Poster Presentations (Mon, 26 Sep, 09:30−12:00)
Diagnostic/Biomarkers

1409 POSTER

Highly Sensitive Detection of MicroRNa and MRNa From FFPE Tissue and Blood Samples by Expression Microarray

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Background: Gene expression profiling of readily available clinical samples, such as blood or FFPE tissue, is a promising method to discover novel diagnostic markers. As RNA is subjected to degradation even in properly-collected tissue samples, it is more difficult to obtain intact RNA from FFPE or body fluid samples for diagnostic analysis. ***3D-Gene***™ is highly sensitive gene expression microarray, featuring the unique micro-columnar structure on the platform substrate and the beads agitation system during the hybridization reaction. Using ***3D-Gene***™, we achieved highly sensitive and reproducible detection of mRNA or miRNA from FFPE tissue samples.
Material and Methods: Total RNA was extracted from human serum, plasma and frozen or FFPE tissue samples, with the recommended protocol for each sample. For mRNA detection, total RNA was reverse-transcribed to cDNA and labeled with fluorescent dye directly or after the amplification. For miRNA detection, total RNA was labeled with fluorescent dye directly. These pretreated target nucleotides were hybridized to ***3D-Gene***™ while the hybridized buffer containing target nucleotides was agitated by beads during hybridization. The hybridized microarrays were washed and scanned for image acquisition.
Results: The result was highly correlated with the expression profiles from frozen tissue samples. Furthermore, exosomal miRNA from serum or plasma was also detected with high sensitivity and reproducibility. From these analyses of FFPE tissue or blood samples, we found potential miRNA biomarkers for various cancers.

i. Using ***3D-Gene***™, we detected mRNA expression profile from FFPE samples with high reproducibility. We also showed high correlation of the expression profiles between FFPE and frozen tissue samples. Furthermore, microRNA obtained from frozen as well as FFPE tissue samples was reproducibly detected at atto-molar level. Some miRNA biomarkers for various cancers were found from FFPE samples.
ii. Serum and plasma are suggested to contain microsomes in which miRNA is enclosed. miRNA from serum and plasma samples were detected with high sensitivity and reproducibility with ***3D-Gene***™. Some miRNA biomarkers for various cancers were found from patients' serum.

Conclusions: The Application of our ***3D-Gene***™ for the gene expression analysis of clinical samples could bring a formally unexplored venue in the biomarker discovery and diagnostic field.

1410 POSTER

Hypoxia in Epithelial Ovarian Cancer − Remodelling the Epigenome and Paclitaxel Response

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Background: Chemoresistance continually restricts the efficacy of first line paclitaxel based regimes in patients with epithelial ovarian cancer (EOC). This is in part due to paclitaxel's anticipated pharmacological response in a proliferative normoxic environment despite our knowledge of the characteristic hypoxic and senescent nature of such solid tumours inherently known to be chemoresistant.
Evidence is accumulating that chemoresistance pathways may be epigenetically regulated by the atypical expression of the Chromatin Remodeling Polycomb Group (PcG) proteins BMI1 and EZH2. Specifically, the recent identification of oxygen as a mediator in PcGs function and in light of the emergent understanding of their impedance upon anti-mitotic drug function exemplified by Taxol®, warranted investigation into the potential effects of the hypoxic tumour microenvironment on BMI1 and EZH2 and a combined putative role in chemoresistance to paclitaxel in EOC.
Materials and Methods: The ovarian cancer cell lines A2780, OVCAR7 and UPN251 were cultured in normoxia (21%) and hypoxia (1% O2) for 24, 48 and 72 hours followed by western blot analyses of BMI1 (35 kDa) and EZH2 (90 kDa). The effect of hypoxia (1%) on paclitaxel (100nm) response was examined by the MTT viability assay. Chemoresponse was also established following siRNA specific targetting of BMI1 and EZH2.
Results: Western blot analysis demonstrated that 72 hours hypoxic (1%) exposure significantly affected the protein expression of BMI1 and EZH2 in A2780, OVCAR7 and UPN251 compared to their normoxic controls. A2780, the cell line most sensitive to paclitaxel, over-expressed BMI1 and EZH2 in hypoxia, while OVCAR7 and UPN251 showed decreased expression of both PcG proteins. The MTT assay demonstrated that increased exposure to hypoxia (1%) was coincident with increased resistance to paclitaxel (100nm) in the three cell lines. Moreover, siRNA knockdown of both polycomb proteins resulted in increased chemoresistance.
Conclusion: We have previously published that hypoxia, a key feature of the tumour microenvironment alters the global epigenome. This current study further demonstrates that the master chromatin remodellers BMI1 and EZH2 are also altered in hypoxia. Moreover both the hypoxic environment and siRNA selective knockdown of both BMI1 and EZH2 resulted in increased viability and cellular resistance to paclitaxel. We suggest that differential PcG levels induced by hypoxia impact on paclitaxel responsiveness possibly through global epigenomic changes in the transcriptome.

