Biljni lektini u prevenciji i liječenju raka

Plant lectins in cancer prevention and treatment

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Abstract. Plant lectins are specific carbohydrate-binding proteins that are widely distributed in various plant species. They participate in many physiological processes and are capable of modulating the immune response. Recently, greater attention has been drawn to their remarkable anticancer properties. Lectins are associated with cell adhesion, cell proliferation and induction of apoptosis. However, some of the questions related to the molecular mechanism / metabolic pathways and biological effects of lectins are still open. New challenges in the research of lectins are related to their application in nanotechnology and development of glycoproteomics. In addition, glycoproteomics is a powerful tool in the characterization of lectins and will be indispensable for development of lectin based drugs in the near future. This review provides a brief outline of the up-to-date advances in the field of plant lectins, focusing on their complex mechanisms implicated in apoptosis and autophagy. The current applications in cancer treatment are also described.

Key words: anticancer agents; lectins; plant lectins

Sažetak. Biljni lektini su specifični glikoproteini koji su široko rasprostranjeni u različitim biljnim vrstama. Sudjeluju u mnogim fiziološkim procesima gdje moduliraju imunološki odgovor. Velik interes za ovu skupinu proteina javio se zbog njihove sposobnosti da utječu na protutumorsku aktivnost putem stanične adhezije, stanične proliferacije i indukcije apoptoze. No još su uvijek nerazjašnjena neka pitanja koja se odnose na molekularni mehanizam / metaboličke puteve i biološke učinke lektina. Novi izazovi u istraživanju lektina odnose se na njihovu primjenu u nanotehnologiji te razvoju glikoproteomike. Glikoproteomika je jedan od moćnih alata u karakterizaciji lektina, a u bliskoj budućnosti i neizostavni alat u razvoju lijekova koji se temelje na lektinima. Ovaj pregledni rad ukratko upisuje izvore, strukturu i primjenu biljnih lektina, povezujući njihove složene mehanizme djelovanja u apoptozi i autofagiji. Opisana je primjena lektina u terapiji raka.

Ključne riječi: biljni lektini; lektini; protutumorski spojevi

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ABBREVIATIONS

ATG families – autophagic family proteins;
Bcl-2 – apoptosis regulator protein;
BNIP3 – BCL2/adenovirus E1B 19 kDa protein -interacting protein 3;
ERK – extracellular signal-regulated kinase;
Gal – galactose;
GalNAc – N-acetylgalactosamine;
GlcNAc – N-acetylglucosamine;
HILIC – hydrophilic interaction liquid chromatography;
LC-MS/MS – liquid chromatography-tandem mass spectrometry;
MALDI TOF – time-of-flight mass spectrometer with matrix-assisted laser desorption/ionization;
MALDI TOF/TOF – tandem time-of-flight mass spectrometer systems with MALDI;
Man – mannose;
MRM MS – Multiple Reactions Monitoring Mass Spectrometry;
PI3K/Akt pathway – intracellular signaling pathway;
PNGase – peptide-N4-(N-acetyl-beta-glucosaminyl)asparagine amidase;
PNGase F – amidase that cleaves between the innermost GlcNAc and asparagine residues of high mannose, hybrid, and complex oligosaccharides from N-linked glycoproteins;
Ras/Raf – signal transduction pathway;
Sia – sialic acid.

INTRODUCTION

Lectins are a complex and heterogeneous group of non-enzymatically carbohydrate-binding proteins that specifically recognize and bind reversibly to specific mono- and oligosaharides on cell surfaces, the extracellular matrix, and secreted glycoproteins. More than a hundred of these molecules have been isolated from plants, viruses, bacteria, invertebrates and vertebrates, including mammals. They bind carbohydrates and possess the capability to agglutinate cells or precipitate polysaccharides and glycoconjugates. Lectins are a component of traditional herbs such as dietary and medicinal plants.

Various plants contain different plant lectins related to their molecular specificity. Plant lectins can be classified into three groups based on: i) their overall mature structure, ii) different families, according to some common features, and iii) diversity of carbohydrate-binding specificities. Differences in lectin structure and carbohydrate specificity are related to their different functions[1]. Depending on carbohydrate specificity, major lectins are divided into mannose binding lectins, galactose/*N*-acetylgalactosamine binding lectins, *N*-acetylglucosamine binding lectins, *N*-acetylneuraminic acid binding lectins and fucose binding lectins as shown in Figure 1. However,

Table 1. Overview of the plant carbohydrate-binding motifs according to Ref. 1.

Lectin domain	Carbohydrate specificity	Examples
Agaricus bisporus agglutinin domain	T-antigen	ABA, MarpoABA
Amaranthins	T-antigen	Amaranthin, HFR2
Class V chitinase homologs	Blood group B, high-man N-glycans	RobpsCRP
Cyanovirin domain	High-man N-glycans	CV-N
Euonymus europaeus agglutinin domain	Blood group B, high-man N-glycans	EEA
Galanthus nivalis agglutinin domain	Man, oligomannosides, high-man N-glycans, complex, N-glycans	GNA, ASA II, ASAL, ACA, LOA
Hevein domain	Chitin, high-man, Man, N-glycans	Hevein, UDA, WGA, HFR3
Jacalins	Gal, T-antigen, Man, N-glycans	Jacalin, Heltuba, HFR1
Legume lectin domain	Man/Glc, Gal/GalNAc, (GlcNAc)n, Fuc, Siaa2, 3Gal/GalNAc, complex N-glycans	PHA, ConA, Gleheda, PSA, GSII
LysM domain	Chitin-ologosaccharides	LysM, CEBiP
Nicotiana tabacum agglutinin domain	GlcNAc-oligomers, high-man N-glycans	NICTABA, PP2
Ricin-B domain	Gal/GalNAc, Siaa2–6Gal/GalNAc	Ricin, SNA-I

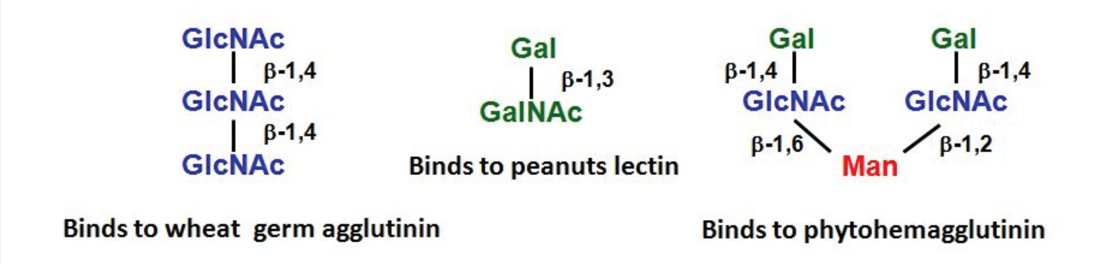

Figure 1. Binding Selectivities of Plant Lectins. The plant lectins wheat germ agglutinin (WGA), peanut lectin (PNA), and phytohemagglutinin recognize different oligosaccharides according to Ref. 3.

