Abstracts for poster presentation

PP112

A quantitative methylation specific PCR assay to determine promoter methylation status of the MGMT gene

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Background: MGMT (O6-methylguanine-DNA methyltransferase) is a DNA repair enzyme that is involved in the repair of damage caused by a variety DNA crosslinking compounds, including alkylating agents. Increased methylation of the MGMT gene promoter region causes diminished or silenced expression of the gene, making cells more sensitive to DNA damage. This relationship has been shown for glioblastomas and alkylating agents such as Temodar® (temozolomide). Approximately 30% to 45% of glioblastoma multiforme (GBM) tumors exhibit MGMT gene methylation. Retrospective studies have shown that detection of MGMT promoter methylation in tumor samples is associated with an increased likelihood of a favorable response to temozolomide.
Materials and Methods: Tissue sections from paraffin-embedded (FFPE) samples were evaluated for MGMT promoter methylation. Quantitative methylation specific PCR (QMSP) was used to determine the number of copies of both the methylated MGMT promoter and the β-actin gene in each sample. The β-actin gene was used as an internal normalization control, and to determine the quality and sufficiency of DNA from the samples.
Results: 135 previous characterized specimens were used for accuracy study. Results of 10 specimens could not be obtained due to low β-actin gene level. Besides 2 specimens with borderline methylation value, 123 out of 125 (98.4%) specimens were concordant. An additional 250 formalin-fixed, paraffin-embedded samples were evaluated with the QMSP assay, with approximately 33% demonstrating evidence of MGMT promoter methylation. Inter-assay and intra-assay reproducibility were determined to be 100% based upon the qualitative methylation result.
Conclusion: The QMSP assay for MGMT promoter methylation status is a robust and reproducible assay in FFPE samples. The observed percentage of positive cases is comparable to published findings in which 30−45% of GBM specimens have been shown to have a methylated MGMT gene promoter. The MGMT DNA methlyation assay may be used to provide information on both prognosis and potential response to chemotherapeutic agents in GBM.

PP102

Monitoring BCG immunotherapy for high risk urothelial cancer of the urinary bladder − a novel biomarker

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Background: Intravesical immunotherapy with *Mycobacterium bovis* BCG is an effective treatment option for high risk superficial urothelial cell carcinoma of the bladder. In spite of extensive research the underlying mechanism remains unclear. Furthermore, outcome can not be predicted individually due to the lack of a suitable marker and is indirectly estimated from clinical parameters such as tumor size, previous second transurethral resection and others. Therefore, patients may be at a risk risk for progression or recurrence due to delayed other treatment.
The purpose of this study was to identify an easy to determine serum marker capable of directly measuring the immune stimulatory effect of BCG to predict individual patients outcome and possibly optimize the therapy protocol.
Materials and Methods: Because sera samples are easily accessible, we screened the circulating pool of immunoglobins from a patient after successful BCG-immunotherapy with a combinatorial random peptide library to identify corresponding target antigen(s). The antigens were then validated as marker for immune activation and clinical outcome.
Results: We selected, isolated, and validated an immunogenic peptide motif from *M. bovis* BCG Heat Shock Protein (HSP)-65 as an immunodominant epitope of the humoral response following BCG-immunotherapy. Increasing IgA and IgG anti-HSP-65 titers predicted specifically a positive patient outcome in a cohort of bladder cancer patients, relative to several cohorts of control patients.
Conclusion: This is the first study to report a serological biomarker capable of directly measuring BCG-immunoresponse and predicting individual outcome. Subsequent studies will determine the value of this candidate marker to modify BCG-based treatment for individual bladder cancer patients.

PP92

Molecular changes predicting response to therapy and prognosis among patients with stage IIIB breast cancer

