UHNMAC News

The University Health Network Microarray Centre Newsletter – Spring 2007

UHN Microarray Centre

ANNOUNCEMENTS

1st Annual Toronto Functional Genomics Symposium

Hosted by the UHNMAC, the theme of this sympsium is genomics, proteomics, and bioinformatics, with an emphasis on array-based technologies. Visit our website, http://www.microarrays.ca/symposium/general.html, for more information or to register.

Prices for most Agilent Services have been lowered

In March 2007, the UHNMAC lowered most Agilent Service prices. Please visit our website, http://www.microarrays.ca/services/Agilentservice.html, for details.

Collaboration with SLRI Microarray Facility

In February 2007, the UHNMAC announced its collaboration with the Samuel Lunenfeld Research Institute Microarray Facility. For more details, please read the Press Release available at http://www.microarrays.ca.

Welcome to the Spring 2007 edition of the UHNMAC News!

This newsletter features a review of a recent publication about research using UHNMAC CpG Island microarrays. The second author of this paper, Jonathan Davies (BC Cancer Research Centre), will be presenting his research at the 1st Annual Toronto Functional Genomics Symposium in June. In addition, the feature report on Lipid Microarrays highlights a technique used by Dr. William Robinson (Stanford School of Medicine), who will also be presenting his research at the Symposium in June.

FEATURE ARTICLE & REVIEW

Weber M, Davies JJ, Wittig D, Oakeley EJ, Haase M, Lam WL, Schubeler D. Chromosome-wide and promoter-specific analyses identify sites of differential DNA methylation in normal and transformed human cells. Nature Genetics, 2005, 37(8):853

Taking a closer look at aberrant methylation in cancer

DNA methylation plays a crucial role in the control of gene activity and nuclear architecture[1]. Alterations in DNA methylation, such as hypermethylation at certain CpG-rich promoters and hypomethylation at repeated DNA sequences, are often found in cancers[2-4]. It is thought that alterations in

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FEATURE REPORT : Lipid Microarrays

The "Omics revolution" has led to the recent emergence of "lipidomics"[1], a field dedicated to the study of lipids and how they interact with proteins and other cellular components. By identifying lipid-associated molecules in a cell or biological fluid, lipid microarrays are a useful tool for increasing the knowledge base of the human lipidome[1,2]. Much like antibody and other protein microarrays, lipid arrays are protein-detecting microarrays. Lipid microarrays consist of various lipid sub-types spotted individually, in an array pattern, on a solid surface. The lipid array provides a protein-lipid interaction profile and can be used to identify proteins of potential therapeutic value[2].

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As always, general questions about microarrays can be addressed to help@microarrays.ca, orders for UHNMAC array products can be placed at orders@microarrays.ca, and questions about any of our services can be addressed to geneservice@microarrays.ca. If you have any suggestions for newsletter articles or questions you'd like addressed, please contact general@microarrays.ca.

Aberrant methylation

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DNA methylation may contribute to carcinogenesis by inactivating tumour-suppressor genes[4]. In this study, chromosomal and promoter-specific methylation profiles allowed Weber *et al.* to approximate the extent and localisation of differential methylation between primary and transformed cells[5]. The significance of the study is two-fold; it was the first to report the use of methylated DNA immunoprecipitation (MeDIP) for identifying changes in DNA methylation in transformed cells and the results of this study suggest that aberrant methylation of CpG island promoters in malignancy may be less frequent than previously hypothesised[5].

MeDIP is a powerful epigenomic technique that allows for the enrichment of methylated DNA using antibodies specific for methylated cytosine to immunocapture methylated genomic fragments. However, the need for whole genome amplification after immunopreciptation can introduce PCR biases, a consideration to keep in mind when using the MeDIP technique[1]. Another study using MeDIP by Zhang *et al.* combined MeDIP and tiling arrays to provide an almost complete DNA methylome of *Arabidopsis*[6].

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By combining MeDIP with CpG island microarray (Human 12k CpG array from UHN Microarray Centre) hybridisation, Weber *et al.* created methylation profiles of all human chromosomes and assessed the frequency of aberrant CpG island methylation in transformed cells. A CpG methylation profile was generated for SW48 colon cancer cells and compared to methylation profiles of primary fibroblast and normal colon mucosa. This analysis showed that methylation levels of most CpG islands were maintained in the SW48 cancer cell line[5]. When comparing SW48 cells with normal colon mucosa, Weber *et al.* identified an almost identical population of unique sequences, suggesting that this methylation was linked to the transformed state. This study also found that the global distribution of methylated cytosine in SW48 cells was similar to that of primary fibroblasts. And, much like the normal fibroblast cells, the highest levels of methylation in the transformed cells occurred at gene-rich chromosomal regions. Several regions of marked hypomethylation were found in transformed cells, however, these were almost entirely in gene-poor regions.