- Plant lectins seem to have great potential as anti-cancer therapeutic agents.
- Lectins can cause cancer cell agglutination and/or aggregation and blocking of further migration.
- Some plant lectins are capable of modulating the immune response in different ways.
- Toxic plant lectins can be used as a supportive therapy to improve health-related quality of life (HRQoL).
- Glycoproteomics is one of the tools in characterizing lectins and the development of lectin based drugs.

genome/transcriptome analyses revealed that plant lectins can be classified into twelve distinct families of evolutionary and structurally related lectin domains which are presented in Table 1.

Many of the characterized plant lectins interact with monosaccharides, but their affinity for simple sugars is lower in comparison with more complex carbohydrate structures such as *N*-glycans[2]. The *Leguminosae* is the best-characterized family of plant lectins. This family includes lectins such as Concanavalin A (ConA), soybean agglutinin (SBA), and lentil lectin. Two other smaller families of plants whose lectins have been characterized are the *Gramineae* (cereals, such as wheat germ) and *Solanaceae* (potatoes and tomatoes).

One example of their mature structure is ConA, as a tetrameric protein which binds specifically α-D-mannosyl, and α-D-glucosyl residues. Another example is peanut (*Arachis hypogaea*) agglutinin, which is homotetrameric non-glycosylated protein (without RIP activity) and shows specificity for the tumor-associated T-antigenic disaccharide Gal*β*1, 3GalNAc. Ricin is classified as both lectin and type II ribosome inactivating protein (RIP) and consists of two parts; an A chain (with *N*-glycosidase activity/RIP activity) and a B chain (hemagglutinating/lectin activity) with the B chain capable of binding different carbohydrates, such as β-D-glucose and β-D-galactose. The list of origin of lectins and their abbreviations is presented in Table 2.

Due to their ability to distinguish carbohydrates in human blood cells, different specific lectins can be used in blood typing to differentiate between blood types[4].

One of the earliest findings was related to the biological role of some lectins as cell surface sugars and its mitogenic stimulation on the surface of the lymphocytes. This property makes their glycosylation useful tools in cancer research, especially for the isolation and characterization of polysaccharides and glycoconjugates as diagnostic tools for the investigation of early cell-membrane alterations and carbohydrate changes that accompany neoplastic processes, and in immunological studies[5].

In the past, numerous lectins were isolated from plants as well as from microorganisms and animals, however, their structure and function as recognition molecules in cell-molecule and cell-cell interactions in various biological systems, have been established during the past two decades.

Despite the disparity in physicochemical and biochemical characteristics, lectins from different sources exhibit common biological activity. They are involved in the strategies of different scientific and practical fields such as agricultural, agro-economy, food production and food science, life

Table 2. List of origin of some lectins

Abbreviations	Name of Lectin	Origin (*eng.*)	Origin (*lat.*)
AAL	*Aleuria Aurantia* Lectin	Orange Peel Fungus	*Aleuria aurantia*
ABA	*Agaricus bisporus* agglutinin	Edible mushroom, white button mushroom	*Agaricus bisporus*
Abrin A	Abrin A	Crab's Eye	*Abrus precatorius*
ACA	*Amaranthus caudatus* agglutinin	Amaranth	*Amaranthus caudatus*
AGG	Gamma-globulin		
AML	*Astragalus membranaceus* lectin	Huáng qí	*Astragalus membranaceus*
AMML	*Astragalus membranaceus* var. Mongholicus lectin	Milk vetch / Huang qi	*Astragalus mongholicus*
ASAL	*Allium sativum* leaf agglutinin	Garlic	*Allium sativum*
ASA I	*Allium sativum* bulb agglutinin I	Garlic	*Allium sativum*
ASA II	*Allium sativum* bulb agglutinin II	Garlic	*Allium sativum*
CEBiP	Chitin elicitor binding protein	Rice	*Oryza sativa*
CMA	Chelidonium majus agglutinin	Greater celandine	*Chelidonium majus*
CML	*Cratylia mollis* lectin	Cratylia	*Cratylia mollis*
Con A	Concanavalin A	Jack bean	*Canavalia ensiformis*
CV-N	Cyanovirin-N	Cyanobacterium Nostoc ellipsosporum	*Nostoc ellipsosporum*
EEA	*Euonymus europaeus* agglutinin	European spindle	*Euonymus europaeus*
Gleheda	*Glechoma hederacea* agglutinin	Ground-ivy	*Glechoma hederacea*
GNA	*Galanthus nivalis* agglutinin	Snowdrop	*Galanthus nivalis*
GS-II	*Griffonia simplicifolia* agglutinin II	Griffonia seed	*Griffonia simplicifolia*
GSA-IA4	*Griffonia simplicifolia* agglutinin	Griffonia seed	*Griffonia simplicifolia, Griffonia (Bandeiraea) simplicifolia*
Heltuba	*Helianthus tuberosus* agglutinin	Jerusalem artichoke	*Helianthus tuberosus*
HFR1	Hessian fly responsive 1	Hessian fly	*Mayetiola destructor*
HFR2	Hessian fly responsive 2	Hessian fly	*Mayetiola destructor*
HFR3	Hessian fly responsive 3	Hessian fly	*Mayetiola destructor*
HEV1	Hevein	Pará rubber tree	*Hevea brasiliensis*
JAC	Jack fruit lectin, Artocarpus integrifolia agglutinin, Jacalin	Jack fruit	*Artocarpus integrifolia*
Jacalin	Jacalin	Jackfruit	*Artocarpus integrifolia*
Lentil	Lentil lectin	Lentil	*Lens culinaris*
LCA	*Lens culinaris* agglutinin	Lentil	*Lens culinaris*
LOA	*Listera ovata* agglutinin	Twayblade	*Listera ovata*
LysM	LysM domain		
MAL	*Maackia amurensis* agglutinin	Amur maackia	*Maackia amurensis*
MarpoABA	*Agaricus bisporus* agglutinin homolog	Edible mushroom, white button mushroom	*Agaricus bisporus*
ML-I	Mistletoe lectin I	Mistletoe	*Viscum album* L.
ML-II	Mistletoe lectin II	Mistletoe	*Viscum album* L.
ML-III	Mistletoe lectin III	Mistletoe	*Viscum album* L.
MLL	Mulberry leaf lectin	White mulberry	*Morus alba*
Nictaba	*Nicotiana tabacum* agglutinin	Tobacco	*Nicotiana tabacum*
NPA	*Narcissus pseudonarcissus* agglutinin	Common daffodil	*Narcissus pseudonarcissus*
PCL	*Pleurotus citrinopileatus* lectin	Citrinopileatus	*Pleurotus citrinopileatus*
PHA	Phytohemagglutinin, *Phaseolus vulgaris* leukoagglutinin	Common bean, kidney bean	*Phaseolus vulgaris* L.
PNA	Peanut agglutinin	Peanut	*Arachis hypogaea*
POL	*Pleurotus ostreatus* lectin	Mushroom	*Agaricus bisporus*
PP2	*Nicotiana tabacum* agglutinin domain	Tobacco	*Nicotiana tabacum*

