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Methylation status of WIF-1 promoter region in breast cancer cell lines and tumour tissues

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Background: Aberrant epigenetic activation and upregulation of the Wnt pathway is a key feature of many cancers. Wnt inhibitory factor-1 (WIF-1) is a secreted antagonist that can bind to Wnt proteins directly and inhibit Wnt signaling pathway. Downregulation of WIF-1 protein expression has been detected in human bladder, breast, non small-cell lung, prostate and gastrointestinal carcinomas. WIF-1 has been reported to be silenced by promoter hypermethylation in human cancer cell lines and tissues.

Objectives of this study: To determine the presence of WIF-1 promoter methylation in breast cancer cell lines, tumour and non-tumorous tissues of breast cancer.

Methodology: Four breast cancer cell lines, MCF-7, T47D, ZR75–1 and MB231, were used. For the breast tissues, 20 surgically resected breast cancer tissues and the corresponding paired non-tumorous tissues taken more than 5 cm from the tumour at a macroscopically normal focus were analyzed (in case where such distance was not present, the non-tumour sample was taken from a distance furthest away from the tumour sample). Both the tumour and non-tumorous tissues were subsequently confirmed histologically. The tissues were snap frozen in liquid nitrogen-cooled isopentane and stored at −70°C until use. High molecular weight DNA was extracted from the breast tissues according to the method previously described. Methylation of the promoter region of WIF-1 was examined by bisulphite DNA modification followed by methylated specific PCR (MSP).

Results: CpG island hypermethylation in the WIF-1 promoter region was observed in 2 (50%) of 4 breast cancer cell lines, MCF-7 and ZR75–1. Analysis of the breast tissues revealed that 7 (35%) of the breast tumour tissues and 10 (50%) of the paired non-tumorous tissues had methylation of the WIF-1 promoter region.

Conclusion: WIF-1 promoter hypermethylation occurred at a high frequency in breast cancer tumour and non-tumorous tissues. This suggests the notion that WIF-1 methylation is an early and premalignant alteration, and supports previous reports that WIF-1 silencing due to promoter hypermethylation is an important mechanism underlying aberrant activation of the Wnt signaling pathway in carcinogenesis.

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Expression microarray analysis of newly-developed chemotherapy-resistant breast cancer cell lines

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Chemotherapy resistance in metastatic breast cancer is a major challenge facing the treatment of this disease. Tumours can harbour a plethora of mechanisms to avoid the effects of chemotherapy agents such as increased membrane pump activity and drug detoxification enzymes. In order to investigate these and other possible mechanisms of chemotherapy resistance, we established novel breast cancer cell lines which were significantly resistant to the chemotherapy drugs doxorubicin, cisplatin and 5-fluorouracil. In contrast to other chemotherapy-resistant cell line models, these models of chemotherapy resistance aimed for a greater degree of clinical relevance by establishing resistant cell lines with concentrations of chemotherapy drug derived from patient's peak plasma concentrations. Secondly, treatment was carried out in discrete cycles, again to mimic the clinical environment. Control cell populations were grown in parallel to ensure that any genetic changes were due to chemotherapy resistance and not random changes, which can often occur in tumour cell lines.

For analysis of gene expression changes associated with in vitro chemotherapy resistance, RNA was extracted from resistant and control cell lines, reverse transcribed into fluorescently labelled cDNA and competitively hybridised to cancer-specific oligonucleotide microarrays. After normalisation and correction for dye bias effects, the expression a total of 17 genes (doxorubicin: 5; cisplatin: 7; 5-fluorouracil: 5) were found to be significantly altered between the chemotherapy-resistant cell lines and the control cell lines.

The development of these chemotherapy-resistant breast cancer cell lines and identification of candidate genes associated with in vitro drug-resistance may reveal novel therapeutic targets in breast cancer.

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Detection of circulating tumor cells in peripheral blood of patients with primary and metastatic breast cancer

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Introduction: The purpose of this study was detection and analysis of disseminated tumor cells in blood of breast cancer patients by expression profiling using disseminated tumor cell (DTC) detection assay.

Material and Methods: Patients with primary breast cancer (n = 178) and patients with metastatic breast cancer (n = 161) were enrolled onto a multicenter clinical performance evaluation study. Five ml blood were used for DTC analysis. The DTC assay consists of immunomagnetic tumor cell selection (targets EpCAM and MUC-1). The immunobead selected cells were used for mRNA isolation and c-DNA synthesis. Breast carcinoma-associated transcripts GA733–2 (EpCAM), MUC-1 and HER-2 were analysed by multiplex PCR. Claudin7 was determined by single-round RT-PCR, while cytokeratin 19 (CK19), mammaglobin 1 (MBGB1) protate-specific ets factor (PSE) and survivin (BIRK5) were determined by nested RT-PCR. PCR products were analysed by capillary electrophoresis with the Agilent Bioanalyzer 2100. Specificity of the RT-PCR was confirmed by examination of blood of healthy donors.

Results: Sensitivity for every single transcript was adjusted to 2 tumor cells per 5 ml blood. In the group of patients with primary breast cancer (n = 174) we archieved an overall detection rate of 20%. In patients with metastatic disease 93 of 161 (58%) were positive. The detection rate of tumor-associated transcripts in primary breast cancer was 18% (18/101) for lymph node negative and 24% (18/76) for lymph node positive patients. Tumor-associated transcripts were heterogenously expressed in positive samples. Expression rates for MUC1, GA733–2, and HER-2 were 71%, 26%, 32%, respectively. Survivin, CK19, Claudin 7, MGB1, and PSE transcripts were detected at a frequency of 29%, 19%, 35% and 10%.

Conclusion: Using a new immunomagnetic tumor cell enrichment method we have established a c-DNA bank of peripheral blood samples from patients with primary breast cancer. Recently microarray strategies confirmed established and identified new informative molecular markers for the detection of micrometastatic cancer cells. In the present study we describe the application of a panel of 8 genes overexpressed at high levels in metastatic breast cancer for the identification of tumor cells in peripheral blood. HER-2, Survivin as a unique member of the inhibitor of apotosis protein family, as well as PSE identified in circulating breast cancer cells may serve as a prognostic indicators of tumor progression and could represent valid targets for new individualized therapeutic interventions.

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Analysis of tamoxifen-resistance related genes by Taqman low density array technology in clinical samples

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Objective: Identifying distinct gene expression signatures of predictive diagnostic value is one of the challenges of genomics research. The study was performed to evaluate if a set of genes found to be differentially expressed in a tamoxifen-resistant and -sensitive human breast cancer xenograft model by means of Affymetrix GeneChip® analysis (Mol Cancer Ther 2005; 4(1):151–68) can be confirmed in clinical samples from breast cancer patients responsive to tamoxifen or developing a recidive under tamoxifen therapy. For this purpose we employed a new and efficient PCR technique combining Low Density Array with TaqMan® RealTime RT-PCR from Applied Biosciences.

Samples and Method: Samples from two independent animal experiments with the tamoxifen-sensitive human mammary carcinoma xenograft MaCa 3366 and its tamoxifen resistant subline MaCa 3366/TAM were used. Furthermore, we included 23 clinical samples including primary breast tumours of patients shown to be recurrent or to remain disease-free under tamoxifen treatment. We applied TaqMan® Low Density Array methodology to analyse the differential expression of 11 genes significantly up- and down-regulated in the tamoxifen-resistant tumour xenograft as compared to the sensitive one as determined by Affymetrix chips.

Results: Using TaqMan® Low Density Arrays we could show a high correlation of the xenograft data derived from Affymetrix chips and from Low Density Arrays. Several genes in the clinical samples of the recurrent