7.2 to 8.5 years, p = 0.0001–0.0008). In patients with CHEK2 mutations, breast cancer occurred 2.1 to 3.8 years earlier than in patients without mutations in CHEK2 gene. However, the identified differences were found not significant (p = 0.4802 and 0.2060). Among remaining 125 patients with bilateral breast cancer, who had not germline mutations in BRCA1, BRCA2 and CHEK2 genes, 85 had family history of neoplasm and 40 had not. The women with bilateral breast cancer and family history of breast cancer only were not diagnosed for earlier occurrence of bilateral breast cancer. However, the age of women with metachronous breast cancer and with family history of breast and ovarian cancer or ovarian cancer only was different from the age of women with no such family history (a difference of 7.7 years, p = 0.0608).

The age of women with family history of neoplasm other than breast and ovarian, at which bilateral breast cancer was diagnosed, was significantly lower than the age of patients with no such family history (a difference of 5.2 years, p = 0.0169).

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The role of steroid sulfatase (STS) and organic anion transporter polypeptide B(OATP B) mRNA expression in predicting the clinical outcome in human breast cancer

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Introduction: Steroid sulfatase (STS) is the enzyme responsible for hydrolysing biologically inactive estrogen sulfates to active estrogens. Therefore it plays a significant role in supporting the growth of hormone-dependent tumours of the breast, endometrium, and prostate. There is little evidence as to what controls its expression in vivo. OATP-B is a member of family of membrane transporter proteins that regulates the uptake of steroid sulfates through cell membranes.

This study examines mRNA expression of STS and OATP-B genes located on chromosome X (location Xp22.32) and chromosome 11 (location 11q13) respectively. Our objective was to determine, using quantitative PCR, whether the mRNA expression levels of these genes were positively correlated with clinical outcome in human breast cancer.

Methods: A total of 153 samples (120 tumour tissues and 33 normal tissues) were analysed. The levels of transcription of STS and OATP-B were determined using real-time quantitative PCR. The mRNA expression was normalized against CK19. The levels of expression were analyzed against tumour's stage; grade, nodal status, local relapse, distant metastasis and survival over a 10 year follow up period. The levels were also analysed against hormone receptors status including ERα, ERβ, and HER1–4.

Results: The levels of STS mRNA were significantly higher in malignant samples compared with normal breast tissue samples (p = 0.031). They were also higher in node positive disease (p = 0.0222). STS mRNA expression increased with increasing tumour grade but this did not reach statistical significance. We also noted an increase in levels correlating with tumour stage using TNM classification. This became statistically significant when we compared stages TNM1 and TNM2, TNM2 and TNM3, and TNM3 and TNM4 ($p \leqslant 0.00001$, 0.0017, and 0.02 respectively). Furthermore, STS expression levels positively correlated with progression of disease as levels were significantly higher in samples of patients who developed metastasis, local recurrence, or died of breast cancer when comparing to those who were disease free for > 10 years (p = 0.0036).

We found no significant correlation between levels of STS expression and ERα/ERβ status. The levels positively correlated with HER1 and HER3 receptors.

The levels of mRNA expression of OATP-B were higher in malignant tissue compared to normal tissue, this however did not reach statistical significance (p = 0.4045). Levels were also higher in node positive disease (p = 0.0672). Expression levels increased with increasing tumour grade, this became statistically significant when comparing grade 1 to 2, and grade 2 to 3 (p = 0.0271, 0.0289 respectively). We also observed an increase in levels correlating with TNM tumour staging, this however did not reach statistical significance. There was no significant correlation between OATP-B expression levels and clinical progression of breast cancer. We found no correlation between STS and OATP-B expression levels.

Discussion: This study demonstrates a compelling trend for STS transcription levels to be higher in cancerous tissues. These levels were even higher in patients who developed progressive disease. OATP-B expression levels correlated with the grade and stage of the disease but not with clinical outcome. These results suggest that STS mRNA has a significant potential as an independent predictor of clinical outcome in patients with breast cancer.

