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.

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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.

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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