III, were candidates to this study of gene profile, associated to response to primary chemotherapy. Thirty eight pt were included in a training set and another four, in an independent validation set. Median age of pt was 51 years, the majority of them presented large lesions with a mean diameter of primary breast tumor and axyllary lymph nodes of 91.1 mm. All pt (except two) received 4 courses of doxorubicin and cyclophosphamide (AC) therapy at 60 and 600 mg/m^2, respectively, in a routine basis protocol. Median duration of chemotherapy was 67 days and mean administered dose of doxorubicin was 96.1%. Response to chemotherapy was clinically evaluated and based on RECIST guidelines, 34 pt, who presented at least a 30% reduction in tumor dimension, were considered responsive, and eight, were considered resistant. Samples obtained from tumor biopsies before chemotherapy, were hand dissected and only samples composed of at least 80% malignant cells, were further processed. RNA was extracted, amplified and gene expression analysed using cDNA microarrays glass slides, containing 657 sequences, printed in three or six replicates. cDNA microarray platform, complying with MIAME format, was submitted to the Gene Expression Omnibus (GEO) data repository (GPL 1727).
Results: Seventeen genes were differentially expressed between responsive and resistant tumors, however, hierarchical clustering was not able to discriminate the groups. cDNA microarray gene expression data was confirmed by quantitative real time PCR measurements and Spearman rank correlation between these assays was significantly positive for five of seve genes analyzed. A classifier was designed using multiple transcripts and a trio was identified comprising: EMILIN1, FAM14B and PBEF. The classifier error was 5.41% and sensitivity to detect responsive tumors was 100%, both in the training set and in a small validation set, which means that all patients, who presented an objective response, were identified.
Conclusions: Our results suggest that a trio of genes might distinguish responsive tumors to doxorubicin based therapy.

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Down regulation of the DNA Double strand breaks repair genes in early stage breast cancer

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Of the many types of DNA damage, the Double-strand breaks (DSBs) are the most dangerous, because of the intrinsic difficulty of their repair as compared with other types of DNA damage. In this study we have analyzed genes expression of the main genes involved in DNA Double Strands repair pathways in 20 early stage (pT1cN0M0) breast cancers.
A total of 13 genes implicated in both homologous recombination (HR) and Non-homologous End Joining (NHEJ) mechanisms were analyzed on a 7700 Sequence Detection System (Applera Italia) by Quantitative Reverse Transcription Polymerase Chain Reaction, using MGB probes chemistry. For each case matched pathologically normal breast tissue and tumor were analyzed. Expression of the target gene was normalized by the expression of the housekeeping gene GAPDH and for each gene mRNA levels were determined as relative expression (RE) in the tumor as compared with matched normal tissue (RE in the normal equal to 1). Thus RE value < 1 indicate reduced expression and RE >1 indicate an increased expression. A substantial reduction in mRNA relative expression was found for the majority of the genes tested.
Approximately 70% of the tumors showed down regulation for MRE11, RAD52, BRCA1, G22P1, and XRCC5. ATR, NSB1, RAD50 and RAD54, and Artemis were down regulated in approximately 60% of the cases. The up-regulated genes were RAD51 (70% of the cases), BRCA2 (60%) and ATM (50%). The majority of the cases showed RE value from 0.5 to 1.5 corresponding to a 50% down or up-regulation in the tumor as compared with matched normal tissue. However, a subgroup of breast cancer (approximately 50% of the cases) showed a marked reduction of RE expression levels for ATR, NSB1, MRE11. For these genes, RE value were between 0.062 e 0.315 corresponding to reduction in mRNA levels from 94% to 70% as compared with matched normal breast tissue.
Overall our data suggest a substantial down-modulation of both mechanisms involved in DNA double strands break repair in a relative early stage of breast cancer progression. Moreover our data indicates a marked down-regulation of the MRE11 complex (MRE11/NSB1/RAD50) in at least half of the tumors. This complex plays a role in each of the aspects of chromosome break metabolism acting as a break sensor, in the activation and propagation of checkpoint signalling pathways and in promoting recombination between sister chromatides.