Abbreviations	Name of Lectin	Origin (*eng.*)	Origin (*lat.*)
PSA	*Pisum sativum* agglutinin	Pea	*Pisum sativum*
RBA	Rice bran agglutinin	Rice bran	*Oryza sativa* L.
Ricin (RCA)	*Ricinus communis*, Anti-B4-blocked ricin	Castor bean	*Ricinus communis*
Ricin A	*Ricinus communis*, Anti-B4-blocked ricin	Castor bean	*Ricinus communis*
RLL	*Russula lepida* lectin	Russula rosea	*Russula lepida*
rML	Recombinant mistletoe lectin	Mistletoe	*Viscum album* L.
RobpsCRA	Chitinase-related agglutinin homolog	black locust	*Robinia pseudoacacia*
Saracin	*Saraca indica* lectin	Ashoka	*Saraca indica*
SBA	Soybean agglutinin	soybean	*Glycine max*
SBL	Soybean lectin	soybean	*Glycine max*
SNA	*Sambucus nigra* agglutinin	Elderberry, European black elderberry	*Sambucus nigra*
STL	*Solanum tuberosum* lectin	Potato	*Solanum tuberosum*
TML	*Tricholoma mongolicum* lectin	Paimo mushroom	*Tricholoma mongolicum*
UDA	*Urtica dioica* agglutinin	Stinging nettle	*Urtica dioica*
VAA	*Viscum album* agglutinin	mistletoe	*Viscum album*
VCA	*Viscum album* coloratum agglutinin	Korean mistletoe	*Viscum album* L. *var.* coloratum
VFA	*Vicia faba* agglutinin, Broad bean lectin	Broad bean, fava bean	*Vicia faba*
WGA	Wheat germ agglutinin	Wheat	*Triticum aestivum* L.

science, health science, pharmaceuticals, etc. Many plant lectins showed anticancer properties *in vivo*, and *in vitro*, thus they have a potential for use as a therapeutic agent in the malignant neoplastic disease treatment. This review discussed today's main research interest of plant lectins as the next generation of anticancer drugs.

PRODUCTION, PURIFICATION AND PROTEOMIC APPLICATION OF LECTINS

Two main ways, enabling the production of lectins, a) isolation from their natural sources by chromatographic procedures, or b) production by recombinant DNA technology as shown in Table 3. The yields of animal lectins are usually low in comparison to the yield of plant lectins such as legume lectins[6].

Isolation of lectins integrates different purification techniques, such as precipitation (using acids, organic solvents and salts) and chromatographic methods such as affinity chromatography (AC), ion-exchange chromatography (IEX), hydrophobic interaction chromatography (HIC) and gel permeation (GF)[7]. Lectin affinity chromatography (LAC), often uses the immobilized lectins such as ConA in separation and isolation of glycopeptides that express *N*-linked structures and high-mannose glycans[7-11].

Recombinant DNA technology has been used for cloning and characterizing newly pure and sequence-defined lectins. Recombinant lectins are often produced in *Escherichia coli* and by post-translational modified recombinant lectins are produced in eukaryotic organisms. These recombinant lectins may have different applications such as *i*) in cancer diagnostics and/or therapy, *ii*) antimicrobial, antiviral and anti-insect agents, or *iii*) in microarray for glycome profiling[12]. Although recombinant lectins can be synthesized, due to high costs and low yield, more acceptable production is isolated from plant sources. This especially applies to the isolation of high-yield *Phaselous* cultivars cvs. French bean 35 (Table 3).

Current methods to quantify lectin levels in foods and other matrix analyses are based on immunosorbent assay (ELISA), which mainly relies on specific monoclonal antibody or pre-labelled well-known lectins, or toxicity tests. Wang et al.[14] suggested a new strategy to detect the specific carbohydrate binding capability of lectins based on enzyme-linked adsorbent assay applying different monosaccharide–polyacrylamide conju-

Table 3. Yields of animal and plant lectins (from different *Phaseolus* cultivars) obtained by chromatographic isolation and plant lectins produced by recombinant DNA techniques modified according to Ref. 13.

Plant lectins from *Phaseolus cultivar* (yield mg/100 g seed)				
Source	**Chromatographic purification**	**Yield**	**Sugar specificity**	**Reference**
Anasazi bean	Affi-gel blue gel, Mono S and Superdex 200	13	N.F.	82
Dark red kidney bean	DEAE-cellulose and Affi-gel blue gel	107	N.F.	83
Escumite bean	AC	163	N-acetyllactosamine-type glycans	84
Extralong autumn purple bean	Blue-Sepharose, Q-Sepharose, Mono Q and Superdex 75	35	Galactose	85
French bean 12	SP-Sepharose, Affi-gel blue, Q-Sepharose, and Superdex 200	4.8	N.F.	86
French bean 35	Blue-Sepharose, Q-Sepharose and Superdex 75	1100	N.F.	87
Red kidney bean	Affi-gel blue gel and CM-Sepharose	27.5	Lactoferrin, ovalbumin, thyroglobulin	88
Animal lectins				
Acropora millepora (coral) plasma fluid	Mannose AC	0.7 mg/100 ml plasma	N.F.	89
Aristichthys nobilis (bighead carp) gills	DEAE-Sepharose, Sephacryl S-200 and Superdex 200	9.4 mg/100 g	N.F.	90
Bubalus bubalis (Buffalo) heart tissue	Ammonium sulfate precipitation and Sephadex G50	0.97 mg/100 g	N.F.	91
Capra hircus (goat) heart tissue	Ammonium sulfate precipitation and Sephadex G50, Lactosyl-Sepharose 4B AC	1.09 mg/ 100 g	Galactose	92
Holothuria scabra (sea cucumber) coelomic fluid	Ultrafiltration and Phenyl-Sepharose	1.6 mg/100 ml	N.F.	93
Macoma birmanica (marine bivalve) foot muscles	Ammonium sulfate precipitation and N-acetylglucosamine Sepharose 4B	4.5 mg/100 g	N.F.	94
Nemopilema nomurai (jellyfish)	SP-Sepharose and BSM- Toyopearl	0.35 µg/100 g	N.F.	95
Plant lectins produced by recombinant DNA techniques				
Natural source of lectin	**Yield (mg/L culture medium)**	**Genetically modification in cells**		**Reference**
Allium sativum (garlic) leaf	5	cDNA was cloned into NdeI and BamHI restricted plasmid pET19b and expressed in *E. coli* strain BL21 (DE3) cells		96
Artocarpus incise (breadfruit)	16	cDNA was cloned into the pET-25b(+) and expressed in *E. coli*.		97
Artocarpus incise (breadfruit)	18–20	cDNA was cloned into EcoRI/XbaI restricted plasmid pUC57 and expressed in *E. Coli*		98
Glycine max (Soybean)	0.1	cDNA was cloned NcoI/NdeI/BamHI restricted plasmid PET-3d and expressed in *E. coli* strain BL21(DE3)pLysS		99
Nicotiana tabacum (tobacco) leaves	6	cDNA was cloned EcoRI/NotI restricted plasmid and expressed in *E. coli* strain top10F		100
Oryza sativa (rice) roots	14.6	cDNA was cloned into NdeI/BamHI restricted pET 3D plasmid and expressed in *E. coli* strain BL21 (DE3) cells		101
Pisum sativum (pea)	2–5	cDNA was cloned into HindIII/PstI/BamHI restricted plasmid and expressed in *E. coli* strain W3110		102
Polyporus squamosus fruiting bodies	4–7	cDNA was cloned into NdeI/BamHI restricted plasmid and expressed in *E. coli* strain Nova Blue (DE3)		103