Recently, other studies have been published that attempt to quantify the extent of DNA methylation at CpG island promoters and repeated DNA sequences in cancers[2,3]. A study by Nishiyama *et al.* provides the first report of a DNA sequence (NBL2) that can be either extensively hypermethylated or hypomethylated in cancer[2]. This study suggests that tumourigenesis-linked DNA methylation changes are much more flexible than commonly realised. Nishiyama *et al.* hypothesise that CpG island promoters that become hypermethylated in earlier stages of cancer may subsequently undergo demethylation during waves of tumour progression-linked DNA demethylation. In addition, a study by Lujambio *et al.* concluded that DNA hypermethylation contributes to the downregulation of microRNAs in human tumours[7].

The study by Weber *et al.* concludes that the combination of MeDIP with CpG island microarray hybridisation allows for the identification of epigenetically silenced genes in cancer cells and provides the first epigenomic map of DNA methylation in the human genome[5]. The results of this study found that the global pattern of CpG island methylation is conserved between primary and transformed cells and that the number of hypermethylated CpG island promoters in transformed cells was unexpectedly low. A more comprehensive analysis including non-CpG island promoters may be required to conclusively determine whether or not preferential localisation of aberrantly methylated CpG island promoters occurs in chromosomal regions with differential methylation. Additional studies should reveal further insights into

the dynamics and hierarchy of epigenetic regulation during normal development and disease[5].

Jonathan J. Davies (second author) will be presenting his research at the 1st Annual Toronto Functional Genomics Symposium on June 19, 2007

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Lipid microarrays

from page 1

Protein overlay lipid blots (colloquially termed “Fat Westerns”) have previously been used for biochemical investigations on lipid selectivity[1,3] and could be considered a precursor of lipid microarrays. The main advantage of lipid arrays over protein overlay lipid blots is the ability for lipid arrays to simultaneously assess the protein-lipid interaction between hundreds of arrayed lipids and a complex mixture of proteins from a biological sample. In addition, lipid arrays are simple, chemiluminescence-based assays and array production can be automated[4].

Lipids form many structural features of cells and are critical members of cellular signal transduction pathways[2]. They are also important targets of immune responses in several microbial and autoimmune diseases[4]. For example, autoimmune responses directed against phospholipids and gangliosides contribute to the pathogenesis of systemic lupus erythematosus and Guillain-Barre syndrome, respectively[5]. Lipid microarrays have recently been used to investigate the hypothesis that myelin lipids may be target autoantigens in individuals with multiple sclerosis (MS)[4]. Kanter *et al.* generated lipid microarrays comprised of the lipid sub-types found in the myelin sheath and evaluated samples from MS and control patients using these arrays[4]. Based on the results, Kanter *et al*. were able to detect lipid-specific antibodies against sulfatide, sphingomyelin and oxidized lipids in cerebrospinal fluid from MS patients[4].

The manufacture of lipid microarrays involves some challenges. Modified lipids can be immobilised to functionalised solid surfaces, such as epoxy and carbonyl diimidazole, by covalent attachment. Despite being a laborious process, chemical modification of the lipids doesn’t usually affect their functionality as most lipid molecules have long fatty acid chains that can be used for chemical derivatisation[6]. Due to their

amphiphilic nature, lipids can also be immobilised non-covalently to hydrophobic solid surfaces or porous surfaces. For example, polyvinylidene fluoride (PVDF) membranes attached to microscope slides have been used for arraying lipid solutions[4]. It is believed that the hydrophobic part of the lipid anchors itself to the hydrophobic PVDF array surface, thus the lipid molecules are oriented such that the polar regions, such as the sulfate group or glycan molecule, are accessible for protein binding[4]. The advantages of non-covalent immobilisation for lipid array production include: elimination of the derivatisation of the ligand molecule, immobilisation of a larger amount of probe molecules on the porous substrate, and porous substrates may provide a 3-D hydrophilic environment similar to solution phase for biomolecular interactions to occur[1]. The difficulty of producing high-concentration lipid solutions for use in conventional pin or ink-jet spotters has provided the impetus to develop a polydimethyl siloxane (PDMS) flow spotter for the patterning of lipid microarrays[2]. By forming lipid spots through continuous flow, high-density arrays can be made with more dilute lipid solutions. In addition, flow deposition offers improved spot formation and allows spots of different concentrations to be printed, since flow rates and deposition times could be uniquely varied for each spot.

Since many lipids do not exist in isolated form but rather aggregate in bilayers and membranes, it is likely that lipid bilayer and cell membrane arrays may gain popularity in the future. Simon *et al*. have published a paper about the formation and stability of a suspended biomimetic lipid bilayer on silicon submicrometer-sized pores[7]. In addition, cell membrane microarrays have also emerged as a tool that facilitates the study of ligand-receptor interactions and cell-cell signalling[9]. Cell membrane arrays consist of lipid bilayers containing biological molecules of interest supported on a solid substrated[9].

William Robinson (Stanford University School of Medicine) will be presenting his research at the 1st Annual Toronto Functional Genomics Symposium, June 18-19, 2007

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