-2/neu ($p = 0.031$) were statistically significant factors related to the node metastasis in T1 breast cancer.

Conclusions: The presence of LVI, increased bcl-2 expression, and HER-2/neu overexpression were related to the increased incidence of ALNM in T1 breast cancer. LVI was the most predictable factor of ALNM.

394 Poster

Is there any negative impact on histologic assessment of breast masses and sentinel nodes marked with blue dye?

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Introduction: Blue dye is widely used in breast surgery nowadays. In sentinel node biopsy, combined method with radiolabeled material and patent blue dye is the most accepted method.

Methylene blue dye is also used as a safe and cost effective martial for marking non palpable breast masses before surgery and it's use in sentinel node biopsy has been reported to be effective and accurate for sentinel node identification in some studies. But there is debate about possible adverse effect of blue dye on histology and immunohistochemistry evaluation in tissues that are colored with blue dye. So we studied this effect in non palpable breast masses that were marked with methylene blue dye before surgery and sentinel nodes that were detected by blue dye or combination method in our center.

Materials and Method: Pathology slides of 56 masses from 49 patients that methylene blue dye was used as marking method before surgery for them were considered for effect of methylene blue dye on permanent pathology of breast masses and 28 sentinel nodes that were assessed by frozen section were considered for effect of patent blue dye on frozen section assessment.

Two pathologists reviewed slides separately and reported if there was any adverse effect on slide that interfered with assessment. They also reviewed Imunohistochemistry samples and reported probable difficulties.

Results: From 56 masses that were assessed, 4 of masses were malignant one of them insitue ductal carcinoma, 3 atypical ductal hyperplasia, 2 sclerosing adenosis, 10 fibrocystic change, 25 fibroadenomas (3 of them mied type and one with phyloid features), 2 tubular adenomas, one epithelialized liomyoma, 2 intraductal papillomas, one foreign body granuloma, 2 tubular adenomas and 5 epithelial hyperplasia without atypia. Both pathologists did not find any adverse effect due to blue dye in histologic assessment of breast tissue or mass in these 56 excisional biopsies.

From 28 lymph nodes that were sent as sentinel node biopsy, 12 were positive for tumoral involvement in frozen section that 2 of them were micro metastasis. All of these lymph nodes were proved to be metastatic in permanent section. In one case that the frozen section did not found any metastatic tumoral cells in lymph node, tumoral cells were found in permanent section and it was not due to dye interference but because of size of tumor nest that was small.

Conclusion: Injection of blue dye (patent blue or methylene blue) do not have adverse effect on pathology and immunohistochemistry assessment and it can be used for marking non-palpable breast masses and also sentinel node biopsy in breast cancer patients even when frozen section is going to be done for them.

Thursday, 25 March 2010 18:15–19:15

POSTER SESSION

Pathology and biological markers in breast cancer

395 Poster discussion

Valid PCR quantification of mRNA from 16 year old formalin-fixed, paraffin-embedded breast cancer tissue: a methodological study comparing manually trimmed sections and whole tissue sections

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Background: Archival formalin-fixed, paraffin-embedded tissue (FFPE) constitutes a biobank of tumors of all sizes, often linked to clinical studies of great statistical power and long follow-up time. Gene expression analysis on RNA from FFPE has been considered impractical due to the extracted mRNA being fragmented and chemically modified. Furthermore, the technique has been time consuming and characterized by a low grade of automation.

In addition, FFPE often contains an admixture of normal tissue, premalignant changes and invasive cancer. This has called into question the specificity and interpretation of results from analysis of the total amount of collected RNA.

Material and Methods: Two FFPE blocks from each of 21 breast carcinomas, diagnosed 15−17 year ago, were chosen. From each block a whole slide section and a manually trimmed, tumor enriched section (discarding surrounding non-invasive tissue) were prepared. mRNA was isolated with a silica bead-based, fully automated technique developed by Siemens (Siemens Healthcare Diagnostics, Deerfield, IL; not commercially available) including an integrated xylene/ethanol-free deparaffinization step. Tumor content defined as invasive carcinoma with interposed stroma was estimated stereologically from Hematoxylin-Eosin stains. Eluates were analyzed with kinetic RT-PCR for 1 housekeeping gene RPL37A and 3 target genes (ESR1, PGR and HER2). Raw data (C_T values) for target genes were normalized to RPL37A, and relative expression levels calculated and compared to immunohistochemical data.

