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.