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Dot by dot: Analyzing the glycome using lectin microarrays

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Abstract

The glycome, i.e. the cohort of carbohydrates within a cell or tissue, plays a key part in diverse biological interactions involved in health and disease. Glycans are structurally complex and notoriously difficult to analyze. Lectin microarrays, a sensitive and high-throughput method for glycomic profiling, provide a global view of the glycome. In recent work, this technology has been successfully applied to a wide range of studies, from identification of glycan-based stem cell markers to the detection of pathogens and early diagnosis of disease. This review focuses on advances in the field of lectin microarrays.

Introduction

Glycans are complex biopolymers present throughout nature. Points of structural divergence include linear and branched polymers, a diversity of glycosidic linkage points and anomeric orientation. Structural diversity is further amplified by the heterogeneity of monosaccharide building blocks and additional chemical modifications (e.g. acetylation, sulfation, phosphorylation). Glycan structures expressed on the cell surface are recognized by lectins, a class of proteins that bind specifically and reversibly to glycans. Native lectin-glycan interactions control a vast range of biological processes including normal cellular differentiation, proliferation, apoptosis and inflammation [1]. Changes in mammalian glycosylation are associated with malignant transformations [2, 3], tumor progression [4, 5], and cancer metastasis [6–8]. Understanding the relationship between the structure of specific glycans / glycoconjugates and their biological functions remains one of the most challenging tasks in glycobiology. Herein we describe the use of lectin microarrays, a new glycomic technology, to meet that challenge.

The Lectin Microarray

A lectin microarray is composed of a panel of lectins with distinct glycan-binding properties that are printed on a solid support (Figure 1). Lectins are often multivalent binders of glycans, with monovalent binding interactions in the millimolar to micromolar range but physiological interactions up to 2-orders of magnitude higher [9, 10]. In lectin microarrays, lectins are immobilized in a high-density matrix presenting a multivalent display. A variety of microarray slide surface chemistries has been used to manufacture these arrays including N-hydroxysuccinimidyl (NHS) esters [11], epoxides [12], biotin-streptavidin [13], and 3D

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hydrogels [14]. The arrays are then interrogated with fluorescently labeled analytes, resulting in pattern of lectin binding. Structural information about the glycome is obtained by annotating the data using the known glycan-binding specificities of the lectins. Our group was one of the first to present lectin microarrays to the scientific community in 2005 [15]. This early study employed nine plant lectins with diverse binding properties to demonstrate the ability of this platform to profile protein glycosylation using standard dyes and scanners. Hirabayashi *et al* introduced a more sensitive version of this technology, using an evanescent-field fluorescence-detection system to obtain picomolar sensitivity [12]. Since these early studies, the technology for lectin microarrays has improved.

Current lectin microarrays often contain more than 80 glycan binding probes, expanding the carbohydrate structures observed [16, 17]. These probes include recombinant lectins and anti-glycan antibodies, in addition to the plant lectins that comprised the original set of lectin probes [17, 18]. Recombinant lectins address several issues associated with plant lectins, including seasonal depletion of natural sources, inconsistency between batches and the presence of glycosylation. In addition, they allow the incorporation of fusion tags, which can be used to control protein orientation [19]. We developed an *in situ* method for orienting recombinant lectins containing a glutathione *S*-transferase (GST) fusion domain [20, 21]. Oriented lectins exhibited a significant signal even at nanomolar concentrations of analyte using traditional Cy3/Cy5 scanners. This range of detection is similar to that seen with evanescent wave detection-based scanners [22]. Recombinant lectins also introduce the possibility of engineering glycan binding proteins. To analyze the full structural diversity of the glycome will require further expansion of lectin microarrays. There is an estimated minimum of 7,000 epitopes in the human glycome and only ~100 probes currently on these arrays [23]. Engineering of lectins with novel specificity, which natural lectins don't possess, is the next step in the evolution of this technology. Hirabayashi and colleagues have taken the first steps towards this goal, using a directed evolution approach to create a 6-sulfo-galactose specific lectin from a galactose-specific earthworm lectin [24].

Another advancement in lectin microarray technology was the introduction of dual-color analysis by our laboratory in 2007 [25]. Inspired by the ratiometric technology used in DNA analysis, this strategy uses orthogonally labeled samples mixed with a fluorescently labeled common reference to calibrate glycan expression. The dual-color strategy enables subtle differences in glycan expression to be detected that are invisible in single-color analysis. For example, Lec8 cells, a WGA resistant Chinese Hamster Ovary (CHO) cell line, cannot be discriminated from the parent CHO cells using single color analysis as demonstrated by our laboratory and others [26]. In contrast, dual-color analysis clearly reveals the lower WGA binding of Lec8 [25]. Many of the studies illustrated below utilize these advancements for glycomic profiling with lectin microarray technology.

