Development and Applications of Lectins as Biological Tools in Biomedical Research

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Abstract: As a new and burgeoning area following genomics and proteomics, glycomics has become a hot issue due to its pivotal roles in many physiological and pathological processes. Glycans are much more complicated than genes or proteins since glycans are highly branched and dynamic. Antibodies and lectins are the two major molecular tools applied for glycan profiling. Though the study of antibodies and lectins started at almost the same time in 1880s, lectins gained much less attention than the antibodies until recent decades when the importance and difficulties of glycomics were realized. The present review summarizes the discovery history of lectins and their biological functions with a special emphasis on their various applications as biological tools. Both older techniques that had been developed in the last century and new technologies developed in recent years, especially lectin microarrays and lectin-based biosensors, are included in this account. Med. Res. Rev., 36, No. 2, 221–247, 2016

Key words: lectin; hemagglutinin; lectin histochemistry; lectin microarray; lectin-based biosensor

1. INTRODUCTION

It has been more than 100 years since the first lectin was discovered in 1888.[1] Due to their specificity on erythrocytes, Landsteiner presumed the actions of plant hemagglutinins were analogous to antibody reactions;[2] however, they are neither catalytic nor immune in origin, and could hardly be deemed as antibodies. Therefore, a general term *lectin* based on the Latin word *legere* was proposed to represent all sugar-specific agglutinins of nonimmune origin.[3] Numerous lectins have been isolated since the application of affinity chromatography in 1960s.[4–7] As new functions and characteristics were unveiled, more and more researchers got intrigued and became involved in this field in the last century.

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The most important and valuable finding about lectins is that they could specifically and reversibly recognize and bind carbohydrates. This property endows lectins with an enormous potential and value in the study of glycoproteins and facilitates the understanding of mechanisms of many physiological and pathological processes. For example, the adhesion of bacteria or viruses to the host cells is a prerequisite for the initiation of infections. The adhesion process of *E*scherichia *coli* on buccal epithelial cells was found to involve sugar-specific reactions since the adhesion could be inhibited by mannose and pretreatment of *E. coli* with Con A could cripple its adhesion on epithelial cells.[8] On these grounds, mannose was deduced to be the common receptor for *E. coli*. Based on the assumption that adhesion of pathogenic organisms to the host is mediated by the reaction between lectins and carbohydrates, Sharon and colleagues pretreated mouse bladders with methyl α-mannoside to prevent the infection reaction initiated by a mannose-specific *E. coli* strain, and it turned out to be effective.[9] Besides, many other bacteria were also found to adhere to host tissue by the lectin-carbohydrate reactions, though the specific sugars might be different.[10]

Lectins have found multiple applications in biological research, both as agents and tools. As evidenced from numerous reports, lectins could be used as potential anti-insect,[11,12] antifungal,[13,14] antiviral,[15] and anticancer[16,17] agents. The applications and mechanisms of action as biological agents are briefly listed in Table I. Many reviews presenting descriptions of lectins from different aspects have been reported. For example, there are a number of papers in the literature focused on the summary of properties of lectins produced by a certain group of organisms, like the reviews on microbial[18–21] and plant lectins.[22–24] Other reviews may emphasize on the demonstration of the relationship between lectin expression and diseases[25–28] or the application of lectins in cancer research and treatment.[29–32] Though a variety of applications of lectins have been reported in different research[33–36] and review papers,[37–39] the application of lectins as biological tools has not yet been systematically addressed before and this issue will be elaborated in the present review.

2. MILESTONES IN THE HISTORY OF LECTIN EXPLORATION

The earliest description of hemagglutinin dated back to 1888 in the doctoral thesis of Peter Hermann Stillmark.[40] The first pure hemagglutinin, concanavalin A (Con A), from jack beans was obtained by James B. Sumner in 1919.[40] Lectin/hemagglutinin was initially found to precipitate glycogen and agglutinate erythrocytes. It was subsequently proposed for the first time by Sumner and Howell in 1936 that the hemagglutinating effect of lectins on erythrocytes may be the consequence of reactions with the stromata of the erythrocytes and the important constituent on the stromata were most likely carbohydrates.[1]

In 1940s, hemagglutinins were found to distinguish between various blood types by specifically agglutinating blood cells.[41] This unique property of lectins prompted the early investigations on the antigens involved in the ABO blood group system.[40] The observation that the agglutinating effect of lima bean lectin on type A blood cells and *Lotus teragonolobus* lectin on type O cells could, respectively, be inhibited by α-N-acetyl-D-galactosamine and α-L-fucose directed Morgan and Watkins to the deduction that α-N-acetyl-D-galactosamine and α-L-fucose were the sugar determinants conferring A and H (O) blood groups.[40]

The mitogenic activity of lectins was reported by Peter C. Nowell in 1960.[42] This finding refuted the belief that lymphocytes were not capable of mitosis. The involvement of sugar binding in mitogenic activity was demonstrated by the report that the mitogenic activity of Con A could be abolished by the addition of sugars; the mitogenic activity of soybean agglutinin (SBA) on lymphocytes could only be activated when the subterminal galactose and

Table I. Applications of Lectins as Biological Agents

Activity	Lectins	Targets	Mechanisms
Anti-tumor	Con A (from *Jack beans*)	Hepatoma (Mice-P)[155]	Binds with tumor cells in a mannose-specific manner and induces autophagy
	Mistletoe lectins	Pancreatic cancer (Mice-P),[156] head and neck cancer (human-N),[157] breast cancer (human-P)[158]	Induce apoptosis or inhibit protein synthesis
Anti-HIV	BanLec (from banana)[15]; cyanovirin-N (from *Nostoc ellipsosporum*)[159]	HIV virus (in vitro)	Bind to the glycosylated HIV-1 envelope protein (gp 120) and block cellular entry
	Lectins (from leek, stinging nettle, and HHA)[160]	SARS-CoV (in vitro)	Interfere with the glycans on the spike during virus entry and virus release
Anti-insect	AJL (from *Arisaema jacquemontii blume*)[161]	*Bactrocera cucurbitae* larvae	Decreases the activity of acid phosphatase and alkaline phosphatase
	MuBL and MuHL (respectively isolated from *Myracrodruon urundeuva* bark and heartwood, respectively)[162]	*Aedes aegypti*	Increase larval mortality
Anti-fungal	A lectin from *Curcuma amarissima Roscoe*[163]	*Fusarium oxysporum*, *Exserohilum turicicum*, and *Colletotrichum cassiicola*	Not explained
	TEL (*Talisia esculenta*)[164]	*F. oxysporum*, *Colletotrichum lindemuthianum*, and *Saccharomyces cerevisiae*	Crosslinks chitin and prevents cell expansion at tip of the growing hyphae
Anti-bacterial	Ch-salectin (cloned from *Crassostrea hongkongensis*)[165]	*Escherichia coli*, *Vibrio alginolyticus*, *Bacilllus thuringiensis*, and *Staphylococcus aureus*	Binds to a sialic acid containing protein fetuin and inhibits the growth of bacteria
	HSL (*H. scabra* lectin)[166]	*Staphylococcus* sp., *Serratia* sp., *Proteus* sp., *Proteus* sp., etc.	Binds to the glycoconjugte moiety on the surface of bacteria and enhances the phagocytosis rate

N-acetylgalactosamine residues of glycoproteins on the cell surface were exposed by the action of neuraminidase.[43] Many other lectins were also found to be mitogenic.[21,44,45]

