on important tumour-related aspects of the eukaryotic cell, for example its cell division cycle. In the last decade many of the key regulators of the cell cycle have been identified. The role of the retinoblastoma protein family in regulating the activity of the E2F transcription factors (potent inducers of the G1-S transition of the cell cycle) is now well established. Activation or repression of a series of genes is essential for a smooth passage through each cell cycle phase. Among the target genes of E2F transcription factors are cell-cycle control genes (for example, genes encoding cyclin E and the pocket protein p107, a relative of the retinoblastoma tumour-suppressor gene product pRb) and proteins involved in the actual duplication of the genome (for example DNA pola, TK and CDC6). So far, five E2Fs have been cloned that can bind members of the pocket protein family (pRb, p107 and p130). We set out to identify new target genes of E2Fs by generating monoclonal NIH3T3 cell lines that overexpress a particular E2F species. We used DNA microarrays to screen for fluctuations in mRNA levels of known and unknown genes and found that one major target of E2F1 was insulin-like growth factor-2 (IGF-2). NIH3T3 cells overexpressing E2F1 and growing under low serum conditions show very low levels of IGF-2 expression. A plausible explanation for the frequently observed programmed cell death in E2F1-overexpressing cells after serum withdrawal now becomes apparent. It is known that IGF growth factors can inhibit the apoptotic signals in cells. The fact that IGF-2 levels drop as a consequence of E2F1 overexpression might hamper the resistance of the cells to apoptosis. It might therefore be that overexpression of E2F1 triggers apoptosis and levels of the rescuing IGF-2 are too low to prevent the cells from undergoing apoptosis. During the conference we will present our progress with the new glass-based arrays we currently apply to find more E2F target genes, to study cell cycle-related phenomena and to identify tumour-specific expression profiles.

Khan, Javed

cDNA microarrays detect activation of a myogenic transcription program by the PAX3-FKHR fusion oncogene

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cDNA microarrays allow large-scale analysis of the effects of oncogenic transcription factors on gene expression. Alveolar rhabdomyosarcoms (ARMS) are aggressive soft tissue tumours of striated muscle in which 60% have a reciprocal translocation involving chromosomes 2 and 13, [t(2;13)(q35;q14)]. This results in a novel fusion transcription factor, PAX3-FKHR. Using retroviral transduction we have introduced both PAX3 and PAX3-FKHR into NIH3T3 cells and used a mouse cDNA microarray containing 2,200 elements to investigate their effects on gene expression. Introduction of PAX3-FKHR resulted in increased expression of several genes, including that encoding the myogenic transcription factor myogenin, as well as other muscle-specific genes. In contrast, PAX3 failed to induce these genes and repression of several genes was noted, some of which were also downregulated by PAX3-FKHR. This study demonstrates the potential of cDNA microarrays to elucidate the pathways of malignant transformation by oncogenic transcription factors.

Khimani, Anis H.

MICROMAX™ microarray system I: a complete system for high throughput gene expression analysis and drug discovery

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A complete system containing high density microarrays on glass slides and all necessary experimental reagents has been developed. Utilizing the TSA™ (Tyramide Signal Amplification) technology, the system offers significantly improved sensitivity which reduces sample requirement by 10-50 fold and uses fluorescence-based detection. MICROMAX™ Microarray System I spotted with 2,400 known human genes provides the opportunity to monitor gene expression between two comparative cell lines or tissue samples. In addition to following global changes in gene expression patterns in basic research, the system also offers the potential of detecting changes in gene expression for diagnostic applications. We have used the system to analyze gene expression in human post-mortem brains. Samples of parietal cortex of patients with Rett syndrome, an autism-related disease that affects females exclusively, were compared to age-matched tissue samples from normal controls. Several genes were up- or down-regulated in the diseased samples. These studies highlight the potential of the MICROMAX™ system to measure gene expression in complex biological samples.

Klimecki, Walter

A 3-dimensional microarray system for parallel genotyping of single nucleotide polymorphisms

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Motorola is developing a high-throughput, single nucleotide polymorphism (SNP) analysis system which is based on microarrays of raised, hydrophilic gel pads. This three-dimensional microarray system offers distinct advantages over flat microarrays, including a well-characterized, covalent linkage of probes to the gel polymer and a diversity of functional groups available for biomolecule attachment, within an aqueous microenvironment, which is conducive to enzymatic activity. The SNP analysis system assays the DNA polymerase-mediated extension of an anchored oligonucleotide probe which is hybridized to a polymorphic site in an amplified target DNA sample. Assays are analysed for fluorescence signal in a confocal laser slide scanner. This SNP scoring system is currently being used to genotype a population of 1,000 individuals within a larger effort to identify genetic determinants of obesity-related phenotypes in humans. We will present data to illustrate both the overall performance of this SNP genotyping system, as well as specific results from this pilot study.