N.F. – not found; AC – affinity chromatography

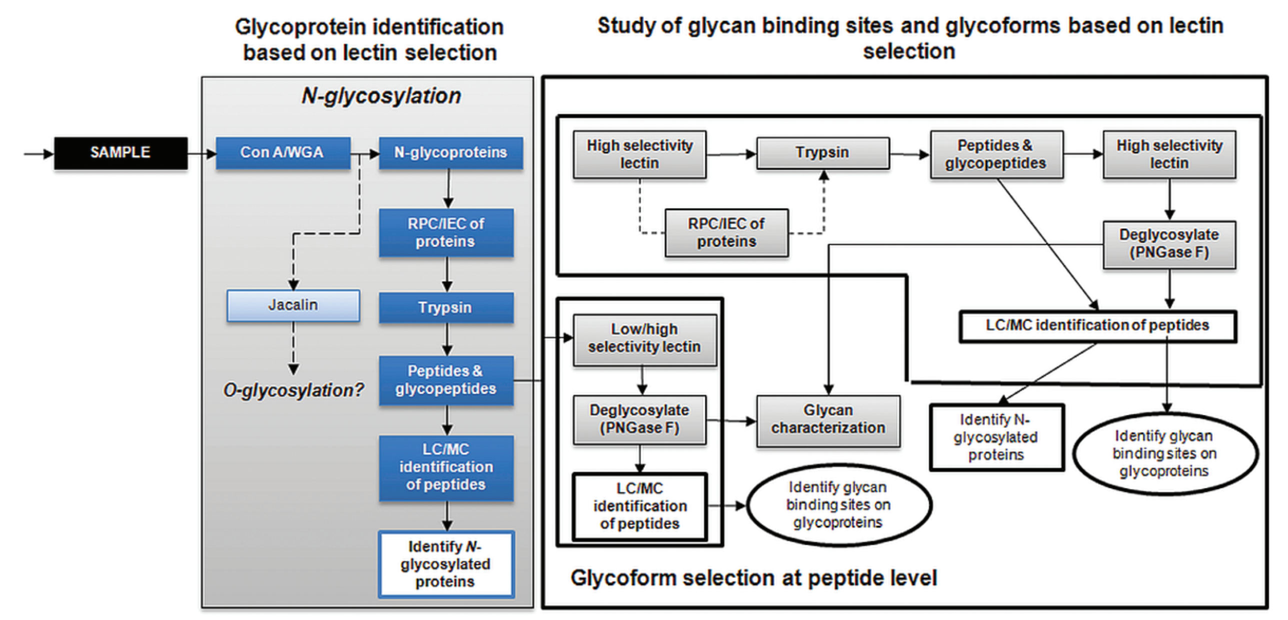

Figure 2. Glycoproteomics based on affinity chromatography at the protein level with immobilized lectin columns modified according to Ref. 7.

gates as capturing agents for screening lectins in biological samples.

In summary, plant lectins have application in LAC, blotting, affinity electrophoresis, immune-electrophoresis as well as in microarrays, as in evanescent-field fluorescence-assisted lectin microarray[15].

Proteomic strategies to quantitative analysis of plant lectins include the use of chromatographic or electrophoretic strategies combined with mass spectrometry (LC-MS/MS, MALDI-TOF MS or MALDI-TOF/TOF MS). The workflow often involves a combination of LAC, tryptic digestion, ion-pairing HILIC, and precursor ion-driven data-dependent MS/MS analysis with a script to facilitate the identification and characterization of occupied *N*-linked glycosylation sites[16,17] (Figure 2).

Proteomic approach was used in investigation of quantitative differences in aberrant glycosylation of target glycoproteins between noncancerous group and patient group with carcinoma such as adenocarcinoma lung cancer (ADLC)[18], liver cancer[19] developed by cooperatively using comparative lectin-capturing, targeted mass spectrometry (MRM MS), and antibody/lectin sandwich ELISA. This different proteomic approach can be useful for identifying and verifying biomarker candidate involved in aberrant protein glycosylation[7].

SOME IMPORTANT ANIMAL AND HUMAN LECTINS

Twelve structural families of lectins are known to exist in mammals where carbohydrates bind to another structure such as protein–protein, protein–lipid or protein–nucleic acid. Although they have other functions, their main function is generally related to the recognition molecules within the immune system, as direct first-line defense against pathogens, cell trafficking, immune regulation and prevention of autoimmunity[20].

C-type lectins with C-type lectin domain-containing proteins (CTLDs) are characteristic of mammals. Their seven subgroups are based on the order of the various protein domains in each protein[3]. Changes in the amino acid residues that interact with the carbohydrate alter the carbohydrate-binding specificity of the lectins. A calcium ion bridges the protein and the sugar through direct interactions with sugar hydroxyl groups as shows Figure 3. These proteins function as adhesion and signaling receptors in many immune functions such as inflammation and immunity to tumors and virally infected cells. A large class found in animals includes collectins, selectins, endocytic receptors, and proteoglycans that can play an important role in cellular functions[21,22].