The preferential agglutinating property of lectins on malignant cells was discovered in 1960s. Aub et al.[46] initially intended to study the nature and changes of cell surfaces during malignancy by comparing the response of tumor and normal cells to the same enzyme (lipase) that included phospholipase-D (PL-D), porcine pancreatic lipase, and a lipase prepared from wheat germ. However, difference was only found in the presence of wheat-germ lipase that tumor cells were closely attracted to each other while normal cells remained separate. When

the wheat-germ lipase was heated up to 75°C at which temperature the activity of lipase should be completely destroyed, the clumping effect of wheat-germ lipase remained intact, indicating the clumping effect was actually caused by certain impurities in lipase preparation. Three years later, this functional impurity (hemagglutinin) was isolated and characterized by Burger and Goldberg.[47] Most importantly, they found that the agglutinating effect could be reversed by N-acetyl-D-glucosamine (GlcNAc), indicating that GlcNAc was a competitive factor with the site responsible for the attachment of the agglutinin to the cell surface. Many other lectins were subsequently found to possess similar functions to differentiate tumor cells or subpopulations. For instance, the major immature thymocyte subpopulation could be separated from the immunocompetent minor subpopulation by selective binding of peanut agglutinin (PNA).[48] This selective effect was attributed to a Gal1β1,3GalNAc α2,3-sialyltransferase that was capable of masking the PNA binding sites and comparatively more extensively expressed on immunocompetent subpopulations.[49] For some time, PNA binding was deemed as a classic technique for defining the cortical and medullar regions of the thymus.[49]

The availability of affinity chromatography in 1960s and the advent of recombinant techniques in 1980s made a large number of pure lectins available[40]. The 1970s and 1980s were the golden era for lectin research during which time the physicochemical properties, amino acid sequences, and 3D structures of lectins were gradually unraveled and elucidated.[40,50–52] Lectins were intensively studied and numerous papers describing purification of new lectins,[53,54] binding properties of lectins,[9] utilization of lectins in the study of membrane glycoproteins,[55,56] antimicrobial activities of lectins,[56] etc. were published. The antitumor activity of lectins isolated from *Ricinus communis* seeds was reported in 1977.[57] Since 1980s, many other lectins were also extensively reported to possess antitumor activity, though only few of them have been applied to clinical trials.[58,59] With the advent of the 21st century, high throughput lectin microarray and highly sensitive lectin-based biosensor have been developed. However, new functions and applications of lectins are still expected and will be constantly discovered in the future. A diagram summarizing the important findings of lectins is presented in Figure 1.

3. THE DEVELOPMENT OF LECTINS AS BIOLOGICAL TOOLS

A. Lectins as Tools for Targeting Tumor Markers

Early detection of tumor metastasis is very important for the survival of cancer patients. In most cases, the metastases are not detectable until they have reached a certain size. Then it is usually too late and difficult to control the tumor progression. Besides early detection, a more complete understanding of tumor metastasis is highly critical. The applications of lectins in the detection of tumor metastasis and study of the mechanisms of tumor metastasis have both been reported in the past decades.[60,61] More and more research has unraveled that the process of tumor metastasis is always mediated by changes of glycoproteins on the cell membranes, which may facilitate migration of cancer cells to secondary sites.[62] Alterations in cellular glycosylation could be studied by lectins and monoclonal antibodies.[63,64] Lectins are superior to monoclonal antibodies in the recognition of terminal, internal, or branched sugar residues, making lectins valuable in the study of glycosylation.

The binding with lectin isolated from *Helix pomatia* (HPA) was reported to be correlated with local axillary lymph-node metastasis of breast cancers in 1984.[65] The same authors subsequently published a 20-year retrospective study that revealed a relationship between HPA binding and survival as well as cancer recurrence time in breast cancer patients, presenting the potential of HPA to predict long-term survival of breast cancer patients.[66] Around 80% of primary breast cancers could be stained by HPA and overall, 79% of the metastases were

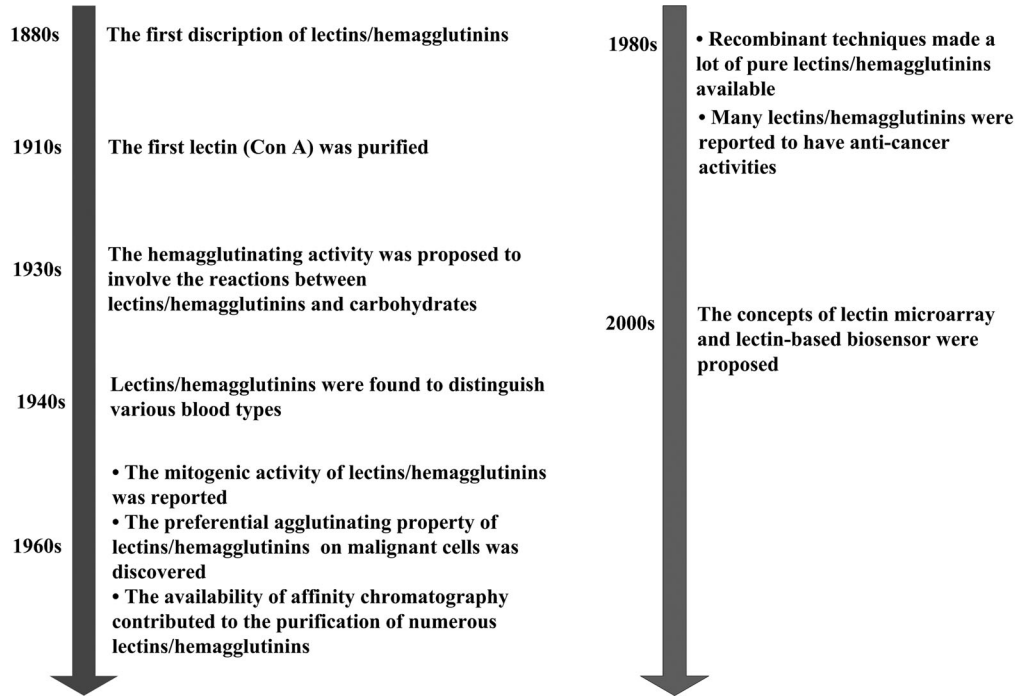

Figure 1. A diagrammatic sketch of millstones in the history of lectin research.

