Huang, Tim Hui-Ming

CpG island arrays: an application toward deciphering epigenetic signatures of breast cancer

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Tremendous progress has recently been made in the development of high-throughput microarray technologies for monitoring genetic alterations and expression profiles of genes in cancer cells. Such technological advances appear to be lacking, however, for another common molecular alteration, namely DNA methylation, in cancer. We have recently developed a novel technique, called differential methylation hybridization[1] (DMH) that provides for the first time, an opportunity to conduct genome-based methylation analysis in breast cancer. The first part of this innovation was the generation of over 1,000 CpG island fragments (or tags) as hybridization templates arrayed onto nylon membranes. The second part involved preparation of radiolabelled amplicons, representing a pool of methylated DNA from the test genome. These amplicons were prepared from 28 paired normal and breast tumour tissues and used as probes in array-hybridization. Positive hybridization signals identified by the tumour amplicon, but not by the normal amplicon, indicate the presence of hypermethylated CpG island loci in breast tumours. Close to 9% of these tags exhibited extensive hypermethylation in the majority of breast tumours relative to their normal controls, whereas others had little or no detectable changes. Pattern analysis in a subset of CpG island tags revealed that CpG island hypermethylation is associated with histologic grades of breast tumours. Poorly differentiated tumours appeared to exhibit more hypermethylated CpG islands than their well or moderately differentiated counterparts (P<0.041). Our early findings lay the groundwork for population-based DMH study and demonstrates the need to develop a database for examining large-scale methylation data and for associating specific epigenetic signatures with clinical parameters in breast cancer.

1. Huang, T.H.-M., *et al. Hum. Mol. Genet*. **8**, 459-470 (1999).

Hui, Lijian

Analysis of gene expression patterns in hepatocellular carcinoma

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Hepatocellular carcinoma, the second leading cause of cancer death in China, is responsible for over 130,000 deaths every year. Until now, there have been very few screening tests for early diagnosis of hepatoma, which often delays early treatment. We have developed a modified representational difference analysis (RDA) technology and identified more than 200 upregulated genes in hepatoma, among which over 60% were later confirmed as truly upregulated genes in additional hepatoma samples. Approximately 20%–30% of these upregulated genes had detectable expression differences between normal and hepatoma samples in a mini-cDNA array based on the approximately 200-gene set. We built a cDNA array that represents a set of 11,000 human genes, including approximately 5,000 previously reported and 6,000 new EST clusters. By applying this cDNA array we further identified more than 800 upregulated genes in clinical hepatoma samples that were not detected by the previous RDA method. Combining the two methods significantly facilitated the discovery of hepatoma-related genes, as the cDNA array provides a broader view of gene expression level and subtractive cloning offers greater sensitivity in detection of low-abundance gene transcripts.

Hummel, Rene´

The combination of RFDD-PCR gene profiling and cDNA arrays provides a strong gene profiling technology

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Display Systems Biotech has developed a new approach to select for interesting groups of genes and directly link them to microarrays. We have combined a new differential display technique called Restriction Fragment Differential Display PCR (RFDD-PCR) with microarray technology. This method is based on ligation of specially-designed adaptors to cDNA fragments, obtained by restriction enzyme digestion with a four-base cutter, followed by PCR amplifications with a series of specific primers. The use of specific PCR primers and touch-down PCR makes the RFDD-PCR analysis highly reproducible. In addition, having an internal confirmation of each gene fragment at two independent positions in a profile further eliminates the possibility of false positives among differentially expressed genes.

Furthermore, the sequence information from the ends of each RFDD-PCR fragment allow identification of known genes without isolating the fragments based on their correspondence to specific cDNA fragments in your gene profile. Through the development of a fully integrated bioinformatics package in this system, we have successfully identified cDNA fragments without sequencing. To our knowledge, this is the first time this has been possible with a gene expression profiling method.

We have identified a range of downstream applications for this technology. For example, through the re-amplification of RFDD-PCR fragments with two modified universal primers (primers having a coupling technology attached to the 5´-end) we are able to generate fragments that can be covalently coupled to glass chips. These geneARRAYs can be constructed with a density of between 2-20,000 spots per chip. This makes them extremely versatile as verification tools and for re-screening of new samples. Potential applications include their use in analysing patient samples, in drug discovery and optimisation, and as diagnostic tools.

Isaksson, Anders

Multiplex analysis of nucleic acid sequences by amplification of padlock probes on DNA arrays

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Methods are needed to identify large numbers of nucleic acid sequence variations and to clarify their biological significance. We are developing a method for identification and quantitation of DNA and RNA sequences using padlock probes. The two ends of these linear DNA probes can hybridize next to each other on a target strand. Only when the ends are correctly matched to the target, however, can they be joined by ligation, converting the probes to circular molecules. Specific nucleic acid sequences can be detected by scoring circularized probes. We have shown that padlock probes are sufficiently specific to distinguish single-nucleotide variation in total genomic DNA. If only circularized probes can be amplified, it may be possible to perform highly multiplex analysis, because only intramolecular reactions are detected. Such a method would be an alternative to genotyping methods relying on multiplex PCR. We are applying a rolling-circle replication mechanism to amplify circularized probes and achieve localized signal amplifica-