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.