HPA-positive irrespective of the sites which may be the brain, lymph node, lung, or liver. However, though the majority of primary breast cancers are HPA-positive, both HPA-positive and HPA-negative metastatic tumors could be found in the same individual, indicating that the binding profile of metastatic tumors may evolve to a different form.[67]

HPA binding is also associated with the metastasis of other cancers, such as lung cancer,[68] melanoma,[69] prostate cancer,[70] and colon carcinoma.[71] About 90% of 96 patients with metastatic adenocarcinoma of the lung expressed HPA-binding glycoproteins.[68] HPA showed selective binding on metastatic (HT29) and nonmetastatic (SW480) human colorectal cancer cells.[72] Glycoproteins from HT29 cells that could be recognized by HPA were isolated by a HPA affinity chromatography column and analyzed by MS. They included glycoproteins involved in cell adhesion and migration (integrin $\alpha 6$, integrin αV, and annexins) as well as proteins that participated in antiapoptotic pathways (Hsp-70, Hsp-90, and TNFR-1).[72]

Besides HPA, many other lectins were also demonstrated to be capable of discriminating cancer cells from normal cells. They can directly characterize biomarkers or indirectly facilitate the detection of other biomarkers. TIMP1 (tissue inhibitor of metalloproteinase 1) is a glycoprotein which is aberrantly glycosylated with terminal addition of β-1, 6-N-acetyglucosamine (β-1, 6-N-GlcNAc) in colorectal cancer cells.[73] However, its low abundance in serum limited its clinical applications. A research group solved this problem by enriching the glycosylated TIMP1 isoform via the utilization of phytohemgagglutinin-L_4 (PHA-L) that could specifically recognize β-1, 6-N-GlcNAc, followed by trypsin digestion and immunoenrichment by monoclonal antibody.[73] It achieved a sensitivity at attomolar levels.[73] *Sambucus nigra* agglutinin (SNA) and PNA in combination with antibodies were applied to the detection of a breast cancer biomarker CA 15- 3.[74]

Among various types of oligosaccharide modifications, fucosylation is demonstrated to be one of the most important types in carcinogenesis and cancer metastasis.[75] Fucosylated

haptoglobin (Fuc-Hpt) is identified to be a serologic marker for pancreatic cancer.[76] The expression of α1, 2-fucosylation of a prostate-specific antigen (PSA) was elevated in prostate cancer patients when compared with that in patients with benign prostatic hyperplasia (BPH).[77] Alpha-1-proteinase inhibitor (API) isolated from the sera of healthy women and that from women diagnosed with breast or ovarian cancer were significantly different in fucose and GlcNAc content.[78] Fucosylation of alpha-antitrypsin (F-AT) demonstrated the potential of predicting unresponsiveness of ovarian cancer patients to chemotherapy.[79] Among patients with various types of advanced malignancies, those detected with highly fucosylated serum α_1-acid glycoprotein (AGP) were more likely to have a poor prognosis after surgery.[80] Besides, alteration in fucosylation of alpha-fetoprotein (AFP) is found to be closely related with hepatocellular carcinoma (HCC).[75] In such a case, lectins that could recognize fucosylated glycoproteins are very useful. In a short conclusion, tumorigenesis and developments are indeed accompanied with complicated alternations of glycosylation and lectins are very useful biological tools to characterize them.

B. Affinity Chromatography Using Immobilized Lectins

The first Con A column was designed by Edwin H. Donnelly and I. J. Goldtein in 1970.[81] Con A was polymerized by the treatment of glutaraldehyde and finely dispersed before mixing with Bio-Gel P-10. The mixture was loaded into a column which was then prepared for use. The carbohydrate specificity of cross-linked Con A was demonstrated to be intact and showed tremendous potential in fractionating different polysaccharides and glycoproteins by selectively binding to the sugar residues.[81] The interaction between lectins and carbohydrates is noncovalent, but involves hydrogen bonds, as well as hydrophobic, electrostatic, and van der Waals interactions.[82] The binding affinity between lectins and carbohydrates is very weak, with dissociation constants varying from approximately 10^{-3} to approximately 10^{-6} M[83] and bound proteins could be easily detached using a competitive binding substance. Besides, the interaction between lectins and carbohydrates is not catalytic, which avoids the modification of the ligand on target proteins during the purification process. Several types of Sepharose-based affinity chromatography media with immobilized lectins are commercially available. For example, Con A-Sepharose and Lentil Lectin Sepharose 4B column are provided by GE Healthcare Life Sciences. Among the various lectin chromatography media, Con A-Sepharose and wheat germ agglutinin (WGA) agarose affinity chromatography are the most commonly used due to their stability and comparatively low price.

Since a lectin may not be very specific, two or more glycans can bind with a certain lectin affinity column.[84] Therefore, a serial type of lectin affinity chromatography (SLAC) could be employed to further isolate them. In the study of O-glycosylation sites on proteins from the human blood serum, a combination of Con A (specific to mannose) and Jacalin (specific for GalNAC in O-glycosylation and mannose N-type glycans) columns was selected to isolate O-glycosylated peptides.[84] A series of lectin columns composed of Con A, *Pisum sativum* (PS) lectin, WGA, phytohemagglutinin E4 (PHA-E4), and phytohemagglutinin L4 (PHA-L4) was designed to compare the changes between prostate carcinoma (PCA) and BPH in sugar-chain structures of serum PSA.[85] PSA was separated into seven fractions by the lectin column and the relative amounts of each fraction were compared, with results showing that the relative level of a multiantennary complex type with branched GlcNAc β (1$\rightarrow$4) mannose was significantly increased in PCA.[85] *Wisteria floribunda* agglutinin affinity chromatography was utilized to lead a more detailed N-glycome pattern analysis of bovine lactoferrin (bLF) by separating the N-glycan pool into three epitopes.[86] AFP is clinically applied as a serum marker for the diagnosis of HCC. Though the increased serum level of AFP is demonstrated to be

correlated with HCC and is employed for tumor detection, sensitivity and specificity of this method is unsatisfactory.[87] Therefore, the study of AFP glycoforms is proposed to increase the sensitivity and specificity. A variety of lectins have been employed to isolate and characterize the glycoforms of AFP. For example, by the difference of affinity to Con A, AFP is separated into AFP-C1 and AFP-C2; by *Lens culinaris* agglutinin (LCA), AFP is separated into AFP-L1, AFP-L2, and AFP-L3; and by erythroagglutinating phytohemagglutinin of *Phaseolus vulgaris* lectin (PHA-E), AFP is separated into AFP-P1, AFP-P2, AFP-P3, AFP-P4, and AFP-P5.[88] AFP-L3 is one of the glycoforms that have been widely studied. It can be recognized by LCA and appears to be uniquely produced by cancer cells.[89] According to the report of Debruyne,[87] AFP-L3 detection (sensitivity: 80–90% and specificity: more than 95%) displayed a much more promising result than total serum AFP detection (sensitivity: 40–65% and specificity: 76–96%) in clinical applications. Other than the application in biomedical and clinical research, lectin chromatography could also contribute to the development of other areas. For example, lectin affinity chromatography is utilized to isolate and characterize the polysaccharides that cause membrane fouling in membrane bioreactors for wastewater treatment.[90]

Lectin affinity chromatography has been well commercialized and researchers who have little experience could also easily proceed by following the manufacture's instruction. Many prepacked, reusable individual column agarose gel kits that include washing/carbohydrate elution buffer, gel, and column holder can be purchased from EY Laboratories.

Based on the same principle, carbohydrate affinity chromatography is also developed to isolate lectins. The binding specificity of the target lectin should be learned in advance so that a proper carbohydrate affinity column can be chosen.

