310 POSTER

MALDI-TOF serum protein profiling for the detection of breast cancer using independent validation

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Background: With a lifetime risk currently estimated 1 in 9, breast cancer is among the most common diagnosed malignancies in women. Proteomic expression profiling generated by mass spectrometry has been suggested as a potential tool for the early diagnosis of cancer. The objective of our study was to assess and validate the feasibility of this approach for the detection of breast cancer.
Methods: In a randomized block design pre-operative serum samples obtained from 63 breast cancer patients and 73 controls were used to generate high-resolution MALDI-TOF protein profiles as a calibration set. The median age of the patient and control group was respectively, 52 (20−81) and 57 years (39−87). The MALDI-TOF spectra generated using WCX magnetic beads assisted mass spectrometry (Ultraflex) were smoothed, binned and normalized after baseline correction. After pre-processing of the spectra, linear discriminant analysis with double cross-validation, based on principal component analysis, was used to classify the protein profiles. Consequently, the classifier constructed on the first 2 plates was applied on the spectra of an independent validation set. This validation set consisted of serum samples from 29 breast cancer patients and 38 controls. The median age was 59 years (26−87) and 57 years (24−71) for the patient and control group respectively.
Results: Double cross-validatory analysis carried out on the protein spectra of the calibration set yielded a total recognition rate of 86%, a sensitivity of 88% and a specificity of 84% for the detection of breast cancer within the calibration set. The AUC of this classifier was 90.3%. When this classifier was applied on the spectra of the independent validation set a total recognition rate of 80.9%, a sensitivity of 72% and a specificity of 89% were found.
Conclusions: The use of a randomized block design, but mainly an independent validation set proves that discriminating protein profiles can be detected between breast cancer patients and healthy controls. The high sensitivity and specificity indicate that serum protein profiles could be an promising option for the detection of breast cancer.

311 POSTER

Early discovery of genomic correlates of drug response by high-resolution gene copy number profiling

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Cancer is a highly heterogeneous disease in terms of the genetic profile and the response to therapeutics. An early identification of a predictive genomic marker in drug discovery may help select patients that would respond to the drug in clinical trials. Here we outline our strategy for early identification of genomic correlates of response, which utilizes high-resolution comparative genomic hybridization (CGH) coupled with drug sensitivity screening. The resolution and reproducibility of CGH is optimized by using high-density genotyping arrays that interrogate 114K SNPs distributed across the genome at a median distance of 24 kb. The data are analyzed using an internally developed bioinformatics software package that enables identification of gene copy number changes correlating with a pre-defined class label (drug sensitivity/resistance). Additionally, the software also permits identification of recurrent aberrations and produces complete information on all genes residing within the region of aberration. This, together with convenient visualization features, facilitates target discovery.
We applied this methodology to discover genomic correlates of sensitivity to a novel small-molecule antagonist of Bcl-2 family proteins recently discovered at Abbott Laboratories and shown to induce tumor regression in mouse models. The compound, ABT-737, was tested in a panel of small-cell lung carcinoma, leukemia, and lymphoma cell lines and revealed differential inhibition in each of the cancer types (10 nM mM). The CGH screen of these lines followed by Fisher's exact test analysis revealed a previously unknown amplification on 18q21−22 that was associated with the sensitivity of SCLC cell lines to Bcl-2 antagonists. The region of gain contained several genes known to be critical mediators of apoptosis. Expression microarray profiling showed that the genes residing in the amplified region of 18q are also overexpressed in the sensitive lines relative to the resistant ones. Analysis of SCLC tissue microarrays by FISH revealed that the gain of 18q21.22 is a frequent event in SCLC. Thus, our findings suggest that 18q21−22 copy number will be a clinically relevant predictor for sensitivity of SCLC to Bcl-2 family inhibitors. This genomic marker may have a broader application in cancer, as it leads to overexpression of genes associated with apoptosis evasion and chemoresistance. Taken together, our data demonstrate the potential value of high-resolution CGH for identification of genomic biomarkers.

312 POSTER

Proteomic profiling of invasive cancer cells reveals a novel prognostic marker for human breast cancer

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Unlocking the mysteries of metastasis, a major cause of cancer mortality, is essential for the development of novel therapies and diagnostic methods. Cell surface constituents are the key players in interactions needed for a metastatic dissemination of cancer. We are using subclones of the MDA-MB-435 human carcinoma cell line as a model for the metastatic spread of cancer. One clone metastasizes consistently to the lungs whereas the other fails to grow at any distal site in athymic mice. To study the metastatic process we biotinylated the cell surface proteins and isolated them from the post-nuclear supernatant with magnetic streptavidin beads. Isolated proteins were analyzed with 2D gel electrophoresis and mass spectrometry. Galectin-3 was found to be expressed only on the surface of the metastatic cell line. The expression level of this protein is known to correlate with cancer aggressiveness and metastasis. MHC class I antigen, on the other hand, was down-regulated in the metastatic cell line compared to the non-metastatic cell line. This down-regulation is thought to be associated with tumour invasion and development. We have also identified novel cell surface proteins that could contribute to the formation of distant metastases. In addition, we have discovered two novel splice variants of one of the novel cell surface proteins. These splice variants were expressed as GFP-fusion proteins in order to follow their intracellular localization and effect on cell proliferation, invasive growth and tumour formation in mice. The expression of these splice variants affected the localization of the endogenous protein. In addition, we have analysed the localization and levels of this protein in the array of human breast carcinoma samples (n = 350). This analysis revealed a significant correlation between the expression and localization of our protein and the survival of the patients indicating this protein as a novel prognostic marker for human breast cancer.

313 POSTER

Comparison of molecular determinants of angiogenesis and lymphangiogenesis in lymph node metastases and in primary tumours of patients with breast cancer

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Background: Angiogenesis and lymphangiogenesis are complex processes, driven by multiple factors. In primary breast tumours (PTs), VEGFA, -C and -D are the most important (lymph)angiogenic factors. Recently, the induction of lymphangiogenesis in axillary lymph node (LN) metastases of patients with breast cancer was described. The aim of this study was to compare the molecular determinants of (lymph)angiogenesis in LN metastases and in PTs of patients with breast cancer.
Materials and Methods: RNA was isolated from FFPE tissue sections of a metastatically involved and uninvolved LN and the PT of 26 lymph node positive patients. By qRT-PCR, the expression of 12 (lymph)angiogenic markers was measured. The expression was correlated with tumour cell proliferation, angiogenesis and lymphangiogenesis, quantified by tumour cell proliferation fraction (TCP%) and (lymphatic) endothelial cell proliferation fraction [(L)ECP%]. TCP%, ECP% and LECP% were assessed on IHC double-stains for CD34/Ki-67 and D2−40/Ki-67, respectively.
Results: In involved LNs, the relative gene expression levels of PROX1 ($p < 0.001$) and FGF2 ($p = 0.008$) were decreased and the expression levels of VEGFA ($p = 0.01$) and PDGFB ($p = 0.002$) were increased compared to uninvolved LNs. The expression of most markers was increased in PTs compared to involved LNs. In metastatically involved LNs, the expression of VEGFA correlated with ECP% ($r = 0.54$, $p = 0.009$) and LECP% ($r = 0.76$, $p < 0.001$). In PTs, VEGFA correlated only with ECP% ($r = 0.74$, $p < 0.001$).