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Background: Patients with T4 tumors of any type, with or without lymph node involvement, and without metastases (T4 N0−2 M0), are classified with stage IIIB breast cancer which is considered one of the worst stages of prognosis. Characterization of molecular mechanisms associated with such a disease could help with patients' stratification and management. In the present study, we examined alterations in survivin-p53-pERK1−2 expression and CyclinD1-h-prune gene copy number among stage IIIB breast carcinomas, in order to determine their association with clinico-pathological parameters and patients' prognosis.
Materials and Methods: Paraffin-embedded samples from 53 consecutive stage IIIB patients underwent immunohistochemistry and FISH analysis. Chi-square and Fisher's exact tests were used to evaluate correlation with treatment responses [complete clinical response (cCR), partial clinical response (cPR), pathological complete response (pCR), major pathological response (MpR; corresponding to pT0–pT1 classification after primary chemotherapy)] and survivals.
Results: Overexpression of survivin, p53, and pERK1−2 as well as amplification of h-prune and cyclinD1 were evaluated for association with several histological tumour characteristics: estrogen and progesterone status, HER2 amplification, Ki67 proliferation index. No statistically significant correlation was observed, with the exception of an inverse distribution of positive pERK1−2 and Ki67 expressions [absence of pERK1−2 staining in 16/42 (38%) Ki67+ cases vs. 4 (15%) pERK1−2+ tumours in 26/42 (62%) Ki67− cases]. The Ki67 and HER2 parameters were significantly associated with better clinical response rates [5/7 (71%) cCR vs. 11/35 (31%) cPR and 8/8 (100%) cCR vs. 29/45 (64%) cPR, respectively], whereas pERK1−2 expression was significantly associated with worse clinical response rates [0/8 cCR vs. 5/45 (11%) cPR]. Univariate analysis showed a significant association to better survivals in breast cancer cases with absence of h-prune amplification, pERK1−2 immunostaining, and survivin expression. After multivariate analysis, pathological response to primary chemotherapy and survivin expression remained the only parameters closely correlated to prognosis.
Conclusion: Although our study is retrospective and based on a relatively small number of patients, our findings provide some important indications about the prediction of the response to therapy and the role on prognosis in stage IIIB breast cancer patients.

PP22

TGFB1−509C>T and IL10−92C>A polymorphic variants in relationship to breast cancer progression and response to neoadjuvant chemotherapy

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Background: Genetic polymorphisms are responsible for inter-individual variation and diversity and have been recently considered as the main

genetic markers involved in the development and progression of breast cancer. The aim of our present study was to determine the association of combinations of TGFB1−509C>T (rs1800469) and IL10−592C>A (rs1800872) genotypes with the clinicopathological parameters and response to neoadjuvant chemotherapy.
Materials and Methods: Case patients were newly diagnosed breast cancer patients T1−4N0−2M0 (n = 186; age from 20 to 79 years) which were cared in Tomsk Cancer Research Institute. The healthy women (n = 190; age from 30 to 75 years) from Western Siberian region were used as the control group. Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism method.
Results: Frequency of combination TGFB1(C/C) and IL10(C/A) genotypes was significantly higher in non-aggressive phenotype of breast cancer as defined by the absence of axillary lymph node metastasis ($p < 0.05$). A significant difference in distributions of genotypic combinations of TGFB1(C/T) and IL10(C/C) between women with multicentric and unicentric breast cancer was found (52.6% and 27.3% respectively; $p < 0.02$). We have revealed that high frequency of TGFB1(C/C) and IL10(C/A) variants was observed in patients with basal (hormone receptor-negative and HER2-negative) tumor against breast cancer patients with luminal subtype tumor (estrogen receptor-positive) ($p < 0.0004$). In contrast, 10.2% of luminal subtype patients were found to carry the combination of homozygous TGFB1(T/T) and IL10(C/C) genotypes compared to 2.3% of basal subtype patients ($p < 0.09$). In addition, this combination of TGFB1(T/T) and IL10(C/C) genotypes was related to a favorable response to neoadjuvant chemotherapy ($p < 0.05$).
Conclusion: Our data suggest that the genetic variants of TGFB1−509C>T and IL10−592C>A are associated with the progression of breast cancer. The combination of homozygous TGFB (T/T) and IL10(C/C) variants may be a potential prognostic marker for response to neoadjuvant chemotherapy.

PP61

Detection of breast cancer markers in serum by surface-enhanced laser desorption/ionisation time-of-flight mass spectrometry (SELDI-TOF-MS)

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Background: Breast cancer is one of the most frequent and deadly cancers worldwide. Although the survival of patients has increased over the last decades, many patients die from metastatic relapse. Progresses in screening or early diagnosis will improve survival of breast cancer and reduces breast cancer-related morbidity. Although breast cancer biomarkers offer a promising means of detecting this disease at the earliest and most treatable stages there has never been any good serum tumor markers for early detection. The purpose of this study was to identify and evaluate a proteomics approach to search for new biomarkers in serum of breast cancer patients.
Materials and Methods: Blood samples of 50 women with breast cancer (CA) and 50 healthy women (CTRL), matched to the age, were drawn prior to surgery. We used SELDI-TOF-MS for protein profiling with three different active surfaces of the protein chips: cationic exchanger (CM-10), hydrophobic surface (H50) and a strong anion exchange surface (Q10) with different binding properties. Data were analyzed by multivariate statistical techniques and artificial neural networks.
Results: SELDI-TOF-MS could discriminate between serum of breast cancer patients and healthy women. We could generate a statistic significant ($p < 0.001$) panel with 15 biomarkers resulting of multiple peaks with different molecular weights. The diagnostic pattern could differentiate CA from CRTL with specificity of 77% and sensitivity of 85% in serum.
Conclusion: In this study a new biomarker panel in serum was successfully generated to allow breast cancer patients to be discriminated from healthy women. This promising approach provides a high sensitivity and specificity by a less invasive method similar to mammography that is used in screening programs. Therefore this study could also exemplify SELDI-TOF-MS as a potential screening method to detect breast cancer and for high-throughput biomarker discovery.