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p21 as a target for breast cancer therapy: the role of p53 status in its efficacy

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$p21^{waf1/cip1}$ has emerged as an important but pleiotropic regulator of differentiation, cell cycle progression, senescence, and apoptosis, and this molecule has been suggested by us and others as a molecular target in breast cancer treatment. Recent reports have shown that doxorubicin differentially activates Akt in some breast cancer cell lines, including p53-mutant T47D cells, and that activated Akt increases cytosolic expression of p21. We undertook this study to investigate whether p21 is conveying anti-apoptotic effects in breast cancer as a function of p53 status. We find that doxorubicin treatment of T47D, a p53-mutant human breast cancer cell line, results in a dose-dependent decrease in both p21 levels and the anti-apoptotic protein, XIAP, with a concomitant increase in PARP cleavage (indicating apoptosis). No changes in the apoptosis-related proteins CAS (cell apoptosis susceptibility) and Apaf-1 were seen with doxorubicin treatment. Similar results were found in a p53 mutant renal cell carcinoma cell line, 786-O. To determine whether p21 is conferring the anti-apoptotic effect seen with lower dosage of doxorubicin treatment, we used an RNAi approach. Down regulation of p21 with siRNA did not change PARP cleavage or expression levels of XIAP, CAS, and Apaf-1 as compared to cells treated with doxorubicin alone. However, p21 down regulation enhanced apoptosis induced by lower dosage of doxorubicin in the p53-wt renal cell carcinoma cell line, ACHN. We are currently investigating this effect in p53-wt breast cancer cell lines. Due to the fact that p21 is a downstream target of p53 in the DNA repair pathway, our results suggest the anti-apoptotic function of p21 is dependent on p53 status and are consistent with previously published data showing that p21 accumulation after doxorubicin treatment only occurs in p53 wild-type breast cancers. More importantly, these results suggest caution when choosing p21 as a therapeutic target in breast cancer therapy. The genetic composition of tumors, such as p53 status, should be carefully considered in selecting the therapeutic regimen.

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Analysis of genetic alterations in plasma DNA from breast cancer patients: a possible molecular biomarker in early detection and prognosis of breast cancer

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Introduction: Genetic alterations are associated with the development of breast cancers, which are the most common malignancy in women. Tumor metastasis is the major cause of death in cancer patients. In the process of metastasis, tumor cells disseminate from the original site through the circulatory system and establish the secondary tumors in distant organs. Therefore, increasing levels of circulating tumor DNA was found in the bloodstream of cancer patients and apoptotic and necrotic cells are a major source for plasma DNA. Plasma DNA may be an indicator for cancer. Early diagnosis and identification of molecular tumor markers are the main topics of clinical cancer research. Molecular tumor markers described in plasma include oncogene amplifications, and microsatellite alterations, such as loss of heterozygosity (LOH) and microsatellite instability (MSI). It was postulated that identifying the genetic alterations in plasma may play an important role in the cancer diagnosis and prediction of cancers.

Patients and Methods: A total of 116 cases were analyzed in our study, including 34 non-metastatic patients with breast cancer, 41 metastatic patients with breast cancer originally, and 41 anonymous individuals without tumor, with previously identified breast cancer-specific microsatellite grade I markers, such as LPL, TP53, and D16S413, and grade II marker, D17S855, which located in the intron of the BRCA1 gene, using ABI 3100 capillary genetic analyzer. Additional 42 plasma samples, including 13 non-metastatic patients with breast cancer, 16 metastatic patients with breast cancer originally, and 13 anonymous individuals without tumor, were also used to analyze MYCN oncogene amplification with real time quantitative PCR using LightCycler instrument.

Results: LOH/MSI was detected in 6 of 41(14.6%) anonymous individuals with non-tumor disease, in 14 of 34(41.2%) non-metastatic patients, and in 18 of 41 (43.9%) metastatic patients. The frequency of LOH/MSI of plasma DNA was significantly lower in anonymous individuals