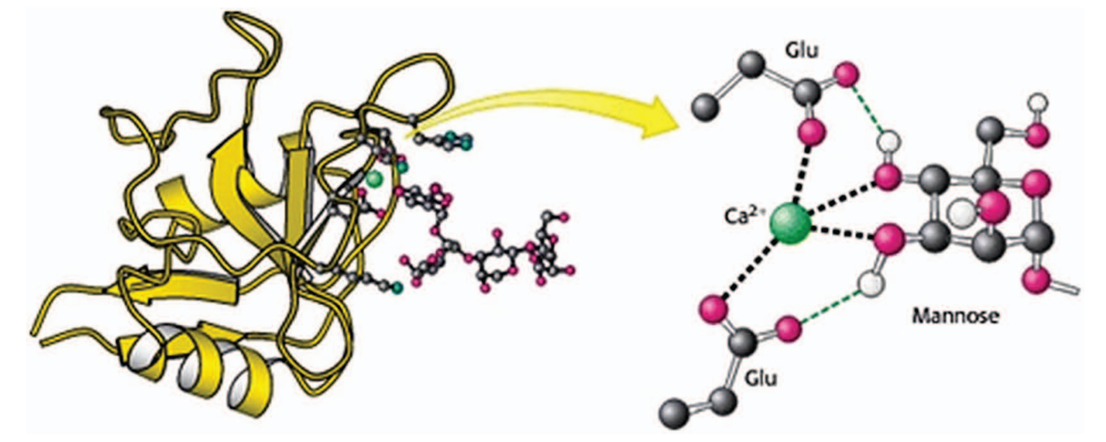

Figure 3. Structure of a C-Type, carbohydrate-binding domain from an animal lectin according to Ref. 3.

All selectins are single-chain transmembrane glycoproteins that share similar properties to C-type lectins due to a related amino terminus and calcium-dependent binding on immune-system cells to the sites of injury in the inflammatory response[23]. The L, E, and P forms of selectins bind specifically to carbohydrates on lymph-node vessels, the endothelium, or activated blood platelets, respectively. P- and E-selectins are highly expressed on the luminal plasma membrane of vascular endothelial cells at sites of inflammation, therefore, can be smart targets for the delivery of anti-inflammatory drugs[24].

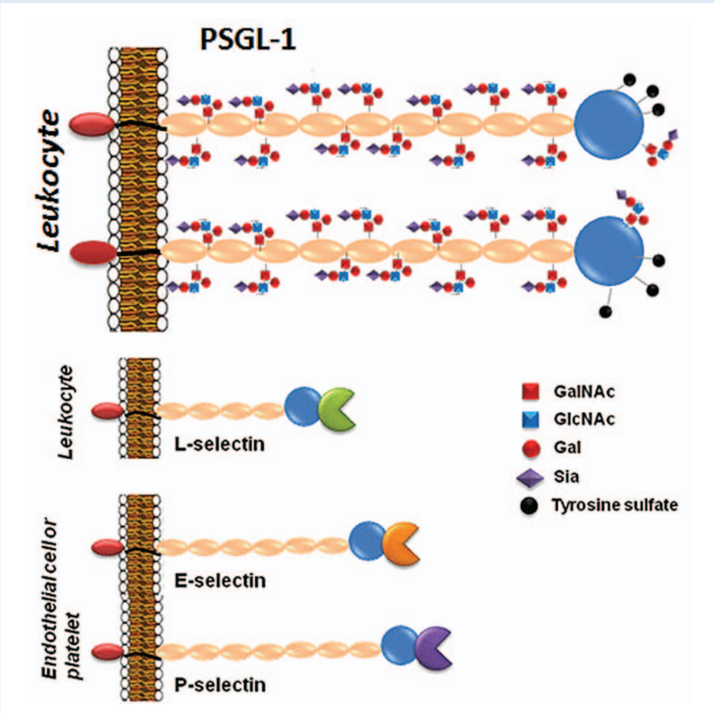

Figure 4. Scheme of selectins and their common ligand PSGL-1 modified according to Ref. 26.

New therapeutic agents that control inflammation may emerge from a detailed understanding of how selectins bind and distinguish different carbohydrates[25]. P-selectin glycoprotein ligand 1 (PSGL-1) is the only transmembrane glycoprotein characterized at the molecular, cellular and functional levels, and which is comprised of extracellular, transmembrane, and cytoplasmic domains[26] (*see* Figure 4). PSGL-1 is one of the promising selectin inhibitor, which has entered clinical trials[26,27].

The ability of viruses to infect specific cell types is partially certain by the ability of these viruses to bind particular structures or receptors on the surfaces of cells. In some cases, these receptors are carbohydrates.

Viral infections often coincide with platelet activation. Increased levels of E-selectin on the endothelial cell surface were found in Dengue virus-infected patients[28]. In these patients, increased E-selection encouraged enhanced activation of adhesion as well as enhanced activation of the coagulation cascade. The viral protein from influenza virus recognizes sialic acid residues found in cell-surface glycoproteins (hemagglutinin)[29]. P-selectin is an important adhesion molecule in regulating T cell responses which may be important for T cell memory and immunity to influenza virus[30].

Soluble P-selectin levels (sP-selectin) in plasma were higher in hepatitis C patients with low platelet counts. This result indicates that hepatitis C virus infection (HCV) might be directly responsible for a condition of *in vivo* platelet activation in patients with chronic C hepatitis[31]. Also, low platelet and sP-selectin levels are related to the degree of liver disease and thrombosis in patients with cirrhosis[32].

APPLICATIONS OF LECTINS

Plant lectins: possible application in diagnostics and therapy

Due to the large range of natural sources as well as high specificity, lectins are important tools in cell biology and immunology. This part is focused on the anticancer activity of selected plant lectins *in vitro*, *in vivo* and in human case studies. Lectins can penetrate into cells, causing cytotoxicity, apoptosis, cancer cell agglutination and/or aggregation, and inhibition of tumor growth. Several studies demonstrated a strong correlation between specific lectin-binding patterns and their biological effects in various tumors.

Agglutination is primarily done by binding to the glycoprotein receptors on cell membranes, resulting in blocking further migration. Thus, they can affect cancer cells, by modulating the status of the immune system by altering the production of various interleukins, certain protein kinases, and proteins themselves by binding to ribosomes. Plant lectins affect both apoptosis and autophagy by modulating representative signalling pathways involved in Bcl-2 family, caspase family, p53, PI3K/Akt, ERK, BNIP3, Ras-Raf and ATG families[33].

In vitro studies

Although carbohydrates are associated with cell growth and viability, glycosylation also has an integral role in many processes leading to cell death. Glycans, simple or complexed with glycan-binding proteins, can transfer intracellular signals or control extracellular processes and so promote initiation, implementation and resolution of cell death programs.

In vitro studies have shown that plant lectins elicit apoptosis in different cancer cell lines. Plant lectins can modify the cell cycle by inducing non-apoptotic G1-phase accumulation mechanisms and G2/M phase cell cycle arrest and apoptosis[34,35].