C. Lectin Histochemistry and Cytochemistry

Based on the carbohydrate-binding property of lectins, they were applied to the histological staining of specimens as early as 1980s.[91] Basically, lectins can be labeled with a fluorescent dye, such as fluorescein isothiocyanate (FITC), and viewed directly by using a fluorescence microscope, or they are conjugated to enzymes (such as horseradish peroxidase) or haptens (such as biotin and digoxigenin), followed by second incubation with proper substrates to become detectable by light microscopy or electron microscopy with peroxidase, ferritin, or colloidal gold.[92] Biotin is a small molecule that can be easily attached to proteins chemically or enzymatically while not destroying the function of proteins. Biotinylated lectins that bound with specific glycoproteins on the specimens were detected by avidin biotin peroxidase complex (ABC) and finally visualized after incubation with 3.3′-diamiobenzidine (DAB) substrate.[91] Avidin could also be conjugated with colloidal gold[93] and allows the detection of biotinylated lectins under both light and electron microscopes. Lectins conjugated to digoxigenin could be detected by antidigoxigenin antibodies that may be complexed to particles of colloidal gold for electron microscopy.[94] One of the recent applications is that several biotinylated lectins were used to characterize the sections of 93 lung adenocarcinomas patients.[95] The results indicated that the binding of HPA, *Phaseolus vulgaris* leukoagglutinin (PHA-L), and *Ulex europaeus* agglutinin had great prognostic value for the overall and relapse-free survival.[95] WGA and Con A conjugated with horseradish peroxidase allowed the visualization of *Aspergillus* structures in histopathological specimens of infected tissues, such as the brain and lungs.[96] Ten different horseradish peroxidase conjugated lectins were employed to characterize distribution of the glycoconjugated oligosaccharides in placentas from women complicated by altered glycemia in different degrees.[97] Fluorescein-tagged WGA was developed as a fast, reliable, and inexpensive tool for visualization of skeletal muscle fiber and general connective tissue.[98] Lectin histochemistry also exhibited great value in identifying apoptotic spermatocytes and spermatids; with

Table II. Commercialized Lectin-Derived Tools

Types of tools	Representatives	Applications	Companies
Lectins for histochemistry and cytochemistry	Lectin from *Ulex europaeus*-Atto 488 conjugate (19337)	For the detection of α-L-fucose by microscopy, flow cytometry and fluorescence in situ hybridization, etc.	Sigma-Aldrich
	WGA, Alexa Fluor® 488 Conjugate (W11261)	For the detection of N-acetyglucosamine and sialic acid residues by microscopy and flow cytometry	Life Technologies
	Horseradish peroxidase (HRP)-conjugated *Limax flavus* lectin (LFA) (H-5101-1)	For the detection of sialic acid (generic term for derivatives of N-acetylneuraminic acid and N-glycolylneuraminic acid)	EY Laboratories
	DIG Glycan Differentiation Kit (11210238001)	For differentiation between complex and high-mannose chains, differentiation between α(2,3) and α(2,6) linkage of terminal sialic acids, and identification of the core disaccharide Galβ(1,3)GalNAc of O-glycans	Roche
Lectin blotting kits	Lectin from *Glycine max* (soybean) peroxidase conjugate (L2650)	For the detection of N-acetyl-D-galactosamine of cell lysate by Western blot	Sigma-Aldrich
Columns for glycoprotein isolation	Con A lectin resin (89804)	For the isolation of glycoproteins containing alpha-linked mannose and terminal glucose residues	Thermo Scientific
	Lentil Lectin-Sepharose 4B (17-0444-01)	For the purification of a number of glycoproteins and other carbohydrate containing molecules	GE Healthcare Life Sciences
Lectin microarrays	PlexArray® Lectin Array Kit I (BG-L-K001)	For glycoprotein screening (label free)	Plexera
	Lectin Array 40 (GA-Lectin-40)	For glycoprotein screening (fluorescence labeling)	RayBiotech

this method, Gal β1, 3-GalNACα1, α-D-mannose, GalNAc, and L-fucose were found to be the glycoconjugate residues that correlated with the apoptosis of germ cells.[99]

Fluorescent-, hapten- and enzyme-labeled lectins are commercially available (as displayed in Table II). There were several types of fluorescence-tagged lectins designed for Golgi apparatus staining from Life Technologies. Golgi is the organelle that modifies different

macromolecules inside the cells. Glycosylation of proteins is completed in the Golgi by addition of carbohydrates,[100] which makes the Golgi abundant in glycoproteins and easily tracked by different lectins. According to the introduction provided by Life Technologies, WGA conjugates could specifically stain the trans-Golgi in fixed cells.[101] GS-II from *Griffonia simplicifolia*, which demonstrates eminent specificity to terminal nonreducing α- and β-N-acetyl-D-glycosaminyl (GlcNAC) residues of glycoproteins, could be applied to the staining of intermediate-to-trans Golgi.[101] *Helix pomatia* agglutinin (HPA) could track Golgi by specifically binding to α-N-acetylgalactosaminyl residues on the organelle.[101] However, these Golgi staining products described in that handbook are currently discontinued.[101] Nevertheless, many other fluorescence-tagged lectins were commercially available for the detection of cellular carbohydrates. The company EY Laboratories provides very comprehensive lectin products and lectins conjugated with different fluorescent dyes, biotin, colloidal gold, ferritin, and horseradish peroxidase are all commercially available.

These well-labeled lectins are also very powerful tools for the study of membrane trafficking.[102] For example, redistribution of *Galanthus nivalis* agglutinin (GNA) binding glycoproteins triggered by guanosine 5′-O-(3-thio) triphosphate (GTPγS), which perturbs endoplasmic reticulum (ER) Golgi transport, was tracked by GNA.[102] It is interesting to find that after treatment of GTPγS, GNA-containing vesicles tended to accumulate from ER to the perinuclear region of the cells.[102] The application of GS-II-Alexa 594 or GNL-Alexa 647 conjugate together with other methods to the characterization of conserved oligomeric Golgi (COG) depleted cells revealed the importance of COG complex in trafficking and maintenance of glycosylation machinery.[103] Fluorescence-tagged lectins could also be utilized to sort different cells by flow cytometry.[104]

It could be concluded that lectins are very important tools to trace glycoproteins on tissues, single cells, or organelles. However, its applications are not limited to the ones described above. For example, WGA has become a mature and effective tool to trace transsynaptic circuitry in anatomical studies.[105] It could be taken up by neurons and label both first- and second-order neurons.[105] Intranasal administration of peroxidase-conjugated WGA (WGA-HRP) to adult rats suggested that WGA-HRP could be transported from olfactory receptor axons to the brain.[106] Yoshihara et al. first applied WGA cDNA under the control of neuron-type specific promoter elements to visualize neural structures that were anatomically connected and functionally related.[105] This technology was demonstrated to be very successful with great accuracy and reproducibility. By far, WGA transgene has become a genetic tool to visualize neural structures.[107] T2r5-WGA transgenic mice could specifically express WGA in taste receptor cells (TRCs) and served as a powerful tool to trace the neural pathway for aversive sensations.[108] Researchers from Canada and Japan evaluated neural connectivity from the locus coeruleus via transgenic mice containing WGA transgene that was conjugated to a dopamine-β-hydroxylase gene promoter.[107]

D. Lectin Blotting

Lectin blotting is a technology analogous to immunoblotting. The procedures are similar to those of immunoblotting with only a few discrepancies. Lectin conjugation methods described in the "Lectin histochemistry and cytochemistry" section could also be applied here.[109] After electrophoresis, proteins were transferred to nitrocellulose membranes, equilibrated and incubated with biotinylated lectins, followed by washing and incubation with streptavidin–alkaline phosphatase conjugate.[102] The blots will then be developed with BCI/NBT (5-bromo-4-chloro-3-indolyphosphate/nitro blue tetrazolium) substrate.[102] In this process, biotinylated lectins are analogous to the first antibodies in immunoblotting, but biotinylated lectins are much more easily prepared and more economically available. Immunoblotting covers a wider range of

proteins, while lectin blotting is more specific to glycoproteins. With this technology, lectins can help identify certain glycans in certain pathways or organelles semiquantitatively.