Results: RNA was successfully extracted from all sections, and gene expression reliably quantified for the three target genes. Agreement between whole slide and trimmed sections were optimal, indicating that expression levels for ESR1, PGR and HER2 are not strongly influenced by contamination from surrounding tissue. Concordance between RNA- and protein expression was excellent for ESR1 and HER2, making it possible to define RNA tresholds, distinguishing between positive and negative samples.

Conclusions:

- Isolation and quantification of ESR1, PGR and HER2 mRNA from >15-year-old FFPE with kinetic RT-PCR are feasible and reproducible using the automated technology by Siemens, and do not require prior trimming of the tissue.
- High level of concordance between the quantitative RNA expression level and the semi-quantitative protein level for ESR1 and HER2.
- Quantitative expression analysis using kinetic RT-PCR in routinely processed FFPE is feasible and could be adapted in diagnostic testing.

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Role of miR-143 regulating DNA methyltransferases 3A in breast cancer

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Background: MicroRNAs (miRNAs) are 19−25-nucleotides regulatory non-protein-coding RNA molecules that regulate the expressions of a wide variety of genes including some involved in cancer development. In particular, decreased expression of miR-143 has been reported in various human cancers including colorectal cancer and B-cell lymphomas. The aim of this study was to elucidate the role of miR-143 dysregulation in breast cancer.

Material and Method: Expression levels of human mature microRNAs (miRNAs) were compared with paired breast carcinomas and adjacent normal tissues by TaqMan real-time PCR based expression arrays. Decreased expression of miR-143 was further confirmed in breast cancer cell lines and paired breast tumors and normal adjacent tissues by qRT-PCR. Potential targets of miR-143 were defined. The functional effect of miR-143 and its targets was performed in human breast cancer cell lines to confirm target association.

Results: Down-regulation of miR-143 was verified in both human breast cancer cell lines and 80% (12/15) of breast tumors ($P < 0.001$). DNA methyltranferase 3A (DNMT3A), one of a key enzyme involved in DNA methylation, was defined as a potential target of miR-143 by *in-silico* analysis. Overexpression of miR-143 in breast cancer cell lines down-regulated expression of DNMT3A, decreased tumor cell growth by MTT assay and soft agar colony formation assay. DNMT3A was demonstrated to be a direct target of miR-143 by luciferase reporter assay. Inverse correlation between DNMT3A protein and miR-143 was found in tumor and normal breast tissues.

Conclusions: In this study, we show for the first time in breast cancer that miR-143 specifically targeted DNMT3A and the expression of miR-143 was inversely correlated with DNMT3A expression. Our findings demonstrated that down-regulation of miR-143 and up-regulation of DNMT3A are significant changes in breast tumors. These findings indicate a tumor suppressive role of miR-143 in epigenetic aberration of breast cancer.

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Extreme loss of immunoreactive phosphoproteins during routine fixation of primary breast cancer

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Aim: To characterize the changes in immunoreactivity of common biomarkers before and during fixation.

Background: Following surgical resection, breast cancer specimens are routinely X-rayed for evaluation of margin clearance and subsequently fixed in formalin. Very few studies have investigated whether the time elapsed between surgical resection and tissue fixation impacts on immunohistochemically measured biomarkers including phosphorylated proteins, which are subject to intense research scrutiny. Validation studies are therefore warranted.

Material and Methods: Core-cuts taken from the surgical specimen immediately after resection (timepoint A) and after routine X-ray (timepoint B) were formalin-fixed and paraffin-embedded and compared to the routinely fixed resection specimen (timepoint C) (n = 23 sets). The variation in expression of Ki67, p-Akt and p-Erk were investigated by immunohistochemistry using the following antibodies, phospho-Akt (Ser473), phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (Cell Signalling) and Ki67 MIB-1 clone (DAKO), respectively. H scores were used for all markers except Ki67 where percentage of cells staining was recorded.

Results: Ki67 expression remained consistent across all timepoints. There were no systematic differences in p-Akt or p-Erk expression between timepoints A and B but, in the majority of cases, their expression was significantly reduced by at least two fold in resections (C) compared to biopsies (mean A,B). In 7 cases for p-Akt and 9 cases for p-Erk, moderately to strongly staining core-cuts were completely or almost completely negative in the resection specimen. Notably, p-Akt cytoplasmic expression was not decreased on resections compared to core-cuts in contrast to p-Akt nuclear expression. Data will also be shown for ER, PgR and HER2 expression (currently incomplete).