This can be explained by the binding between lectin-tumor cells which depend on lectins with different sugar-binding specificities such as galactosyl-specificity of the mushroom *Pleurotus ostreatus* lectin (POL). The specificity was increased by substitution at the C-2 position of the galactosyl residue with a fucosyl or acetylamino group[36]. Different carbohydrate-binding specificities were studied by Wang et al.[37] on human hepatoma (H3B), human choriocarcinoma, mouse melanoma, and rat osteosarcoma cell lines. In comparison to other cells, POL inhibited more sarcoma S-180 cells[37].

The fresh oyster mushroom *Pleurotus ostreatus* produced the most significant cytotoxicity on human androgen-independent cancer PC-3 cells among the mushroom species tested[38]. Three proteoglycan fractions from *P. ostreatus* mycelia were tested for in vitro and in vivo immunomodulatory and anticancer effects on Sarcoma-180-bearing mouse model. Reduced number of S-180 tumor cells and cell cycle analysis showed that most of the cells were found to be stopped in pre-G0/G1 phase of the cell cycle. Three tested proteoglycan fractions elevated mouse natural killer (NK) cell cytotoxicity and stimulated macrophages to produce nitric oxide[39]. The mechanism of this anticancer effect may be explained by the improvement of the host immune system.

In comparison to some plant lectins, dietary lectins may not be toxic. Overview of selected plant lectins which is important in cell biology and immunology is shown in Table 4.

Vicia faba agglutinin (VFA) is a dietary lectin, with D-glucose and D-mannose sugar specificity, which is present in broad beans. VFA can alter the proliferation of colon cells by aggregation, stimulation of the morphological differentiation and reduction of the malignant phenotype of human colon cancer cells by acting to direct binding to N-glycosylated epithelial cell adhesion molecule (epCAM) or through a pathway involving epCAM[40].

Wheat germ agglutinin (WGA) is *N*-acetylglucosamine binding lectin. Its inhibitory effect is linked to a small decrease in α-amylase secretion

Table 4. Inhibitory effects of plant lectins on malignant cells *in vitro* modified according to Ref. 34.

Lectins	Tumor cells	Type of effect	References
AAL, WGA, MAL, TML, STL	H3B human hepatoma, Jar human choriocarcinoma, and ROS rat osteosarcoma	C/TI	37
LCA, NPA	H3B human hepatoma		
AML	K562 leukemia cell line	C/TI, apoptosis activation of the caspase cascade	104
AMML	Human cervical carcinoma cell line (HeLa)	Apoptosis, Cell cycle arrest at S phase	105
ABA, WGA	LS174T, SW1222, and HT29 human colon cancer	C/TI, CA/A	40
VFA, PNA	SW1222 and HT29 human colon cancer		
VFA	LS174T human colon cancer	C/TI, stimulation of morphological differentiation, reduction of malignant phenotype, CA/A	
Abrin A	Jurkat and CCRF-HSB-2 adult T-cell lymphoblastic leukemia cells	C/TI, CA/A	47
	Molt-4 and HPB-ALL adult T-cell lymphoblastic leukemia cells		
	RPMI 8402 and BALL-1 adult T-cell lymphoblastic leukemia cells	C/TI	
	CCRF-CEM adult T-cell lymphoblastic leukemia	C/TI, *CA/A*	
	BALM-1 Acute B-cell lymphoblastic leukemia		
	NALM6 Acute B-cell lymphoblastic leukemia	*C/TI,* CA/A	
ACA, JAC	HT29 human colon cancer	C/TI	106
Anti-CD64 Ricin A	Acute human myeloid leukemia	C/TI, apoptosis	107
CD22-rec Ricin-a	Daudi and Ramos B-cell lines (Burkitt lymphomas)	C/TI	108
CD22-rec Ricin-a	Chronic B-cell lymphocytic leukemia (B-CLL)		
CD22-rec Ricin-a	Acute B-cell lymphoblastic leukemia (B-ALL)	*C/TI*	
CMA, ConA, LCA, UDA, WGA	Merkel cell skin carcinomas	DC/A/BindCellMemR	109
Con A, GSA-IA4	Hs729 (HTB-153) human rhabdomyosarcoma	C/TI, *DC/A/BindCellMemR*	110
Con A, GSA-IA4, WGA	SK-UT-1 and SK-LMS-1 human leiomyosarcoma	C/TI, DC/A/BindCellMemR	
PHA	Hs729 (HTB-153) human rhabdomyosarcoma and SK-UT-1 and SK-LMS-1 human leiomyosarcoma	C/TI, *DC/A/BindCellMemR*	
PNA	SK-UT-1 (HTB-114) human leiomyosarcoma	C/TI, DC/A/BindCellMemR	
	SK-LMS-1 (HTB-88) human leiomyosarcoma	DC/A/BindCellMemR	
WGA	Hs729 (HTB-153) human rhabdomyosarcoma	*DC/A/BindCellMemR*	
ConA, GSA-IA4, WGA	SK-MEL-28, HT-144, and C32 human melanoma	C/TI	111
PHA	SK-MEL-28, HT-144 and C32 human melanoma	*C/TI*	
JAC, WGA	Adenomatous polyps and colorectal neoplasms	DC/A/BindCellMemR	112
ML-I, ML-II, ML-III	Molt-4 human lymphocyte	C/TI	52
ML-I	Molt-4 human lymphocyte	Ribosome binding/inhibition of protein synthesis, DC/A/BindCellMemR, internalization of lectin, apoptosis	53
ML-II	U937 human monoblastic leukemia	Apoptosis, activation of extracellular signal-regulated kinases, activation of p38 mitogen-activated protein kinase, alteration of cellular signaling pathways	54
	U937 human myeloleukemic	Apoptosis, activation of the caspase cascade	55
	Jurkat T, RAW 264.7, HL-60, DLD-1, primary acute myelocytic leukemic	Apoptosis	56