Lectin blotting has now been widely used in biological research especially in the study of cancers. Together with lectin histochemistry, the relationship between certain alterations of glycosylation and development of metastatic phenotype of cancer could be better characterized. The combination of MS technology and lectin blotting could help identify the type of alliterated glycoprotein. For example, duplicate SDS gels of different breast cancer cell lysates were prepared and the proteins on one of the gels were transferred to nitrocellulose and detected with HPA; referring to the bands detected by HPA blotting, the HPA-binding proteins of interest could be manually excised and further analyzed by MS.[110] With this method, heat shock protein 27 (Hsp27), HnRNP D-like, HnRNP A2/B1, and enonlase 1 (ENO1), etc. were identified and found to be upregulated in metastatic MCF7 and T47D cells.[110] Glycoproteins Hsp27 and ENO1 were, respectively, involved in apoptotic pathway and cell migration, which may facilitate the metastasis of breast cancer cells.[110] Besides, integrin $\alpha 6$ is a common HPA-binding protein found in both colorectal and breast cancer cells.[110] A research group from Japan found cells in G1 phase were more susceptible to influenza virus infection than cells in S/G2/M phase and they attempted to disclose the underlying molecular basis by analyzing the sialic acid on cell membranes, which had been demonstrated to the binding targets of influenza virus.[111] Digoxigenin-labeled *Maackia amurensis* lectin (MAL) and *S. nigra* lectin were, respectively, employed to detect sialic acid with $\alpha 2$-3 and $\alpha 2$-6 linkage by blotting methods.[111] The level of sialic acid in both forms of linkages was found to be indeed higher in G1-phase cells.[111] Though lectin blotting is a comparatively cursory method for the detection glycoproteins when compared with lectin-based biosensor, it is easier to prepare and more commercially available and will be widely adopted in the study of glycoproteins.

E. Microarrays

In total, 50–90% of human proteins are proposed to be glycosylated and the glycosylated surface of cells function as an information center that regulates interactions between and within cells.[112] Glycans could be recognized as "identity cards" and subtle alterations may lead to dramatic biological responses.[112] With the revelation of the importance of glycomics, there is a great demand for the development of easy, economical, and time-efficient methods to unveil its mystery. The rich diversity of saccharidic building blocks and its combination forms contributes to the structural complexity of glycans. Glycan sequences could not be directly obtained from the genome, which make it much more difficult to approach than proteins.[113] Lectins, as natural proteins that can specifically recognize different carbohydrates, are employed as the decoders.[114,115] However, single lectin detection would hardly manage the multifarious information encoded in the glycans. Therefore, the idea of collecting a number of lectins on a small chip for glycan profiling was proposed in response to the needs.

Lectin microarray was first reported in 2005 and has become a research hotspot. The application of lectin microarrays in the study of cancer,[116,117] bacteria,[118] fungi,[119] stem cell,[120,121] sperm,[122] diabetes,[123] etc. has been reported in the last 5 years. Lectin microarrays are generally prepared by immobilizing a series of lectins with well-documented specificity on a solid surface. Analytes that may include glycoproteins, cells, or bacteria are labeled with fluorescent dye or antibody before loading on lectin microarrays. Glycoproteins, cells, or bacteria will bind with the corresponding lectins immobilized on the chips depending on the types of carbohydrate residues. Unbound glycoproteins are removed by washing before the chip is viewed by a confocal-type fluorescence scanner. However, the detection sensitivity may be reduced during the washing process since the interactions between lectins and glycoproteins are weak[83] and may be easily ravaged by washing. The washing process that was performed to reduce the

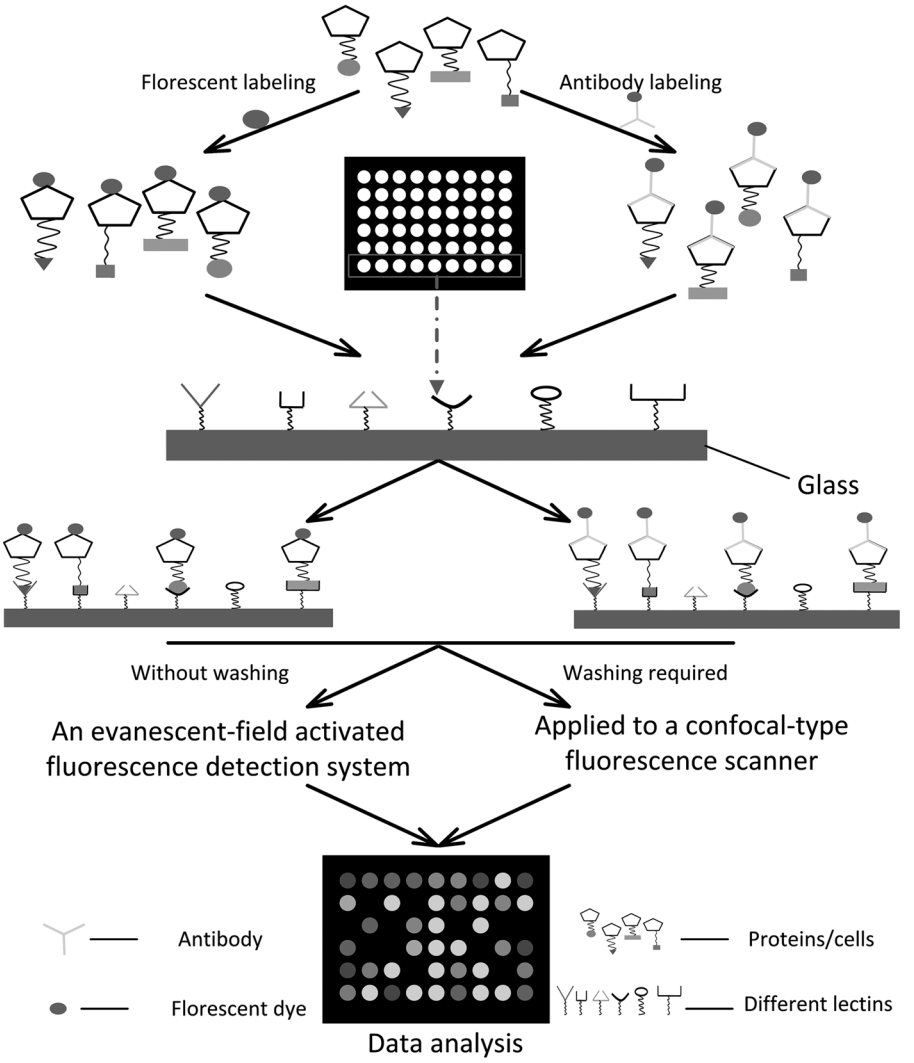

Figure 2. A general scheme of lectin microarray detection. Analytes can be labeled with fluorescent dyes or antibodies and subsequently detected by an evanescent field activated fluorescence detection system or a confocal-type fluorescence scanner.

interference of background could be exempted if evanescent wave activated fluorescence detection method was applied.[124] An evanescent wave will be generated when the total reflection at both interfaces between slide glass and liquid phase and between slide glass and air is achieved.[38,125] The evanescent field is limited to a near space (approximately 100–200 nm) in the liquid phase and enables the direct detection of binding glycoproteins.[125] The general detection procedures are present in Figure 2.