1411 POSTER

Endpoints for Validation of Tumour Markers for Recurrence Risk − Recurrence-free Interval (RFI), Disease-free Survival (DFS), Overall Survival (OS), and Colon-cancer Specific Survival (CCSS) in CALGB 9581

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Background: Tumour markers for recurrence risk reflect the underlying tumour biology and would not be expected to anticipate events unrelated to tumour behavior. DFS and OS are endpoints that may reflect non-colon cancer related events. We examined the validated 12-gene Colon Cancer Recurrence Score (RS) and mismatch repair (MMR) protein status for relationship to endpoints RFI, DFS, OS, and CCSS in CALGB 9581, a negative trial of edrecolomab in stage II colon cancer.
Materials and Methods: Tumour was available for 1361/1738 (78%) patients (pts). Cohort sampling included all pts with recurrence who had banked tissue and randomly sampled pts without recurrence (3:1 ratio). The 12-gene RS (Genomic Health, Inc.) was obtained by immunohistochemistry on primary tumour specimen. MMR status (D= Deficient; I= Intact) was assessed by IHC for MLH1 and MSH2. Univariate weighted Cox proportional hazards models were used to test the association between tumour markers and endpoints based on a Wald-type test statistic constructed using weighted partial likelihood and robust variance estimates.
Results: 690 evaluable patients had 162 recurrences and 192 deaths (96 disease related, 66 not related, 30 unknown). Median follow up was 8 yrs and the recurrence risk was relatively low compared to other studies. Percent of pts with events at 5 years: 15% (RFI), 20% (DFS), 14% (OS) and 8% (CCSS). In pts >70 yrs (35%), only 47% of deaths were preceded by colon cancer recurrence. The continuous RS was significantly associated with RFI (hazard ratio (HR)/ 25 units, 1.52; 95% CI, 1.09–2.12; $p = 0.01$) and CCSS (HR = 1.58, $p = 0.03$) but not DFS and OS (both $p > 0.8$). MMR-D was also associated with RFI (HR, 0.62; 95% CI, 0.39–0.99; $p = 0.04$) but not with other endpoints (all $p > 0.18$). Age (<70 vs. 70+) was the strongest predictor of OS and DFS (both $p < 0.001$) but not RFI or CCSS (both $p > 0.25$).
Conclusions: In stage II colon cancer patients on CALGB 9581, RS and MMR-D were most strongly associated with RFI, an endpoint directly reflecting tumour biology. Among survival endpoints, CCSS was the most sensitive to RS. Other causes of death may explain the insensitivity of DFS and OS to RS. Selection of endpoints for tumour marker studies of recurrence risk should consider the likelihood of events unrelated to tumour behavior.

1412 POSTER

Genetic Markers in Relation to Bevacizumab-induced Hypertension

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Background: There are currently no biomarkers predicting outcome or toxicity associated with bevacizumab treatment for advanced solid tumours.

Here, we combined biomarker studies from several phase III trials with bevacizumab to systematically assess whether genetic variation in VEGFA pathway genes and other genes is associated with bevacizumab-induced hypertension.
Methods: Germline DNA was available from 628 patients diagnosed with advanced primary colorectal (NO16966), pancreatic (AVITA), non-small cell lung (AVAiL), renal (AVOREN) and breast (AVADO) cancer and treated with bevacizumab. Toxicities were identified from clinical trial reports and graded according to common toxicity criteria. Overall, 113 patients had grade 1−4 bevacizumab-induced hypertension (assessed across trials with CTCAE v2−3). A total of 158 single nucleotide polymorphisms (SNPs) located in VEGFA, VEGFA-receptors (FLT1 and KDR) and other genes were selected using a SNP tagging approach and genotyped using MALDI-TOF mass spectrometry. A logistic regression on individual patient data was performed after stratification for cancer type and other covariates.
Results: Ten SNPs were associated with bevacizumab-induced hypertension ($p < 0.05$), but none of these surpassed the threshold for multiple testing ($p < 0.0003$). The three SNPs showing the strongest association ($p < 0.01$) were: rs2305949 in KDR (allelic OR 0.93, 95% CI 0.88–0.98, $p = 0.0059$), rs4444903 in EGF (allelic OR 1.06, 95% CI 1.02–1.11, $p = 0.006$); and rs1680695 in EGLN3 (allelic OR 1.07, 95% CI, 1.02–1.12, $p = 0.008$). Interestingly, rs2305949 and rs4444903 were closely linked to amino acid changes occurring on position 273 and 708 of KDR and EGF, suggesting that these changes may functionally affect both genes and thereby contribute to hypertension. Notably, rs11064560 in WNK1 was also associated with bevacizumab-induced hypertension (allelic OR 1.06, 95% CI 1.01–1.11, $p = 0.02$), thereby supporting previous observations in a limited number of patients [Frey et al. ASCO 2008].ents [Frey et al. ASCO 2008].
Conclusions: Our study represents a large genetic analysis of bevacizumab-induced hypertension using pooled data sets. The genes described warrant further investigation for their potential role in the safety profile of bevacizumab.