Lectins	Tumor cells	Type of effect	References
ML-I	Malignant melanoma	C/TI	58
ML-II, ML-III		*C/TI*	
MLL	MCF-7 human breast cancer cells	C/TI, stimulation of morphological differentiation, reduction of malignant phenotype, DNA fragmentation, activation of the caspase cascade, increase percentage of cells in sub G0/G1 phase	113
	HCT-15 human colon cancer cells		
PHA	SP2 myeloma, Lox-2 Ab-producing hybridoma	CA/A	114
	B-DLCL human large B-cell lymphoma	DC/A/BindCellMemR	115
PNA	Human melanoma cells	DC/A/BindCellMemR	116
RBA	Human monoblastic leukemia U937	C/TI, apoptosis, chromatin condensation/ nuclear fragmentation/DNA release, externalization of membrane phosphatidylserine, DNA ladder formation, G2/M phase cell cycle arrest	117
Ricin	BEL7404 hepatoma	Apoptosis, upregulation of Bak	118
Ricin A	Human A431 epidermoid	Ribosome binding/inhibition of protein synthesis	46
TGF-α-rec Ricin A	A431 human epidermoid cancer	C/TI	
TGF-α-rec Ricin A	H226Br brain metatstatic *var.* human NSCLC squamous cells	*C/TI*	
Saracin	Human T-lymphocytes	Apoptosis, induction of IL-2 secretion	119
SNA	Surgically removed human colon cancer cells	DC/A/BindCellMemR	120
VAA	Murine melanoma and HeLa human cervical cancer	C/TI	121
	A549 human lung carcinoma	Non-apoptotic G1-phase accumulation mechanisms	122
VCA	SK-Hep-1 (p53+), Hep 3B (p53−) hepatic cancer	Apoptosis, down-regulation of Bcl-2/upregulation of Bax, down-regulation of telomerase activity	123
	Human breast cancer	Increased TNF-α, IL-6, IFN-γ and/or IL-4 secretion, Th1- shift in the Th1/Th2 balance	124
WGA	Human pancreatic carcinoma	DC/A/BindCellMemR, internalization of lectin, apoptosis, chromatin condensation/ nuclear fragmentation/DNA release	125
	AR42J rat pancreatic cell line	C/TI	41

Note: *italic* indicate weak effects.

Abbreviations: Cytotoxicity/tumor inhibition – C/TI; cell agglutination/aggregation – CA/A; Direct contact/adhesion/binding to cell membrane or receptors – DC/A/BindCellMemR

in rat pancreatic tumor cell line AR42J[41]. The alterations of the carbohydrate structures of cellular glycoconjugates may be related to goblet cell differentiation in normal, benign and malignant human colorectal tissues[42]. WGA proved highly toxic to human pancreatic carcinoma cells *in vitro*, primarily to sialic acid residues, with lectin internalization. Cytotoxic effect was found in leukemia cells, several human breast cell lines, skin, and liver cancer cell lines *in vitro* as shows Table 4.

Ribosome-inactivating proteins (RIPs) irreversibly inhibit protein synthesis through the removal of one or more adenine residue from ribosomal RNA (rRNA). RIPs type I (approximately 30 kDa) consist of an enzymatically active A-chain, while RIPs type II (approximately 56-65 kDa) consists of chain A and chain B which is specific for galactose-like sugars[43]. Type I is less cytotoxic because this structure does not contain chain B. They are important in several clinical medicine and biomedical research, especially in immunological research and as individual or combined human immunodeficiency virus (HIV) drug therapy as well as anticancer therapy[43]. The RIPs type II such as ebulin I, foetidissimin

II, mistletoe, nigrin b, riproximin have shown anticancer activity *in vitro* and *in vivo*. *Ricin, abrin-a, Sambucus nigra* agglutinin (SNA) and related plant lectins belong to RIPs[44,45].

Recently, these toxins have been investigated in experimental models which elucidate the intracellular trafficking of endocytosed proteins[46]. Transforming growth factor (TGF-α) specifically binds and stimulates phosphorylation of the EGF receptor (EGFR) and activates protein kinase activity during cell signaling. TGF-α is highly expressed in human cancer cells. Synthesis of ricin A conjugate and TGF-α trigger cell proliferation[46], and available levels of EGFR influence the cytotoxic effect on human cancer cells, indicating the involvement of receptor-mediated endocytosis of the conjugate[46].

Lectin-binding specificity as a rule of recognition carbohydrates allows phenotypic and functional characterization of membrane-associated glycoproteins expressed on cancer cells. In comparison to normal lymphocytes, toxin *abrin-a* showed greater cytoagglutination against human cultured cell lines derived from acute lymphoblastic leukemia and adult T-cell leukemia[47].

Lectins (ML-I, ML-II, and ML-III) are the main constituents of mistletoe (MLs) which are responsible for its anticancer and immunomodulatory effects. Nowadays, most researchers focus on investigation of mistletoe lectins, particularly mistletoe lectin I (ML-I). Cytotoxic A-chain inhibits the elongation step of protein biosynthesis by catalyzing the hydrolysis of the *N*-glycosidic bond on ribosomes, resulting in apoptosis or necrosis cell death[48]. Chain B is immunomodulatory, enhancing the secretion of cytokines and activates NK cell[49] which are involved in anticancer activity. This has been observed in 20 mammary carcinoma patients who received ML-I by subcutaneous injections[50]. Recently, the high resemblance between the 3D structure of mistletoe lectin and the shiga toxin from *Shigella dysenteriae* was found, which represents the bacterial origin of this protein[51]. Furthermore, it was suggested that a combination of mistletoe lectin with other forms of recognition receptor ligand substances enhances the immune stimulatory effect.

Several *in vitro* experiments have investigated the anticancer effect of mistletoe extract and its active compounds in breast cancer and predominantly reported their anticancer and cytotoxic effects in cancer cell lines, as presented in Table 5. Mistletoe extracts exhibit substantial cytotoxic effects *in vitro* and none of the studies reported growth stimulation and proliferation of tumor cell lines[52-57].

Viscum album is the European type of mistletoe. Numerous preparations of this plant from different host trees like apple, pine, oak, and others have been used. Also, mistletoe extract preparations are commercially available, including *Israel, Cefaleksin, Lektinol, Eurixor, Iscador, Helixor, Iscucin,* and *Abnobaviscum*[58].The antineoplastic activity of *Viscum album* agglutinin-1 (VAA-1) alone or in combination with other chemotherapeutic drugs, including doxorubicin, cisplatin, and taxol, was evaluated in the human lung carcinoma cell line A549. Stronger synergistic effects were noticed using VAA-1 for all drugs tested. Moreover, VAA-1 was able to induce nonapoptotic G1-phase accumulation mechanisms[59]. Recombinant mistletoe lectin alone or in combination with ionizing radiation also showed down regulation of the proliferative activity and cell killing of transforming murine tumor cells[60].

In vivo studies

The cytotoxic and anticancer activity of plant lectins tested on different animal models has been demonstrated in most of the investigations. They are administrated as oral, intramuscular, intrapleural, intraperitoneal, and intratumoral on relevant sites.

Mistletoe lectins are the most studied lectins in preclinical studies and clinical trials. Until to date, the PubMed database alone lists more than 1280 citations for "mistletoe," of which 113 are clinical studies. Preclinical and clinical studies demonstrated immune response, cytotoxicity, proapoptotic effects, antiangiogenesis, and DNA stabilization[61-65].

