Conclusion: P targets T matrix, resulting in T-associated HA reduction. Ph1 studies have shown in vivo activity of P as reflected by elevated plasma HA catabolites and increased T water diffusion and T perfusion by ADC/DCE-MRI. The use of histochemistry as a predictor of P efficacy as well as HA plasma catabolites, histochemistry and ADC/DCE-MRI to monitor response to P will be investigated Phase 2 trials.

PP 19

Highly sensitive detection of microRNA and mRNA from FFPE tissue and blood samples by expression microarray

H. Sudo, T. Satoko, U. Yoji, K. Toshihiko, I. Makiko, N. Osamu, A. Hideo, N. Hitoshi. *Toray Industries, Inc., Kanagawa, Japan*

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.
Materials 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' sera.
Conclusion: 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.

PP 105

Multiple gene signatures: some putative answers on the why and the how

W. Talloen, L. Bijnens. *Janssen Pharmaceuticals group, Beerse, Belgium*

Background: With the completion of the sequencing of the human genome and with the emergence of biotechnologies such as microarrays, we have entered the post-genomic era with much hope to harvest some of the fruits hidden in the genomic text. At the same time, the current difficulties faced by pharma research to discover generally applicable block-buster drugs have lead to think in terms of personalized medicine. Consequently, high hopes are on clinical opportunities for gene-based prediction of illness or drug response using post-genomic tools. The -omics revolution was also warmly welcomed by statisticians as its data properties imposed new and interesting statistical challenges. For example, the quest for biomarkers in the context of personalized medicine has made many statisticians think about classification models that are robust against overfitting for generation of molecular signatures.
Materials and Methods: Here we propose three biological scenarios where multiple gene signatures may outperform single gene markers; (1) inhibition or catalyzation, (2) downstream effects and (3) upstream effects. A simulation study is set up to mimic three different biological scenarios, and each of the three datasets is analyzed using various algorithms including PAM, Random Forest, Support Vector Machines, CART, etc.
Results: The algorithms under study perform clearly differently between the three scenarios.
Conclusion: This presentation discusses how genes can be aggregated into one composite index (i.e., the marker) so as to reflect the underlying biology, as categorized using the three previously proposed biological scenarios.

PP 58

Plasma microRNAs in breast cancer detection

C. Thompson, H. Heneghan, L. Li, R. Leidner. *Case Western Reserve University, Cleveland, OH, USA*

Background: Circulating microRNA (miRNA) expression levels have been proposed as a potential biomarker for cancer detection due to their key role in cancer and stability in the circulation. In fact, a circulating miRNA-based test for colorectal cancer detection is already in clinical trials. However, the field is much less advanced in breast cancer.
Materials and Methods: Using the Illumina human miRNA microarray, we interrogated the expression level of 1145 miRNAs in the plasma of 18 breast cancer patients prior to tumor resection, 17 patients after tumor resection and 20 mammography-screened controls. Controls were matched to pre-resection cases on age and race. We excluded 245 miRNAs due to low expression across all samples. Differences in expression levels between pre-resection cases and controls were assessed via a pooled t-test.
Results: Thirty six of the remaining miRNAs were differentially expressed between pre-resection breast cancer cases and controls ($p < 0.01$). Using a single ratio-normalized miRNA level, with 100% specificity we were able to correctly identify 13% of the cases. Increasing the signature to 2 miRNAs ratios allowed us to correctly classify 50% of cases. Further signature modeling using 6 ratios yielded a test with 89% sensitivity at 100% specificity. This signature held up to random permutation testing ($p < 0.01$). We noted that this signature was better at detecting ER+ breast cancers, where it correctly identified 100% of the ER+ cases. Furthermore, the expression levels were highly correlated with stage (lowest in in-situ cases and highest in stages 3 and 4), and returned to baseline levels in post-resection samples.
Conclusion: Overall, our data provides compelling evidence of the potential of miRNAs to be used as a minimally invasive screening test for breast cancer, conceivably as an adjunct to improve mammographic accuracy. Confirmation of preliminary results in a larger sample size is underway.

PP 76

Biomarker discovery using multiplexed in solution proximity extension assays: a case–control study for early detection of colorectal cancer

S.B. Thorsen, M. Lundberg, E. Assarsson, N. Gee, B.S. Nielsen, I.J. Christensen, H.-J. Nielsen, N. Brünner, J. Stenvang, S. Fredriksson. *University of Copenhagen, IVS, Patobiology, Frederiksberg, Denmark*

Background: One of the challenges in early detection of colorectal cancer (CRC) is the limited success with screening. We here report a study, focusing on discovery of biomarkers for early detection of CRC, using EDTA plasma samples from a case–control group collected from a larger endoscopy study. We demonstrate that the combination of biomarker discovery and molecular technique development is one lead to discover new diagnostic biomarkers for CRC.
Materials and Methods: We have established and validated a high throughput multiplex in solution proximity extension assay (PEA) platform and demonstrate simultaneous quantification of 96 different proteins in 1 μL sample. The PEA employs two primary antibodies, linked to two different DNA strands; upon simultaneous and proximal binding to a target protein the two strands can be connected. The DNA strands now form a PCR amplicon detectable by real-time qPCR. The amplification ability of the DNA strands drive the sensitivity and lowers sample consumption, while supporting multiplexing capabilities based on the oligonucleotide design. The PEA technology possesses all the required qualities for a biomarker discovery tool. From a literature study investigating interesting molecular pathways relevant for CRC, we designed four biomarker panels. In total, we measure 150 different protein markers, of which many have never been reported in human plasma. Using this multiplex PEA discovery tool, we test the biomarker potential for each protein. Our case–control study consists of four groups: 74 stage I-IV CRC patients, 74 adenoma patients, 74 patients with other diseases, and 74 healthy individuals. All patients and individuals have been age and gender matched.
Results: We have previously applied an earlier version of the assay (PLA) and successfully demonstrated detection and quantification of 74 different protein markers in CRC and matched healthy individuals. This demonstrated the feasibility and potential for the assay and identified putative biomarkers [1]. We will present assay validation results and biomarker potential for all panels included in the new PEA high-throughput assays.