

Maillard, Karine

Expression profiling of soft tissue sarcomas

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Soft tissue sarcomas involve the connective tissues of the limbs, girdle, trunk, head and neck. Despite the numerous subtypes of sarcomas (synovial sarcoma, fibrosarcoma and malignant fibrous histiocytoma), tumours in a particular subtype behave in a reasonably consistent fashion, allowing predictions of recurrence and metastasis. Histological and cytogenetic techniques can group most sarcomas; however, the diagnosis can be problematic and must be performed by experts. We are using DNA microarrays to analyse the expression pattern of genes in soft tissue to identify expression signatures specific to the various sarcoma subtypes.

Our DNA expression arrays are based on cDNAs from the UniGene set of ESTs. PCR products from an initial set of 3740 cDNAs were gridded onto poly-L-lysine-coated glass microscope slides at a density of 378 spots/cm². Hybridisations were performed using Cy3- and Cy5-labelled first strand cDNA from cell lines or sarcomas. After washing, the arrays were scanned using a General Scanning Scanarray3000 scanner. The results of this work will be discussed.

Malek, Renae L.

Use of the Rat Gene Index to examine gene expression patterns from Src-transformed rat fibroblasts that exhibit broad differences in metastatic potential

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We are developing a Rat Gene Index to serve as a non-redundant resource for rat genes that will contain information on expression patterns, gene identity and function as well as links to mapping information and orthologous genes in other species. The non-redundant transcript database was generated by assembling cleaned ESTs and non-redundant expressed transcripts (ETs) into tentative rat consensus sequences (TCs). The TCs were searched against a non-redundant amino acid database and assigned a putative identity where possible. ESTs that failed to assemble into TCs were searched individually. Of the more than 100,000 ESTs currently in GenBank, The Institute for Genomic Research has contributed over 50,000 ESTs. The current RGI Release, version 2.0, contains almost 90,000 ESTs which assemble into over 26,000 singletons and almost 14,000 TCs. We have assigned role categories to identified genes to estimate the number of genes represented by a variety of cellular and organismal functions. We have identified several hundred TCs that do not have significant homology to any known gene in several public databases. These TCs may serve as potential candidates for novel genes that have not been previously identified. We have used the Index to identify

tissue-specific TCs and have confirmed the tissue-specific expression of several hypothetical and unknown proteins by Northern analysis. Finally, we have compared gene expression patterns between rat fibroblasts containing various Src-oncogene derivatives using a glass array containing cDNAs from over 4,000 clones selected from the Rat Gene Index.

Mangan, Joseph

The expression profile of *Mycobacterium tuberculosis* infecting the human monocytic cell line THP-1 using whole genome microarray analysis

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Bacterial virulence is dependent on the correct temporal and spatial expression of overlapping sets of genes in response to specific environmental cues. Defining the genes expressed by intracellular pathogens during interaction with host cells will help increase our understanding of pathogenic mechanisms that result in disease and may provide insights into potential vaccine strategies and novel targets for drug design. We have examined the expression profile of *Mycobacterium tuberculosis* infecting the human monocytic cell line THP-1, using whole genome microarray analysis. Our previous protein profiling and proteomics work indicates that the majority of gene modulation occurs early in the infection process, and thus, has led us to examine the early time points during our model of infection. To do this, we developed RNA extraction methodologies, which both protect bacterial RNA from degradation and prevent transcriptional changes associated with bacterial harvesting while simultaneously allowing the removal of the excess macrophage RNA. Fluorescently-labeled cDNA probes made from these RNA samples were hybridised to a microarray containing PCR products representing each ORF of the *M. tuberculosis* genome. Analysis of the *M. tuberculosis* responses during early interactions with the macrophage is presented and the biology of macrophage induced gene expression explored.

Mecklenburg, Michael

XNA on Gold™: a versatile microarray platform

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Microarrays containing thousands, perhaps tens of thousands, of genes provide a unique opportunity to identify vague genetic factors as well as yet uncharacterised open reading frames (ORFs). However, in many instances, a group of 50 to 100 candidate genes is available or can be gleaned from the literature. In these cases, use of low-density, high 'informational content' microarrays can accelerate genetic analysis.

XNA on Gold™ is INTERACTIVA's affinity array biochip. XNA integrates thin and thick film technologies with Streptavidin-based immobilisation in a microarray format. The stability of the chemically bonded self-assembling monolayers (SAMs) combined with the high affinity of the streptavidin linker layer provides a flexible, robust platform for assay development. The wide range of commercially available biotinylated compounds and the diversity of biotinylation reagents makes it possible to immobilise virtually any biomolecule including