Compared with traditional methods applied to the study of glycoproteins, such as high-performance liquid chromatography, mass spectrometry, and capillary electrophoresis, lectin microarrays are more time and labor saving.[126] Besides, the analytical sensitivity of lectin microarrays is much higher than the traditional methods and only a very small amount of sample is needed for analysis.[64] Except for the efficiency and sensitivity, lectin microarrays are also superior in keeping the biological structures intact during the detection process and could

even be directly applied in the detection of whole cells.[127] For example, the interaction between the glycoproteins on the surface of bacteria and lectins could be directly monitored by this technology. Bacteria were labeled by SYTOX Orange nucleic acid stain, subsequently allowed to interact with the lectins immobilized on a chip, and detected by a fluorescent scanner to fast screen out its binding lectins.[128] Lectins are also superior to anticarbohydrate antibodies since the latter could not be applied to a comprehensive glycan profiling due to the rigid specificity.[38]

Lectin microarrays can serve as a powerful tool for both the excavation and surveillance of markers for cancers and other diseases. Many clinical biomarkers are glycoproteins, and single detection of the proteins has been confronted with handicaps in clinical performance. For example, PSA is generally taken as a serum biomarker for prostate cancer; however, it fails to specifically differentiate aggressive cancer (AC) from nonaggressive cancer (NAC).[129] The analyses of its glycosylation may help identify the possible glycosylation isoforms and increase its clinical performance. To detect differences of the glycosylation of PSA between AC and NAC patients, glycoproteins extracted from normal subjects, patients with NAC, AC, and metastasis were first identified by a high-density lectin microarray.[64] Lectins that could bind with PSA were further applied to the verification of glycosylation changes of individual specimens using lectin-based immunosorbent assays.[64] Based on this two-phase analytical platform, PSA-SNA and PSA-Jacalin were found to be potentially useful for the diagnosis of AC.[64] Lectin microarrays also greatly facilitate the discovery of new biomarkers. For example, a possible urine biomarker, fetuin-A for the prediction of the progression of diabetic nephropathy was discovered by applying the urine samples of patients to lectin microarrays.[123]

In addition, lectin microarrays also find great applications in distinguishing cell types. Hiroaki Tateno et al. applied a lectin microarray equipped with 96 lectins to perform a comprehensive glycan analysis of 114 types of human-induced pluripotent stem cells (iPSCs) derived from four types of somatic cells (SCs).[121] Glycan profiles of these iPSCs were compared with that of nine types of human embryonic stem cells (ESCS).[121] As presented in their results, differentiated SCs and undifferentiated iPSCs/ESCs clustered as two distinctively separate groups when analyzed by unsupervised hierarchical clustering, from which they concluded the two following pieces of valuable information: (1) glycan profiles of SCs were originally different from ESCs; (2) upon induction of pluripotency, SCs acquired common glycans that were similar to that of ESCs.[121] Furthermore, the research group screened out α1–2Fuc-specific lectin rBC2LCN to be the best lectin among all examined to distinguish undifferentiated iPSCs/ESCs from differentiated SCs, and recombinant MOA (rMOA) to detect the contamination of MEF (mouse embryonic fibroblast) in human iPSCs.[121] Fluorescently labeled rBC2LCN (available in Alpha Laboratories) has been commercially used as an agent for detection of undifferentiated iPS/ES. A similar research was reported in the same year that with the application of lectin microarrays, *Euonymus europaeus* lectin (EEL), MAL, and PHA-L were found to be capable of distinguishing undifferentiated human embryonic stem (hES) cells from differentiated hES cells.[127] A lectin microarray with 91 lectin probes was designed for a comprehensive glycocalyx profiling of sperms collected from five different mammalian species by Tao and colleagues.[122] Consistent with other reports, human sperms were demonstrated to contain a higher level of sialic acids than nonhuman sperms.[122]

An important finding about the camouflage and exit mechanism of HIV from host cells was reported by the research group headed by Professor Mahal and colleagues through the application of lectin microarrays and other techniques.[130] The researchers compared the glycosylation pattern among the cell membrane and microvesicles (MVs) prepared from of uninfected T-cell line H9, and HIV-1 (MN) CL.4 strain derived from H9 with lectin microarray.[130] They surprisingly found that HIV and MVs shared similar glycopatterns that were rich in high mannose, complex N-linked glycans, N-acetyllactosamine, sialic acid, and fucosylated epitopes while

blood group antigens expressed on uninfected H9 cells were excluded.[130] It was proposed that HIV-1 may disguise itself as an immunomodulatory MV to deceive the immune system and exit the cells by the same mechanism adopted by MVs.[130] The researchers from the same laboratory later applied a similar approach to the study of MVs from six human cancer cells lines and analyzed their glycosylation patterns.[131]

Another interesting application of lectin microarray comes from the report of Professor Joshi who studied glycosylation changes of bLF at different stages of lactation.[132] As a bioactive glycoprotein in bovine milk, bLF is vital for the protection of newborns at the early stage of lactation.[133] It possesses various beneficial properties that include antimicrobial, antiadhesive, anticancer activities, etc.[134] The glycan components in bLF are identified to play important roles in its multiple biological functions.[133] The research group of Professor Joshi characterized the changes of glycosylation of colostrum (day 0 after postpartum), transitional milk (day 6), and mature milk (day 90) by a lectin microarray and found that the intensity of lectin binding was at the highest level in the initial days postpartum and decreased from day 4, indicating the end of colostrum production and loss of diversity in glycosylation.[132] At the end of their paper, authors also emphasized the great potential of lectin microarrays at an industrial scale to offer a more convenient platform to monitor the glycosylation and quality of proteins.[132]

The concept of glycan microarray has also been brought up in 2002.[135,136] The manufacturing of glycan microarrays is similar to that of lectin microarrays. Different glycans are immobilized on the surface of glass by covalent coupling, physical adsorption, or other methods. The detection of ligand binding could be realized by labeling or surface plasmon resonance (SPR).[137,138] Glycan arrays find important applications in clinical diagnostics and profiling of glycan-binding ligands, especially lectins.[139] For example, it is a very powerful tool in the analysis of the influenza virus receptor specificity since it may cover hundreds of glycan sequences and quickly characterize the hemagglutinin (HA) on the influenza viruses.[140] Influenza viruses are classified into A, B, and C types, among which type A virus is responsible for the majority of the known epidemics and pandemics.[141] The subtypes of type A virus are named by two antigens, HA and neuraminidase (NA), which are, respectively, responsible for the process of targeting host cells and the escape of progenitor viral particles by removing sialic acid.[141] Sialic acid linked to galactose by an α-2,6 linkage (SAα2,6 Gal) is mainly expressed by epithelial cells of the human upper respiratory tract, therefore if the HAs on the influenza viruses could specifically bind SAα2,6 Gal, this type of viruses is usually human-adapted; on the contrary, the avian viruses demonstrate the highest affinity to receptors linked with SAα2,3Gal.[142] According to the research of Shinya et al., SAα2,3Gal is only prevalent in the lower region of the human respiratory tract, and that explains why H5N1 that prefers to recognize SAα2,3Gal could not be commonly transmitted among human beings.[142] The species that express both SAα2, 3 Gal and SAα2, 6Gal linked receptors, such as pigs, are susceptible to both human and avian viruses, serving as a mixing vessel to generate reassortant viruses.[143] Under the right conditions, the H5N1 virus has chances to mutate to a strain that could directly affect human beings and be easily transmitted. Therefore, it is critical to profile the antigens of a new mutant influenza virus to estimate the potential of transmissions among humans and glycan microarray is one of the very powerful tools.

