MICROFLUIDIC LECTIN BARCODE ARRAY FOR HIGH-THROUGHPUT GLYCOMIC PROFILING

Y. Shang¹ and Y. Zeng¹,²,*

¹Department of Chemistry, ²Bioengineering Graduate Program, University of Kansas, USA

ABSTRACT

Protein glycosylation holds the key to understanding the molecular mechanisms of cancer and to developing new biomarkers. Here we report on a microfluidic lectin-barcode chip for high-throughput glycan profiling of cancer biomarkers. We have investigated two formats of lectin arrays using a panel of 16 lectins. Various blocking methods have been studied to suppress the interferences caused by non-specific lectin-glycan interactions and the glycan on the detection antibodies, significantly improving the performance of the antibody-overlay-lectin array. Using a biomarker for ovarian cancer, CA125, we demonstrated the feasibility of using the microfluidic lectin barcode assay for glycan profiling of disease biomarkers.

KEYWORDS: Glycomics, Lectin Array, Glycoprotein, Microfluidics

INTRODUCTION

Protein glycosylation has been associated with almost every aspect of malignancy and thus holds the key to understanding the molecular mechanisms of cancer and to developing new biomarkers [1]. Despite its biomedical significance, progress in glycomics has considerably lagged behind genomics and proteomics. While MS-based analyses have shed light on the biological and clinical implications of glycans, low-throughput MS methods have had difficulties in correlating glycosylation aberrations with the pathological status. Similar to DNA and protein microarrays, lectin array offers a simple and high-throughput tool that is complementary to MS, which enables the whole tissue-level studies of human plasma glycocom [2]. However, lectin-based assays suffer from an intrinsic limitation of the lectin-glycan interactions that are much weaker than the antibody-antigen affinity. Microfluidics offers a unique engineering solution to address this limitation because of its advantages in leveraging assay efficiency, speed, sensitivity, and throughput for genomic and proteomic analyses [3]. Surprisingly, very limited progress has been made to develop novel microfluidics-based glycomics analysis techniques. To address this gap, here we aim to develop a microfluidic lectin-barcode chip capable of sensitive and quantitative glycan profiling with high throughput.

EXPERIMENTAL

A multi-channel polydimethylsiloxane (PDMS) microfluidic chip with parallel on-chip elastomeric pumps and assay microchambers was developed to confer precisely controlled reagent delivery and affinity assay conditions (Fig.1A). The PDMS chips were fabricated using the standard multi-layer soft lithography approach. Lectin barcodes were first patterned onto freshly prepared GPS-functionalized glass substrates using PDMS microchannel array devices. Briefly, 0.25-1 mg/mL lectins dissolved in HEPES buffer (10 mM HEPES, 0.1 mM Ca²⁺, Mg²⁺, Mn²⁺) were introduced into each microchannel by vacuum and then incubated at room temperature for 1 to 3 hours in a humid container. Unbound lectins were washed away by flushing the microchannels with 10 μL HEPES buffer for three times. After the last wash, the PDMS patterning chip was peeled off and the lectin barcode assay chip (Fig.1A) was reversibly bound to the patterned glass substrate with the assay chambers aligned to the lectin array. The non-specific active sites on the glass substrate were blocked with a glycoprotein-free blocking buffer. The redundant blocking solution was washed three times with PBS buffer containing 0.1 mM Ca²⁺, Mg²⁺, and Mn²⁺ ions and 0.05% Tween 20 and the lectin barcode assay device was then ready for use. Two formats of lectin array were investigated for glycan profiling: lectin array and antibody-overlay-lectin array, as illustrated in Fig.1B. The first format provides a convenient means to develop and evaluate the...
A panel of 16 lectins was used to create various barcode arrays on the substrate via microchannel patterning. Using standard glycoprotein RNase B and glycan binding lectin, ConA, we optimized the pumping settings that control incubation time and the lectin deposition conditions (Fig. 2A), which shows much faster analysis speed than the conventional assay (60 min. vs overnight). The performance of antibody-overlay-lectin array is largely limited by the interferences from non-specific lectin-glycan interaction and glycans on the detection antibodies.

The performance of antibody-overlay-lectin array is largely limited by the interferences from non-specific lectin-glycan interaction and glycans on the detection antibodies. We have extensively studied surface coating and fluidic conditions for the on-chip assays by labeling the standard glycoproteins with fluorescence dye (FITC). In the second type of lectin assay, the lectin-bound glycoproteins were measured by using biotinylated antibodies and fluorescently tagged streptavidin.

RESULTS AND DISCUSSION

Fig 1. (A) Design of a hybrid microfluidic array chip that integrates eight parallel units each consisting of a three-valve pump and a chamber for lectin barcode assay. The chip was operated by a pneumatic control circuit for simultaneous protein assays. Pneumatic actuation of the assay chamber promotes mixing inside the chambers for fast affinity binding. (B) Schematic of two formats of solid-phase lectin-based glycan assay (from left to right): lectin array and antibody-overlay-lectin array.

Fig 2. Optimization of microfluidic barcode lectin assay using glycoprotein RNase B. (A) Assay time and lectin concentration for patterning. (B) Blocking of nonspecific binding activity for a barcode array of 16 lectins. Inset: fluorescence images of RNase B detection using 16 lectins with or without blocking by hIgG and lactose.
various blocking reagents including BSA, commercial carbo-free and Superblock PBST buffers, human IgG (hIgG) and sugars. Fig.2B shows effective blocking in profiling RNase B using a combination of hIgG and lactose. Differential glycan profiling was also demonstrated by using the antibody-overlay-lectin array for a pair of proteins, RNase A (non-glycosylated) and RNase B (glycosylated), and human transferrin (Htf) (Fig.3A). Strong ConA and SNA signals indicated the presence of high mannose and sialic acid linked α2, 6 to Gal glycans, respectively.

In order to evaluate the feasibility of our method for glycomic profiling of cancer biomarkers, a commercial CA125 protein extracted from ovarian cancer tissue was tested using 16 lectins (Fig.3B). Compared to the low background obtained under the optimized assay conditions, the glycan profile of CA125 showed significantly increased signals for lectins SNA, MAL II, RCA\textsubscript{120}, SBA, VVL, ConA, and LCA, indicating the presence of complex N- and O-linked glycans (e.g., α-Man, Sia\textsubscript{α2–6}Gal/GalNAc, Sia\textsubscript{α2–3}Gal, Terminal Galβ\textsubscript{1–4}GlcNAc\textsubscript{β1}, terminal GalNAc, Fuc\textsubscript{α1–6}GlcNAc). This is consistent with the complex glycan patterns of CA125 reported in the literature [4].

CONCLUSION

We have developed an integrated microfluidic lectin barcode chip and investigated two formats of lectin arrays using a panel of 16 lectins. As a proof-of-concept, we demonstrated rapid detection and differential profiling of standard glycoproteins and glycan profiling of a cancer biomarker CA125. This method would provide a useful tool for high-throughput glycomic profiling of disease biomarkers.

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CONTACT

* Y. Zeng; phone: +1-785-864-8105; yongz@ku.edu