PP97

The human carcinoembryonic antigen (CEA) predicts therapeutic response towards VEGF-targeting therapies in colorectal cancer

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Background: Angiogenesis represents a key element in the pathogenesis of malignancy. Studies from our laboratory have shown that carcinoembryonic antigen (CEA) functions as a major mediator of angiogenesis apart and independently from VEGF. As patients with metastatic colorectal cancer (mCRC) present with various plasma levels of CEA, we have analyzed whether the efficacy of anti VEGF treatment with bevacizumab was dependent upon plasma CEA levels.
Materials and Methods: To analyze a so far undescribed clinical relevance of CEA in angiogenesis, we retrospectively analyzed relevant data of 271 patients with mCRC, who were treated with bevacizumab plus chemotherapy consisting of fluorouracil and leukovorin or capecitabine in combination with oxaliplatin (FOLFOX, XELOX) or irinotecan (FOLFIRI, XELIRI) (n = 145) or − as control − chemotherapy (FOLFOX or FOLFIRI) plus cetuximab (n = 126). It was analyzed whether baseline CEA correlated with the overall response rate (ORR) consisting of complete remission (CR), partial remission (PR) or stable disease (SD) for at least three months according to RECIST (Response Evaluation Criteria In Solid Tumors). Patient cohorts according to CEA plasma levels were generated: <5 ng/ml (n = 36), 6−30 ng/ml (n = 47), 31–100 ng/ml (n = 26), >100 ng/ml (n = 36).
Results: Baseline CEA plasma levels inversely correlated with therapeutic response in patients receiving bevacizumab-based treatment (P for trend <0.001: OR = 0.52, 95% CI 0.36, 0.74). No such association was found in patients receiving cetuximab-based therapy. ORR in patients with mCRC receiving bevacizumab-based treatment in dependence upon plasma CEA levels can be seen as follows: with a CEA plasma level of <5 ng/ml the ORR was 92.7% (No. of Pat. 41), CEA plasma level 6−30 ng/ml the ORR was 80.4% (No. of Pat. 46), CEA plasma level 31–100 ng/ml the ORR was 60.9% (No. of Pat. 23) and CEA plasma levels >100 ng/ml the ORR was 59.0% (No. of Pat. 39).
Conclusion: Bevacizumab has been introduced as a potent anti-angiogenic therapeutic tool in the management of mCRC. CEA plasma levels might represent an important predictor of treatment response to bevacizumab-based treatment.

PP74

Detection of K-RAS oncogene mutations using PCR and pyrosequencing

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Background: Mutations in the K-RAS oncogene are frequently found in human cancers, including colon cancers, lung cancers, pancreatic cancers, and other cancers of the GI tract. In these cancers, K-RAS mutations are often associated with patient prognosis or drug response. Several recent studies have shown that K-RAS mutation status is an important biomarker of response to monoclonal antibody based therapies, i.e., Erbitux™ (cetuximab) and Vectibix™ (panitumumab), which are targeted against the EGFR cell surface receptor. A variety of methods exist to detect K-RAS mutations in tumor samples including both PCR and sequencing based platforms. In this evaluation we have examined the analytical performance of both a PCR based method (DxS) and a pyrosequencing platform (Biotage).
Materials and Methods: DNA was isolated from formalin-fixed, paraffin-embedded (FFPE) specimens and analyzed for mutations in the K-RAS gene using either the ARMS (amplification refractory mutation system) in combination with a Scorpion primer or PCR and pyrosequencing technologies. The ARMS assay detects the 7 most common alterations in codons 12 and 13 of the K-RAS gene. The pyrosequencing method using the PyroMark Q24 software quantifies mutations in codons 12, 13 and 61.
Results: Intra-assay and inter-assay reproducibility for both methods was performed on pooled isolations from a series of tissues previously shown to have both wild-type and mutant versions of the K-RAS gene. These studies showed 100% reproducibility of both assay platforms. The accuracy of the ARMS assay was assessed using a series of previously characterized tissue samples and cell lines. The ARMS assay showed 100% concordance for the samples evaluated. For the pyrosequencing assay a series of sample previously characterized by the ARMS assay were evaluated