1413 POSTER

Expression Analysis and Study of BCL2 and the Novel Member of the Apoptotic Genes BCL2L12, as Promising Biomarkers for Monitoring of Prostate Cancer Cells' Response to Chemotherapy

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Background: Cancer continues to constitute a global public health problem. Chemotherapy is an effective approach for combating this complicated disease; however there is an urgent need of biomarkers for monitoring patients' response to it. *BCL2* gene family members, including the novel *BCL2L12* discovered from members of our research group, are known to be extensively implicated in apoptosis and their aberrant expression has been correlated with cancer progression.
Materials and Methods: The present study aims to reveal any apparent modulations in the mRNA levels of apoptotic genes belonging to the *BCL2* apoptotic gene family, including the recently identified member *BCL2L12*, upon treatment with broadly used chemotherapeutic agents. Any apparent modulations of these genes could reveal their potential role in monitoring chemotherapy response in human malignancies.
The cytostatic action of each drug was evaluated in PC3 and DU145 prostate human cancer cell lines under study, employing the MTT and trypan blue assays. Total RNA was isolated from control and treated with appropriately selected anticancer drug concentrations cells and 2 μg of it were reverse transcribed into cDNA. The expression levels of the genes under study were determined using conventional and Real-Time PCR, employing proper housekeeping genes for normalization.
Results: Increased concentrations and exposure time of the administered chemotherapeutic compounds lead to the reduction of cancer cells' proliferation efficiency. Moreover, important modulations occurred in the mRNA levels of the genes under study, fact that implicates them in distinct biochemical pathways induced upon administration of various anticancer compounds in malignant cells.
Conclusion: Our results could help towards identifying molecules which take part in the response of cancer cells to chemotherapy and could provide valuable information about the potential of novel genes that encode parts of the apoptotic machinery as valuable tools in monitoring cancer patients' response to chemotherapy, ultimately leading to more focused anticancer treatment strategies.
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1414 POSTER

Single Nucleotide Polymorphism Analysis and Outcome in Advanced-stage Cancer Patients Treated With Bevacizumab

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Background: There are no validated biomarkers predicting benefit from bevacizumab (bev) therapy. In an effort to identify such markers, biomarker studies have been integrated into several Phase III trials with bev in an attempt to correlate genetic variability in VEGFA pathway genes with the therapeutic efficacy of bev.
Methods: Germline DNA was available from 1346 subjects diagnosed with advanced primary colorectal (NO16966), pancreatic (AVITA), non-small cell lung (AVAiL), renal (AVOREN) and breast (AVADO) cancer. Overall, 628 subjects received bev. Common single nucleotide polymorphisms (SNPs) located in the hypoxia-inducible factors (HIF-1A and EPAS1), VEGFA, VEGFA-receptors (VEGFR1 and VEGFR2) and several other genes were selected using a SNP tagging approach. A total of 158 SNPs were genotyped using MALDI-TOF mass spectrometry. A meta-analysis of individual patient (pt) data was performed, after stratification for cancer type and other covariates. Genetic associations were assessed using Cox Proportional Hazard Regression for progression-free survival (PFS) and overall survival (OS).
Results: The rs4145836 SNP in EPAS1 was most significantly associated with improved PFS in both bev-treated pts (allelic HR 0.68, 95% CI 0.56–0.82, $p = 0.0001$) and placebo pts, suggesting that this SNP may be a prognostic marker for outcome independent of bev. The rs699946 SNP, located in the VEGFA promoter, was associated with improved PFS in bev-treated subjects with an allelic HR of 1.27 (95% CI 1.08–1.49, $p = 0.003$). No effect was seen in placebo subjects, suggesting that rs699946 may be a predictive marker for favourable outcome with bev treatment. The nearby rs699947 SNP in VEGFA, which has previously been associated with bev treatment outcome in breast cancer [Schneider et al. 2008], was not associated with a PFS advantage in our study. In terms of OS, the rs12505758 SNP in VEGFR2 was most significantly associated with improved OS in bev-treated pts (allelic HR 1.50, 95% CI 1.21–1.85, $p = 0.0002$). No effects for rs12505758 were seen in placebo pts.
Conclusions: Our study represents a large genetic analysis of SNPs in correlation to bev outcome, based on pooled data sets. The observed associations suggest certain genetic loci as potential markers for favourable prognosis, regardless of bev treatment, and prediction of benefit from bev. Further studies will be necessary to assess the potential clinical value of these preliminary associations.

1415 POSTER

Blood Plasma VEGFA Analysis in the AVAGAST Randomized Study of First-line Bevacizumab (bev) + Capecitabine/Cisplatin (cape/cis) in Patients (pts) With Advanced Gastric Cancer (AGC)

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Background: Recent data suggested that high plasma VEGFA (pVEGFA) levels might predict progression-free survival (PFS) benefit in bev-treated pts with metastatic breast cancer (mBC) [AVADO study, Miles et al. SABCS 2010]. Current data in lung, renal, and colorectal cancer indicate a more prognostic than predictive role for pVEGFA [Bernaards et al. ASCO 2010]. The AVAGAST study included prospective evaluation of pVEGFA as a biomarker (BM).