Lectin microarrays and glycan microarrays are both commercially available in the market. For example, lectin microarrays could be purchased from Procognia, GlycoTechnica, Plexera, Raybiotech, and other companies. Notably, Lectin Array Chip Kit from Plexera is reusable for at least ten times. Glycan Array I and Glycan Array II that, respectively, include 28 and 24 kinds of glycans could be purchased from Molecular Biotechnology. Glycan Array 100 that contains 100 most frequently described glycans is available from Raybiotech.

F. Lectin-Based Biosensors

With the application of biosensors, biological interactions between lectins and glycans are transformed into a signal that could be easily quantitated, analyzed, and presented in a user-friendly way. Biosensors are classified into optical, mass, electrochemical, and thermal biosensors depending on the method of signal transduction. A fluorescent biosensor based on Con A was proposed by a research group from Japan in 2000.[144] Fluorescent Con A was obtained by attaching a photoaffinity labeling reagent to the sugar-binding pocket of Con A followed by UV irradiation and subsequent treatment with dithiothreitol (DTT) to yield a mercaptobenzyl site which that modified with an iodoacetylated dansyl group.[144] The principle of detection was that the binding of specific saccharides to Con A will be sensitively monitored by the change of emission intensity and maximum of the fluorophore. The disadvantage of this method is that labeling of lectin is required, which may increase unwanted variability. Effort was devoted to the development of label-free lectin-based biosensors in the next few years. The initial application of electrochemical detection in the study of glycobiology was reported by Professor Joshi and Professor Wang in 2006.[145] PNA was immobilized on a gold substrate coated with a mixed self-assembly monolayer (SAM).[145] The extent of competitive binding between a nanocrystal-labeled sugar and the analyte was monitored by sensitive amperometric stripping voltammetry.[145] Yielded cadmium stripping voltammetric current peak decreased with the increase of affinity binding of the target glycan on the biosensor.[145] This method was highly sensitive and its detection limit was at the micromolar level.[145] Labeling was still required though the labeling of the lectin and target glycans was not needed.

Many label-free technologies, including SPR, quartz crystal microbalance (QCM), and electrochemical impedance spectroscopy (EIS) that have been applied in genomics and proteomics could also be employed in the study of glycomics.[146] SPR is the technique that has been widely used for the detection of molecular interaction and surface phenomena. When the analyte was recognized by ligands attached on the sensing chip, refractive index of the interface will change, altering the angle of minimum reflected intensity. By measuring the change of SPR angle that is proportional to the mass of materials bound, the concentration of the analyte will be available. QCM is based on resonant frequency changing of a quartz crystal, which is influenced by the mass of accumulations.[147] EIS is the most commonly used label-free electrochemical technique. The system is stimulated by small alternating current amplitude and corresponding interfacial characteristic such as resistance is monitored for data analysis.[146] The principles of SPR, QCM, and EIS in lectin-based biosensor are briefly illustrated in Figure 3. A completely label-free lectin-based biosensor was designed in 2007 based on the technique of EIS that two different hemagglutinins PNA and SNA were covalently immobilized on the surface of Cu/Ni/Au printed circuit board electrodes and the binding of glycans with hemagglutinins was monitored by the changes of impedance.[146] Its availability was tested with IF-antigen disaccharide Galα-3GalNAc, asialofetuin, and fetuin and its detection limit was at fM level.[146]

In order to improve the sensitivity of biosensors, many methods to amplify the signal have been developed. The utilization of naomaterials is one of the strategies. Self-assembled monolayers are usually applied to adjust the properties of interface and facilitate the deposition of gold nanopaticles (AuNPs) that can greatly increase the loading of biorecognition elements and their accessibility.[148] Based on the principles, an ultrasensitive lectin-based biosensor was designed for the detection of sialic acids and the detection limit was down to1 aM level, which was much lower than that of other detecting tools, such as HPLC, capillary electrophoresis combined with mass spectrometry, SPR, etc.[148] In detail, a planar polycrystalline gold electrode was first polished and immersed in aminoalkanethiol solution to form a high-density monolayer (the first SAM) on which gold nanoparticles (AuNPs) were linked; subsequently, the 2nd SAM mixed with 11-mercaptoudecanoic acid (MUA) and 6-mercaptohexanol were formed on the

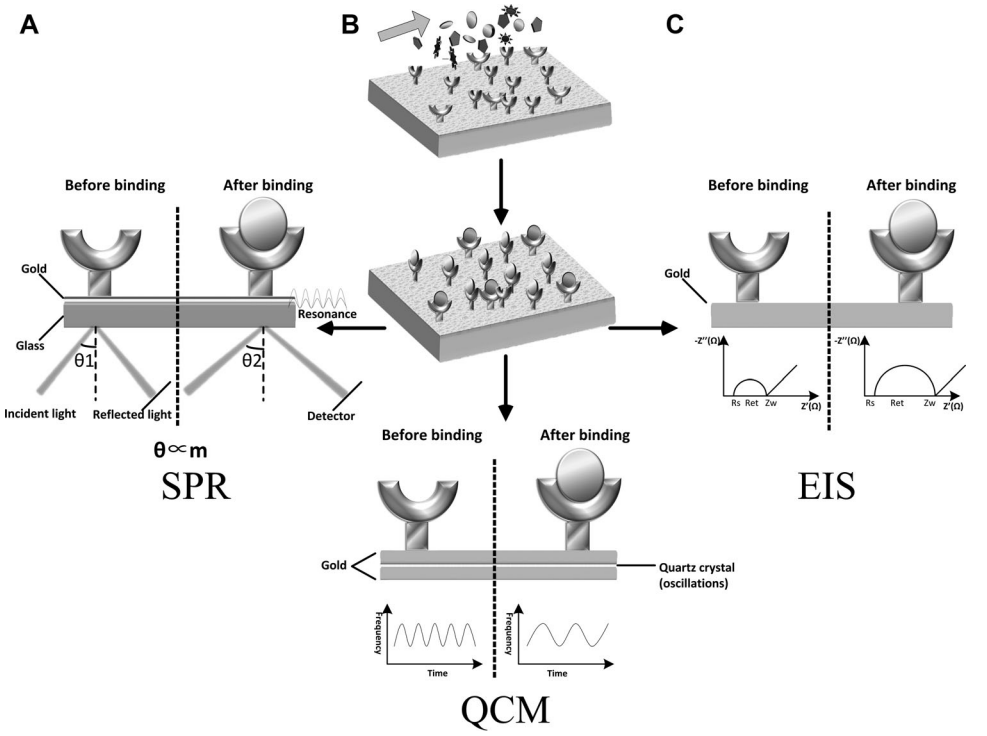

Figure 3. Schematic illustration of the detection principles of lectin-based biosensors. (A) SPR: θ refers to the SPR angle at which the resonance condition is achieved and the intensity of reflected light was greatly reduced. SPR angle alters with the change of refractive index, which is proportional to the amount of proteins attached on the surface. (B) Quartz crystal microbalance: oscillations can be induced under the stimulation of an alternating current. When other variables are removed, the resonance frequency change (Δf) of the quartz crystal is proportional to change of precipitations mass (Δm) on electrode. Sf refers to the sensitivity of the sensor. (C) EIS: the system is applied with a small amplitude alternating current and its dielectric properties that could be interfered with the attachment of analytes are monitored. The data can be present in a Nyquist plot or a Bode plot. Ret, Rs, and Zw, respectively, refer to electron transfer resistance, resistance of the solution, and a Warburg element.

