UHN Microarray Centre

Carbohydrate Microarrays

Glycomics, the functional study of carbohydrates in living organisms, has recently played a greater role in biological research and medical applications[1]. Carbohydrates are important components of glycoprotein and glycolipid cell-surface molecules, which are responsible for processes such as recognition, adhesion, and signalling[2]. These vital functions are known to play a role in growth and development, tissue repair[1], pathogen invasion[3], and tumour progression[4].

Applications of carbohydrate microarrays

Carbohydrate microarrays are emerging as a common technique used in glycomic research, as they can be used to characterise carbohydrate-cell interactions, determine the binding profile of carbohydrate-binding proteins, detect pathogens, and provide high-throughput screening of inhibitors of carbohydrate-protein interactions[1].

Insight into the specificity of cell-surface carbohydrate interaction with antibodies and receptors will advance the development of new therapeutics and diagnostic assays[4]. One study has shown that carbohydrate arrays can be used to monitor the immune response to carbohydrate epitopes at different stages during differentiation, metastasis, or treatment[4]. Huang *et al*. synthesised the cancer antigen Globo H hexasaccharide, an epitope found on the cell surface of breast, prostate, and ovarian cancers, and its truncated sequences[4]. The arrayed saccharides were then used for the fluorescence-based binding analysis of two monoclonal anti-Globo H antibodies and the serum from breast cancer patients, to define the specificity of these antibodies[4]. This study found that the microarray platform was more effective and sensitive than the traditional ELISA method[4].

Another study has found that carbohydrate microarrays are ideal for whole-cell applications as the arrays present carbohydrate ligands in such a way that mimics cell-cell interactions[3]. Pathogen detection experiments can be completed in complex mixtures of cells or protein using the known carbohydrate binding epitopes of the pathogen of interest[3]. Since binding can be observed for low concentration bacterium in heterogeneous solutions, carbohydrate arrays could be used as a fast diagnostic tool[1].

Carbohydrate microarrays have also been used to screen for novel inhibitors of carbohydrate-protein interactions. A study by Bryan *et al*. used carbohydrate microarrays to screen for inhibitors of fucosyltransferases, enzymes critical to the synthesis of inflammation mediators[5]. Such studies could lead to novel therapeutics for inflammatory diseases such as arthritis and colitis.

Recently, a novel microarray technique called comprehensive microarray polymer profiling (CoMPP), has been used to provide insight into the structure and functions of plant cell walls[6]. The CoMPP technique combines the sequential extraction of glycans from various plant tissues and the generation of arrays which are then hybridised with monoclonal antibodies or carbohydrate-binding modules with specificities for cell-wall components[6].

Manufacture of carbohydrate microarrays

Carbohydrate microarrays can be made using standard robotic microarray printing technology. The most common method for preparing carbohydrate microarrays is by covalent attachment of chemically modified carbohydrates to derivatised (chemically modified) glass surfaces[7]. Other methods include non-covalent immobilisation of unmodified carbohydrates on underivatised surfaces, non-covalent immobilisation of chemically modified carbohydrates on underivatised surface, and the covalent immobilization of unmodified carbohydrate on a derivitised surface, a method that is still under investigation[7]. Since carbohydrate-protein interactions are relatively weak, the glycan immobilised on the array should be strongly recognised by the protein and properly oriented and spaced to allow multivalent interactions[7].

The Functional Glycomics Consortium has created a glycan microarray by coupling amine-functionalised glycans to N-hydroxysuccinimide (NHS)-activated glass slides[8]. The NHS-activated surface allows covalent attachment of glycans containing a terminal amine. This glycan array, which represents diverse and biologically relevant structures representing the terminal sequences of glycoprotein and glycolipid glycans, has been used to analyse most major classes of glycan binding proteins (GBP) including antibodies, intact viruses, and mammalian, plant, viral and bacterial lectins[8].

Glycan Binding Protein (GBP) assays

Following the manufacture of carbohydrate arrays, the slides can be used for GBP assays. The slides are incubated in either a one-step procedure with labelled proteins or a sandwich procedure in which the bound GBP is overlaid with a fluorescently labelled secondary antibody or GBPs pre-complexed with labelled antibodies[8]. Surface plasmon resonance (SPR), an optical technique for measuring the adsorption of material (in the case of carbohydrate arrays, the adsorption of GBP) on a metal surface, has also been used as an alternative to fluorescence-based detection methods[9]. Karamanska *et al*. found that SPR imaging of a glycoside array could be used to study plant lectin recognition[9]. The results found by SPR imaging were in agreement with those obtained by fluorescence-based carbohydrate arrays but with the added advantage of label-free analysis[9].

Lipopolysaccaride and glycoprotein microarrays

Lipopolysaccharide (LPS) arrays and glycoprotein arrays are also invaluable tools for glycomics research. Thirumalapura *et al*. published their investigation involving lipopolysaccharide (LPS) arrays for the detection of anti-LPS antibodies[10]. In this study, LPS, a major component of the outer membrane of Gram-negative bacteria, from several bacterial strains were immobilised on nitrocellulose-coated glass slides and hybridised with antibodies[10]. This study found that LPS arrays were about 100-fold more sensitive compared to conventional immunofluorescence assays. Zhao *et al*. used glycoprotein microarrays to screen a variety of lectins to identify glycosylation patterns in sera from normal, chronic pancreatitis, and pancreatic cancer patients[11]. The study suggests that altered glycan structures may have utility for the differential diagnosis of pancreatic cancer and chronic pancreatitis and identify critical differences between biological samples from patients with different clinical conditions[11].

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