Applications of Lectin Microarray Technology

Lectin microarrays have now been used to profile a broad range of biological systems from bacteria to human tissue. Glycosylation of bacterial cell surfaces is a critical factor in symbiosis, pathogenesis, cell-cell interactions and immune invasion. We pioneered the use of lectin microarrays to analyze dynamic changes in bacterial surface glycans [11, 27]. These glycosylation patterns allowed us to clearly distinguish pathogenic from nonpathogenic *E. coli* strains, illustrating a simple method for fingerprinting bacteria and labeling their harmful or harmless nature. Recent work by Yasuda *et al*. has applied this technology to the profiling of lactobacilli [28]. Beyond bacteria, viruses also have glycans on their surfaces. Our laboratory applied our dual-color methodology to compare the glycomes of whole HIV (human immunodeficiency virus) and SIV (simian immunodeficiency virus) virions with host-cell derived microvesicles, small virus-like particles shed from cells [29]. This study found strikingly similarities between the glycomes

(Figure 2), which may be related to a shared site of particle assembly. More recent work from our laboratory focused on the glycosylation patterns of microvesicles from a more diverse panel of human cell lines (T-cells, melanoma, and colon cancer) and the physiological fluid breastmilk. Lectin microarray analysis revealed both enrichment and depletion of specific glycan epitopes, implying a role for glycosylation in microvesicle protein sorting [17]. Taken together this work demonstrates how lectin microarray profiling can lead to new understanding about particle assembly and protein trafficking.

Perhaps the predominant use of lectin microarrays has been in identifying glycan biomarkers, [16, 30–37] some recent examples of which are detailed below. Shinkazi *et al* used lectin microarray technology to discover markers of inflammatory bowel disease (IBD). Lectins with affinity for agalactosyl-IgG, an IBD marker, were identified and their utility was subsequently validated using 410 patient samples [32]. In collaborative work, we utilized lectin microarrays to find glycan-based biomarkers of esophageal cancer (see Figure 3) [36]. Analyzing tissues from patients, we identified a series of biomarkers whose expression were lower in high-grade dysplasia, a precancerous condition with a 59% cancer rate, than in normal tissue. Lectin binding patterns were validated by histology. Initial testing of one of the markers for future use in clinical fluorescence endoscopy found it to accurately identify cancerous tissue. Biomarkers have also been found for liver fibrosis. In recent work Kuno *et al* examined the glycosylation patterns of α 1-acid glycoprotein (AGP), a serum glycoprotein associated with liver fibrosis [38]. They studied a 125 patient samples using an antibody-overlay detection system in tandem with their lectin microarray. Glycoprotein binding to fucose and sialic acid lectins predicted progression to liver fibrosis, providing a potential noninvasive glycan-based diagnostic. Lectin microarrays have also been used to validate non-glycan biomarkers. In a recent study, Hernando *et al* found a microRNA, a small non-coding regulatory RNA, which marked the progression to metastasis in melanoma [39]. The microRNA was found to target the GalNAc transferase GALNT7, and modulation of this gene was tied to metastatic potential. Lectin microarray analysis confirmed the effects of this microRNA on the expression of O-linked glycans. These examples and many more show the power of this system for disease-related glycan analysis.

Conclusions and Future Directions

Lectin microarrays are a powerful technology in glycomics, allowing the high-throughput glycan profiling of samples with varied biological origins. There is the need of a constant expansion of the lectin probes on these microarrays to cope with the number of glycan epitopes in human and other systems. Certain patterns of glycans, such as those with specific sulfation patterns, O-mannosylated, O-fucosylated, and a considerable percentage of glycolipids are not yet represented on current lectin microarrays. The rational design and evolution of novel recombinant lectins will continue to expand the number of available glycan probes and help us to build up a more complete picture of the glycome. As we create this picture, enormous amounts of data will be generated, requiring data storage and analysis. Ideally, an optimized platform, such as an open access database with cross-linked information on lectin specificities should be created for this purpose. In tandem, improved understanding of underlying lectin specificities will be crucial to microarray annotation. Glycan microarray analysis of lectin-binding patterns is beginning to provide us with this information [40, 41]. In view of the important roles glycans have in biological processes, the potential of lectin microarrays as a tool for diagnosis and to uncover glycan-related biology is limitless.

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Highlights

- This review focuses on the advances in the field of the lectin microarray technology.
- Continuous improvements and technological advances of lectin microarrays are described.
- Examples of the application of lectin microarrays to a broad range of biological systems are given.

Figure 1.

Scheme of the lectin microarray technology. Lectins are immobilized onto a NHS-activated glass slide using amine coupling chemistry. Biological samples (as the ones above, represented by glycoproteins, bacteria or viruses) are labeled with a fluorescent tag and bound by lectins via their accessible glycan epitopes. The observed binding pattern provides a comprehensive interpretation of the glycosylation status of the analyte.

Figure 2.

i) Experimental scheme using the dual-color approach; ii) H9 membrane was the common biological reference for comparison between microvesicles (MV) and HIV derived from different T-cell lines [H9 (green line), Jurkat-Tat-CCR5 (Jurkat, blue line), and SupT1 (pink line)] and their respective cell membranes (Mb); iii) Jurkat cells labelled with N-Rh-PE. After growth and fixation, cells were stained with FITC-conjugate DSA and observed under fluorescent microscopy. N-Rh-PE domains (in red) colocalized with domains that were enriched in glycans, as recognized by DSA (in green). Figures were reproduced from [29], with permission.