Conclusions: The delay in fixation in core-cuts taken after post-operative X-ray of resection specimens has no significant impact on expression of Ki67, p-Akt or p-Erk. However catastrophic loss of phospho-staining occurred during routine fixation of most resection specimens: such specimens are grossly unreliable for assessment of p-Akt and p-Erk and possibly other phosphoproteins. The absence of an effect on cytoplasmic p-Akt questions its validity. These findings have profound complications for the assessment of these important proteins in research studies and for potential future measurement for clinical management of breast cancer patients.

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HLA-E and HLA-G tumour expression is of prognostic value for clinical outcome of early breast cancer patients, but exclusively in classical HLA class I tumor-negative patients

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Background: Non-classical human leukocyte antigens (HLA), HLA-E and HLA-G, are known to affect clinical outcome in various tumor types. We examined the clinical impact of HLA-E and HLA-G expression in early breast cancer patients, and related the results to tumor expression of classical HLA class I molecules, as together these cell surface molecules may determine natural killer (NK) cell responses.

Material and Methods: Our study population (n = 677) consisted of all early breast cancer patients primarily treated with surgery in our center between 1985 and 1995. Tissue micro array (TMA) sections of formalin-fixed paraffin-embedded tumors were immunohistochemically stained for HLA-E and HLA-G. For evaluation of HLA-E and HLA-G expression and the combined variable, HLA-EG, a binary score was used. Expression of classical HLA class I expression was previously determined.

Results: HLA-E, HLA-G and HLA-EG were expressed in breast tumors in 50%, 60% and 23% of patients respectively. Remarkably, only in patients with loss of classical HLA class I tumor expression, expression of HLA-E ($p = 0.027$), HLA-G ($p = 0.035$) and HLA-EG ($p = 0.001$) resulted in a worse relapse free period. An interaction was found between classical and non-classical HLA class I expression ($p = 0.002$), suggestive for a biological connection.

Conclusions: We have demonstrated that expression of HLA-E and HLA-G are important factors in the prediction of clinical outcome of breast cancer patients, but exclusively in patients with classical HLA class I negative tumors. HLA-E and HLA-G expression may specifically prevent NK-cell recognition by the host in this subset of tumors. These results provide further evidence that breast cancer is highly immunogenic, but also capable of evading tumor eradication by the host immune system in which both T cells and NK cells play a role.

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Pathological changes after primary chemotherapy in breast operable carcinoma. Correlation with survival after 14 years of follow-up

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Background: There is a lack of standardized criteria to define pathological response to neoadjuvant chemotherapy.

Material and Methods: In a series of 536 breast infiltrating carcinoma stage T2−3/N0−1 treated with primary chemotherapy, we have evaluated the pathological changes associated with chemotherapy. The percent of microscopic reduction of the infiltrating component was calculated and correlated with clinical response and survival after 14 years of mean follow-up.

Results: Changes due to primary chemotherapy and unrelated to tumour stroma were identified in 311 cases (58%) of those 49 (9%) presented completed pathological response (pCR). The mean percentage of pathological changes increased proportionally in each clinical response category: 5.56%; 6.59%; 14.94%; 46% and 87.44% for progression; stable disease; response inferior to 50%; partial response and complete clinical response, respectively. According to the Nielsen's classification those tumours with the greatest percentage of pathological changes were triple negative ones: 49.62%; followed by HER2 positive tumours: 44.46% and finally luminal tumours: 22.6%. In the multivariate analyses the only parameters associated with pathological changes were clinical response ($p < 0.001$); ER negativity ($p = 0.007$) and nuclear grade III ($p = 0.01$). Survival rates were superior for those patients with tumours showing at least 10% of pathological changes (66.9% vs. 52.4%, $p = 0.003$), the greatest differences were seen between pCR compared to non-pCR (91.8% vs. 58.9%, $p < 0.001$). Intriguingly, triple negative tumours were those who benefit most in terms of survival due to the excellent response to chemotherapy: 67.9% compared to 62.2% for HER2 positive tumours and 60.8 for luminal tumours ($p = 0.02$).

Conclusions: measurement of response to neoadjuvant chemotherapy both by clinical and pathological criteria should be as accurate as possible due to their prognostic significance.