Drees et al.[66] reported the reduction of cell proliferation in MAXF 449 cell line, sc/Nude mice using *Abnobaviscum M*. Beuth et al.[67] demonstrated the dose-dependent anticancer activity of *Helixor* using a BALB/c-mouse/BT474 ductal breast carcinoma model. In their *in vivo* experiment, standardized mistletoe extracts harvested from de-

Table 5. Inhibitory effects of plant lectins on malignant cells *in vivo* modified according to Ref. 34.

Lectins	Animal model	Type of effects	References
Anti-CD64-Ricin-A	Acute human myeloid leukemia in NOD/SCID mice	C/TI	107
Con A	B16 melanoma cells in mice	C/TI	71
rML	Human ovarian cancer in SCID mice	C/TI	126
	Chemically induced urinary bladder cancer in mice	C/TI, lower expression of IL-10	127
	Nitrosurea-induced urinary bladder cancer in rat	C/TI, DC/A/BindCellMemR, lower expression of IL-10	128
	Murine melanoma in mice	C/TI, inhibition of metastasis	121
PHA	Non-Hodgkin's lymphoma in mice	C/TI, competition for polyamines	129
	NMRI mice injected with Krebs II lymphosarcoma	C/TI	130
	MCF7 and T47D metastatic human breast cancer lines, SCID mice	DC/A/BindCellMemR	131
	HBL100, BT20, and HS578T human breast cancer lines, SCID mice	*DC/A/BindCellMemR*	
	HT29 highly metatstatic colon cancer, SCID mice	DC/A/BindCellMemR	
	CACO2 colon cancer, SCID mice	DC/A/BindCellMemR	
	HT29 highly metatstatic colon cancer, SCID mice	DC/A/BindCellMemR	
	VAA Urinary bladder carcinoma MB49 in mice	C/TI, reduction of malignant phenotype, *inhibition of metastasis*	132
	VCA C57BL6 mice with B16-BL6 melanoma cells	C/TI, apoptosis, inhibition of angiogenesis, inhibition of metastasis	133
WGA	Colon carcinoma in F-344 rats	C/TI	134
	Human colorectal cancer	Inhibition of metastasis better prognosis/longer survival times	135
MCL	Nasopharyngeal carcinoma (NPC), Nude mice	C/TI, 45 % remission of NPC xenograft tumors	136
SBL	Dalton' lymphoma bearing mice (DL)	Inhibition of tumor proliferation	137
AGG	Nude mice bearing HepG2 xenografts	Reduced tumor growth, increased TUNEL expression, decreased CD-31 and Ki-67 expression	138

Note: *italic* indicate weak effects.

fined host trees ME-A and ME-M (fir tree Abies, ME-A, Helixor ®A, and apple tree Malus, ME-M, Helixor®M) were applied to the breast carcinoma model intratumorally. *In vivo* investigations of the ability of mistletoe extract to improve tumor survival induce apoptosis and necrosis and inhibit cancer cell proliferation in animal models that have yielded inconsistent results. Seifert et al.[68] investigated both the cytotoxic effect and the mechanism of action of two standardized aqueous MEs (ME-A and ME-P obtained from the pine tree) in an *in vivo* the severe combined immunodeficiency model (SCID) mice of B-precursor acute lymphoblastic leukemia (pre-B ALL) cell line (NALM-6). Both MEs significantly improved survival (up to 55.4 days) at all tested concentrations in contrast to controls (34.6 days) without side effects. However, some research showed decreases in the rate of cell proliferation and improvement in tumor survival.

Mushrooms have become popular sources of natural anticancer, antiviral, antibacterial, antioxidative, and immunomodulatory agents. *Pleurotus citrinopileatus* lectin (PCL) [69] and *Russula lepida* lectin (RLL)[70] exerted potent anticancer activity in mice bearing sarcoma 180, and caused inhibition of tumor growth when administered intraperitoneally.

It has also been shown that the chemical modification of polyethylene glycol-modified concanavalin A (PEG-Con A) enhanced the anticancer cytotoxicity of peripheral lymphocytes against melanoma B16 cells in mice[71]. The encapsulation of *Cratylia mollis* lectin (CML) with liposomes lowered its tissue toxicity in the liver and kidney, and improved its anticancer activity in Swiss mice inoculated with sarcoma 180 cell line[72]. Reduction in the tumor size and inhibition of growth represents basic outcomes of used lectin therapy.

The second type of lectins application is combined with conventional anticancer therapy. Toxic lectins can be often used as a supportive therapy to improve health-related quality of life (HRQoL). It has been shown that the use of some types of complementary and alternative medicine (CAM) in breast cancer patients has rapidly increased[73].

In recent years there has been an increase of research studies on mistletoe therapy, including studies of its co-administration alongside chemotherapy to reduce adverse effects and to improve quality of life in breast cancer, ovarian cancer, and lung cancer patients. These clinical trials have not found significant clinical efficacy in terms of tumor control and survival time for patients. However, studies have shown a positive outcome given the HRQoL[61-65].

To design effective cancer vaccines, the best tumor antigens should be combined with the most effective immunogen to achieve better clinical results. Plant lectins can be also applied as immunoadjuvants to enhance antigen-specific tumor activity. Ricin toxin (RTB) was used as immunoadjuvants fused with HPV-16 E7 to prepare an effective vaccine, which could inhibit tumor growth in the lung. The immunization with E7-RTB protein without adjuvant can generate anticancer effects in mice challenged with TC-1 cells. This research confirms the clinical application of therapeutic vaccines with lectins as immunoadjuvants is directed to design effective cancer vaccines[74].

PERSPECTIVE

Plant lectins have been investigated for a long time. However, new research challenges are present. Some of them will be indicated below.

Applications of glycosylated nanomaterials in nanotechnology have gained significant attention in recent years due to their unique structural properties and compatibility in biological systems[75-77]. Strategies for building various types of glyco-nanoparticles (glyco-NPs) and functionalized carbon nanotubes (CNTs)[78] and highlights their potential in targeted drug delivery and molecular imaging as well as their uses in bioassays and biosensors. Glyco-NPs contain a nano-sized metallic core that exhibits carbohydrate ligands on the surface in three dimensions polyvalent displays similar to the glycocalyx structures of cell membranes. The most recent examples of glyco-NPs are as vaccine candidates and probes for assaying enzymes with bond-forming activities. CNTs have attracted great attention in biomedical applications due to their molecular size and unique properties. Introduction of biofunctionalities by integration of carbohydrate with CNTs provide new tools for glycobiological studies[78].

Carbohydrates are crucial for a wide variety of cellular processes ranging from cell-cell communication to immunity, and they are altered in disease states such as cancer and inflammation.

Development of glycan analysis towards high-throughput analytics are new challenges in the fields of glycomics and glycoproteomics. These include advances in applying separation, mass spectrometry, and microarray methods to the fields of glycomics and glycoproteomics. These new bioanalytical techniques influenced the progress in understanding the importance of glycosylation in biology and disease[79-81].

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