gold nanoparticles; and finally the SNA I lectin was covalently linked to the second SAM by standard amine coupling chemistry.[148] Charge transfer resistance (R_{ct}) will increase with the binding of glycans on lectins due to the formation of additional layer and the subtle change in R_{ct} will be detected and subsequently transferred into the information of the analyte concentration. Fetuin and asialofetuin that contained different amounts of sialic acid were chosen to detect the biosensor and it responded to the change of the amount of glycan in a quantitative way.[148]

Lectin-based biosensors could be extensively used in many areas, including medical diagnosis, environmental monitoring, and even antiterrorism[149] in the future. Lectin-based biosensors could facilitate quick characterization of pathogenic bacteria, saving the time of culture and tedious sample preparations required in traditional methods.[37] Zhang et al. fabricated Con A (specific for mannose) and SNA (specific for sialic acid) based biosensors and applied them to the comparison of mannose and sialic acid expression level between normal and cancer cells derived from human lung, liver, and prostate.[150] Their finding indicated that mannose was highly expressed in both normal cells and cancer cells, while sialic acid was more abundantly expressed in cancer cells.[150] Besides, SNA-based biosensors could not only manage the quantitative detection of cancer cells, but also detect the amount of sialic acid on each cell surface,

which is very important for assessing cancer status progression.[150] Some biosensors are reusable after removing the complex formed on the surface with the optimal regeneration solution.[151]

Compared with lectin microarray, lectin-based biosensor is label-free with higher sensitivity and could be applied for quantitative analysis. Nevertheless, lectin microarray that is equipped with a number of lectins could realize the screening of glycosyl property of a target in a high-throughput way. Therefore, lectin microarrays may be applied to the glycosyl characterization of certain unknown glycoproteins, while lectin-based biosensors could be more properly used in the accurate measurement of known biomarkers. However, lectin-based biosensors are only limited to the laboratory level and no commercialized lectin-based biosensors are currently available.

3. CONCLUSIONS AND PERSPECTIVES

The present review summarizes the exploration history and utilization of lectins as biological tools. From the initial finding of agglutinating blood cells to the discovery of recognizing the carbohydrates on the cell membrane and development as powerful tools in the study of glycans, the multiple functions of lectins were gradually unveiled by numerous researchers across a century. The introduction of different technologies derived from lectins is the focus of the review. It is interesting to find that all of the techniques described above rely on the unique property of lectins as carbohydrate recognizing molecules though the forms of applications may be different. For easy reference, the origin and carbohydrate specificity of lectins described in the present review are summarized in Table III.

Commercialized representative products of the various lectin-derived technologies are presented in Table II. Application of lectins is expected to be more diversified and approaches to adapt them for industrial or clinical utilization should also be addressed in the future. Following the application of WGA transgenes,[108] other lectin transgenes may also be developed as comprehensive and systematic genetic tools in other aspects of biological research. Currently, there are researchers aiming to apply lectins as targeted imaging agents in fluorescence endoscopy to detect the alteration of glycoproteins on epithelial cells that transited from Barrett's esophagus to adenocarcinoma with dysplasia.[152] The experiment was tested on specimens immediately excised from patients and conducted in a way that exactly imitated a clinical endoscopic surveillance: esophagi were intubated with a clinical fluorescence endoscope and fluorescence images were taken after the esophagi were sprayed with fluorescein-labeled WGA.[152] Though this technology is not mature for clinical application, such innovative idea will give inspirations for other researchers and facilitate the diversification of lectin applications.

Though the importance of glycomics has received considerable attention, its development is yet limited by the structural complexity of glycans and lack of effective technologies. Antibodies and lectins are the two primary biological tools applied to the profiling of glycans. Due to the similarities in molecular interactions, they are always compared. As indicated by Bengt Danielsson, antibodies recognize whole O-antigen while lectins recognized individual mono- or oligosaccharides.[37] Therefore, antibodies are more specific, which is both an advantage and a shortcoming. Due to its rigid specificity, a certain antibody could only be applied to the exact antigen it is designed for and it is always time-consuming and expensive to isolate.[37] Since glycans are highly dynamic, the application of antibodies may be confronted with great difficulties. It is also problematic to choose or prepare antibodies when the targets are unknown.[153] Different from antibodies, lectins have comparatively weak affinity and low specificity, capable of binding several different ligands. Besides, lectins are superior to antibodies in stability and availability. Due to these advantages, lectins are gaining more attentions as a potential powerful tool that may make great contribution to the dynamic developments of glycomics.

Table III. Summary of Lectin Origin and Sugar Affinity

Lectins	Origin	Sugar affinity
Con A[167]	Jack bean	Mannose
Lima bean lectin[168]	Lima bean	N-acetyl-alpha-D-galactosamine
L. teragonolobus lectin[40]	*Lotus tetragonolobus*	α-L-fucose
SBA[43]	Soybean	Galactose and GalNAc residues
PNA[169]	Fruits of *Arachis hypogaea*	Gal-β(1-3)-GalNAc
WGA[170]	Wheat germ	GlcNAc and sialic acids
Pea lectin[171]	*Pisum sativum*	Mannose and glucose
Lentil lectin[172]	Lentil	α-D-mannose and α-D-glucose
PHA-E[173]	Red kidney bean	Biantennary galactosylated *N*-glycan with bisecting GlcNAc
PHA-L[174]	Red kidney bean	Sialic acid
PHA-L_4[175]	Red kidney bean	β-1, 6-N-GlcNAc moiety of N-linked glycan
Jacalin[176]	Jackfruit	Galβ1,3GalNAc
MAL[177]	*Maackia* seeds	Siaα2-3Galβ1-4GlcNAc
EEL[177]	*Euonymus europaeus*	Galα1-3Gal
UEA I[95]	*Ulex europaeus*	L-fucose
SNA[178]	*Sambucus nigra*	Neu5Ac α2,6Gal and α2,6GalNAc
Lectin GS-II[101]	*Griffonia simplicifolia*	Terminal nonreducing α- and β-N-acetyl-D-glycosaminyl residues
Wisteria floribunda agglutinin[179]	*W. floribunda*	*N*-acetylgalactosamine beta 1 residues
GNA[180]	Snowdrop bulbs	Manα(1-3)Man-containing oligosaccharides
Helix pomatia lectin (HPA)[110,181]	*H. pomatia*	O-GlcNAc and GalNAc residues
Lectin rBC2LCN[104]	Recombinant protein	Fucα1-2Galβ1-3GlcNAc-containing glycans
Recombinant MOA (rMOA)[121]	Recombinant protein	Galα1-3Galβ1-4GlcNAc-containing glycans

By far, though great achievements have been made in the study and applications of lectins, there are still deficiencies to overcome. Lectin-derived technologies greatly benefit from the abundance of lectins and the number of lectins that have been discovered has reached more than 300 by far.[154] However, not all of them were all well characterized for their carbohydrate specificity. Besides, compared with the great variety of glycoproteins, the number of lectins may be far fewer than what is needed by far. Most importantly, there is a lack of a systematic summary of these lectins to which researchers could easily refer when they want to choose them properly for glycan study.

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