fluorescence quenching, ELISA, quartz crystal microbalance, fluorescence microscopy and enzyme inhibition assays, as well as immunohistochemistry.
Results: Aptamers were selected for both enzymes, and showed high affinity binding, in the low nanomolar range, and selectivity for their respective targets, as characterised by fluorescence quenching and ELISA. Enzyme inhibition assays demonstrated the capability of the aptamers to successfully inhibit their cognate enzymes, both in direct assays against the enzyme in the presence of its substrate, and in functional assays in tissue where the enzymes are naturally expressed, demonstrating their therapeutic potential. Fluorescent labelled aptamers were shown to successfully stain the enzyme in immunohistochemistry experiments, and were able to bind to the enzyme in quartz crystal microbalance assays, demonstrating the diagnostic potential of the aptamers. Following small modifications, these aptamers also demonstrated stability in serum and urine, exhibiting potential for *in vivo* use as inhibitors, or for analysis of biological material in diagnostic assays, including immunohistochemistry and as recognition units in biosensors.
Conclusions: Aptamers against tumour enzymes can prove a great alternative to antibodies and small molecule inhibitors, offering greater affinity, specificity and temperature stability, no immunogenicity, and great flexibility in a variety of modifications and uses.

Sunday 27 June 2010 14:35–16:05

Presidential Session
Presidential Session III

45 Use of somatically acquired genomic rearrangements as biomarkers in solid tumours

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Background: Somatically acquired genomic rearrangements are a feature of the majority of cancers and make attractive biomarkers for monitoring disease burden in cancer patients.
Material and Methods: Next-generation sequencing was used to rapidly identify genomic rearrangements in tumour samples obtained from three cancer patients (two with breast cancer and one with osteosarcoma). Somatically acquired rearrangements were confirmed by successful PCR and sequencing of the breakpoints in tumour but not matched normal genomic DNA samples. Highly specific quantitative PCR assays utilizing dual labeled probes were designed to a selected subset of rearrangements. Plasma or serum samples were obtained from each patient to investigate whether patient specific rearrangements could be detected. For one patient, serial serum samples were obtained in order to assess disease burden at multiple time points during treatment.
Results: Tumour-specific DNA rearrangements were successfully detected in plasma samples from each patient. PCR assays for rearrangements were able to detect a single copy of the tumour genome in many milliliters of plasma without false positives. Serial dilutions of templates indicate that results are quantitative through a 1000-fold dynamic range with amplification remaining in the linear range. Disease status, drug responsiveness and incipient relapse could all be monitored with a high degree of accuracy.
Conclusions: This proof-of-principle is the first concrete demonstration of the transforming power of whole cancer genome sequencing to personalise medicine in the oncology clinic. It is applicable across all types of solid tumours, adaptable to many therapeutic regimens and potentially capable of implementation within centralised molecular diagnostics laboratories.

46 An integrative structural and functional approach to pancreatic cancer gene discovery

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Background: Pancreatic cancer is among the most deadly of all cancers. Five year survival is less than 5%, and most patients succumb to the disease in three to six months after diagnosis. Despite its lethality, very few therapeutic options exist including virtually no targeted therapies. A better understanding of pancreatic cancer etiology is warranted.
Methods: Copy number alteration (CNA) was determined across seventy cell lines and early-passage xenografts using high-resolution Agilent 244K CGH arrays. Expression profiling was performed in parallel using Agilent catalog 44K arrays. Additionally, publicly available whole exome sequencing data were mined in order to identify genes showing both CNA and mutation. Identified candidate cancer genes were then simultaneously functionally interrogated using a pooled shRNA lentiviral library and carrying out a competitive growth screen across a panel of ten cell lines sampling the genetic diversity of pancreatic cancer.
Results: From the CGH, sequencing and expression data, we identified 147 genes with considerable structural evidence implicating them as candidate oncogenes or tumour suppressors. Among these, classical addictive oncogenes were identified by those shRNA hairpins depleted in the functional screen in cell lines harboring amplification and/or overexpression of the targeted candidate oncogene. Gatekeeper tumour suppressor genes were identified by those shRNA hairpins enriched in cell lines expressing target genes and also showing no phenotype where expression was already low. Positive control "known" cancer genes were included in all steps of analysis, and encouragingly this integrative approach to cancer gene discovery recovered nearly all of the well known pancreatic cancer genes. Additionally we have identified many novel candidate cancer genes which we are further characterizing.
Conclusions: We present here one of the richest compilations of structural data characterizing pancreatic cancer. We expand on our rich structural data to identify cell context specific oncogenes and tumour suppressors. This strategy naturally lends itself towards discovery of cancer genes ripe for targeted therapy, which is conspicuously absent from pancreatic cancer treatment regimens. In conclusion, we report an integrative structural and functional approach to identifying novel therapeutically attractive pancreatic cancer genes.

47 Molecular signatures of long non-coding RNAs in breast cancer patients

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Background: Of the ~3.3 billion bases of the human genome, only about 2% code for proteins. Since very recently, the remaining 98% have been considered to be 'junk' and functionless. However, large transcriptomic studies have shown that around 90% of the genome is actively transcribed of which a significant fraction may be functional and contribute to a previously underestimated regulatory layer of non-coding RNAs (ncRNAs). In several studies it has been shown that the expression of small ncRNAs, like miRNAs, is associated with diseases including cancer. However, the large group of long ncRNAs has drawn less attention despite their genome-wide distribution. These long ncRNA genes may contribute substantially to regulatory features ranging from epigenetic control and transcriptional regulation.
Material and Methods: For this pilot study we selected 25 breast carcinomas from a larger cohort of 920, representing the five clinically relevant tumour expression subclasses as well as normal breast tissue from breast reduction operations. Total RNA from these samples was analyzed utilizing the custom nONCOchip®, developed by the RNomics group at the Fraunhofer Institute for Cell Therapy and Immunology, Leipzig, Germany. The nONCOchip® covers both, experimentally identified cancer related nc RNAs of oncogenes (STAT-3), tumoursuppressor genes (p53), and cell cycle controlled genes, as well as known or predicted non-coding RNAs from public databases. The array includes in total 243,000 probes, with over 60,000 newly identified transcripts.
Results: The expression analyses of long non-coding RNAs showed that various long ncRNAs are expressed in breast tumours. Preliminary results using unsupervised clustering based on the non-coding RNA expression revealed groups of tumours with distinct expression patterns of long ncRNAs.
Conclusions: The growing list of ncRNA genes influencing carcinogenesis is striking. Long ncRNAs show distinct patterns of expression, divide the tumours in groups different from mRNA expression subgroups, and are likely to be involved in mRNA regulation structures. The identification of clinically relevant ncRNAs may open up for the development of new biomarkers and therapeutically tools that attack diseased cells.
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(*LOB and KR contributed equally to this study and should be regarded as joint first authors).