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Investigation of ICAM-1 Genetic Markers (+241G/A and 469 A/G) in patients with breast cancer

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Background: ICAM-1 is an adhesion molecule which plays a key role in leukocyte recruitment and migration. ICAM-1 also acts as a co-stimulatory molecule for T cell activation and effector function. In this investigation, the frequency of ICAM-1 genetic markers (+241 G/A and +469 A/G) were analyzed in breast cancer patients and healthy control group.
Method: 269 patients and 231 female healthy controls were genotyped for polymorphism in Exon 4 of ICAM-1 gene (+241 G/A) using an Allele Specific Polymerase Chain Reaction (AS-PCR). Exon 6 (**+**469 A/G) dimorphism in ICAM-1 gene was also investigated in 250 patients and 184 healthy controls using a PCR− Restriction Fragment Length Polymorphism (PCR-RFLP).
Results: There was a significant decrease of GG genotype at position +241 G/A, in patients in comparison to the healthy controls (85.5% vs. 93.5%, respectively; P = 0.003). The frequency of G allele at this position, among patients and controls were also found to be 92.8% and 96.7%, respectively. Accordingly, the decrease in the frequency of G allele in patients was statistically significant (P = 0.004). There were no significant differences in allele or genotype frequencies between patients and controls in the case of **+**469 A/G polymorphism.
Conclusion: Data of this investigation conclude that polymorphism at position +241 of ICAM-1 gene may be associated with the susceptibility to breast cancer. To confirm this finding, the protein expression and assessment of sICAM-1 in these patients are under investigation.

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Thomsen-Friedenreich antigen – prognostic factor in primary breast cancer tissue, expression on disseminated tumor cells and target for immunomagnetic enrichment

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Introduction: The Thomsen-Friedenreich (TF) antigen is a specific oncofetal carbohydrate epitope (Galb1−3GalNAca-O-epitope) expressed on the surface of various carcinomas. It mediates endothelium adhesion and tumor invasion. The presence of disseminated tumor cells in the bone marrow of breast cancer patients (DTC-BM) indicates worse prognosis at all stages of the disease. We therefore examined the expression of TF in primary breast cancer tissue and on DTC-BM. Additionally, TF was examined as a target for the immunomagnetic enrichment of DTC-BM.
Methods: TF expression was examined immunohistochemically on Tissue Micro Arrays (TMA) of 265 breast carcinomas from patients with known bone marrow status, using the semiquantitative immunoreactive score (IRS). The prognostic impact together with that of DTC-BM was calculated by Kaplan-Meier and Cox-regression analysis. Second, bone marrow of 25 patients screened positive for DTC-BM was double stained for cytokeratin (CK) / TF and TF / MUC1 by immunofluorescence. Third, immunomagnetic enrichment with anti-TF antibody and Cytokeratin (CK) staining was done on bone marrow samples of 48 patients.
Results: TF expression was demonstrated on 136 / 169 evaluable TMAs. Median IRS score was 2 (0−12). 68 of the 265 patients (25.7%) showed DTC-BM. TF positivity correlated with HER2 negativity (p = 0.048), but not with other histological parameters or DTC-BM. After a follow up of 60.5 months (7−255), the presence of DTC-BM showed prognostic significance for OS (p = 0.032), whereas TF negativity was significant for DFS (p = 0.032), DDFS (p = 0.021) and OS (p = 0.026). Double staining experiments on DTC-BM showed co-expression of TF and CK in 98% of the cells. After immunomagnetic enrichment, 31/48 pts showed DTC-BM, increasing positivity rate from 20.8% to 64.6%.
Discussion: TF seems to be a prognostic factor in breast cancer. It shows nearly complete expression on DTC-BM and is by this a suitable marker for immunomagnetic enrichment of those cells. Antibody based therapy against TF and vaccination studies showed efficacy *in vitro* and in animal models.