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Anti-tumor and anti-viral activities of *Galanthus nivalis* agglutinin (GNA)-related lectins

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Abstract *Galanthus nivalis* agglutinin (GNA)-related lectin family, a superfamily of strictly mannose-binding specific lectins widespread among monocotyledonous plants, is well-known to possess a broad range of biological functions such as anti-tumor, anti-viral and anti-fungal activities. Herein, we mainly focused on exploring the precise molecular mechanisms by which GNA-related lectins induce cancer cell apoptotic and autophagic death targeting mitochondria-mediated ROS-p38-p53 apoptotic or autophagic pathway, Ras-Raf and PI3K-Akt anti-apoptotic or anti-autophagic pathways. In addition, we further discussed the molecular mechanisms of GNA-related lectins exerting anti-viral activities by blocking the entry of the virus into its target cells, preventing transmission of the virus as well as forcing virus to delete glycan in its envelope protein and triggering neutralizing antibody. In conclusion, these findings may provide a new perspective of GNA-related lectins as potential drugs for cancer and virus therapeutics in the future.

Keywords GNA-related lectins · Anti-tumor · Anti-viral · Drugs

Abbreviations

CBAs	Carbohydrate binding agents
Con A	Concanavalin A
CV-N	Cyanovirin-N
DC-SIGN	Dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin
EGFR	Epidermal growth factor receptor
ERK	Extracellular signal-regulated kinase
FADD	Fas-associated protein with death domain
GNA	*Galanthus nivalis* agglutinin
GRFT	Griffithsin
HHA	*Hippeastrum hybrid* agglutinin
LNL	*Liparis noversa* lectin
MEK	Mitogen-activated protein kinase
MMP	Mitochondrial membrane potential
mTOR	Mammalian target of rapamycin
MVL	*Microcystis viridis* lectin
NF–κB	Nuclear factor–κB
OJL	*Ophiopogon japonicus* lectin
PCD	Programmed cell death
PCL	*Polygonatum cyrtonema* lectin
PI3K	Phosphatidylinositol 3 kinase
POL	*Polygonatum odoratum* lectin
ROS	Reactive oxygen species
SVN	Scytovirin
SFL	*Sophora flavescens* lectin
TDL	*Typhonium divaricatum* lectin
TNF-α	Tumor necrosis factor-α
UDA	*Urtica dioica* agglutinin

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Introduction

Plant lectins are a class of highly diverse non-immune origin, carbohydrate-binding proteins, which contain at least one non-catalytic domain for selective recognition and reversible agglutination of cells. The non-catalytic domain is also responsible for precipitating polysaccharides and glycoconjugates through the free glycans or sugars on glycoproteins and glycolipids without altering the structure of carbohydrate [1]. According to their serological relationships, sequence similarities and evolutionary relationships, plant lectins can be classified into 12 different families, including Amaranthin, *Agaricus bisporus* agglutinin, Cyanovirin, Chitinase-related agglutinin, *Euonymus europaeus*

agglutinin, *Galanthus nivalis* agglutinin, Hevein, Jacalins, Lysin motif, Legume lectin, Nictaba and Ricin-B families [2]. Among the above mentioned 12 plant lectin families, GNA-related lectin family has drawn a rising attention for scientists due to their remarkable anti-tumor and anti-viral activities compared to other families [3, 4].

The first GNA-related lectin was termed *Galanthus nivalis* agglutinin (GNA), originally isolated from snowdrop bulbs and described as a lectin with a specificity towards Manα(1-3)Man-containing oligosaccharides [5–7]. After the identification of GNA, similar lectins were isolated and characterized from many other plant species belonging to different families of the Liliopsida (monocots), including Amaryllidaceae, Alliaceae, Orchidaceae, Araceae, Liliaceae and Bromeliaceae [8]. Accordingly, GNA and related lectins were classified into the so-called "monocot mannose-binding lectins". In the last decade, it has been found that proteins with GNA domains occur not only in monocots, but also in dicots, fishes and fungi, therefore it is now referred to this lectin family as "GNA-related lectins" [9].

Molecular structure analysis clearly indicates that the subunits of all GNA-related lectins possess conserved three-dimensional structures with GNA, in spite of different composition of their amino acid sequences (Fig. 1a and b) [8]. GNA is a homotetramer (50 kDa) of four non-covalently associated monomers of 12.5 kDa. GNA monomer exhibits a ß-barrel structure and comprises three subdomains (I, II, III), and each subdomain contains three or four strands of anti-parallel ß sheet, which interacts with each other by loop [10]. Each subdomain of GNA monomer contains one conserved motif "Gln-X-Asp-X-Asn-X-Val-X-Tyr", the conserved residues Gln, Asp, Asn, and Tyr bind O2, O3 and O4 of mannose through a network of four hydrogen bonds. Another conserved hydrophobic residue Val interacts with C3 and C4 of mannose through hydrophobic interactions (Fig. 1c) [11]. In addition, additional amino acid residues in the vicinity of this primary binding site allow the formation of extra H-bonds with other sugar residues of the glycan structure creating a more extended binding site. This strongly increases the affinity for a particular complex carbohydrate structure, while the affinity for simple sugars is usually much weaker [12].

The conserved motif "QXDXNXVXY" is crucial for mannose binding, however, one or several amino acid mutations could be happened to conserved motif and thus forms a novel sugar-binding motif, which might bind other types of sugars. For instance, *Polygonatum cyrtonema* lectin (PCL), a typical GNA-related lectin could bind mannose in the conserved motif but sialic acid in the mutated motif with more strongly binding capability (Fig. 1b) [13]. Importantly, the diversification of sugar-binding types would make GNA-related lectins have more possible opportunities to recognize more types of sugar-containing receptors on the surface or cytoplasm of cells and may activate more cell signaling pathways [14, 15].

GNA-related lectins display diverse biological activities, such as the anti-tumor properties of PCL [3, 16–18], *Liparis noversa* lectin (LNL) [19], *Ophiopogon japonicus* lectin [18, 19] and *Polygonatum odoratum* lectin (POL) [20] as well as the anti-HIV activities of GNA, *Hippeastrum hybrid* agglutinin (HHA) [21, 22] and PCL [11, 13], anti-HSV-II effects of *Ophiopogon japonicus* lectin (OJL) [19], POL and *Typhonium divaricatum* lectin (TDL) [23]. All these bioactivities of plant lectins are associated with their carbohydrate specificities intensively [24–26]. Furthermore, GNA-related lectins have been suggested as one of the promising agents against insect pests and have been engineered successfully using in a variety of crops including wheat, rice, tobacco, and potatoes. Some GNA-related lectins such as ASAL, GNA, PTA, LRA, ZAA, DOA and GEA could effectively inhibit pest or pathogens [27–30]. These studies indicate

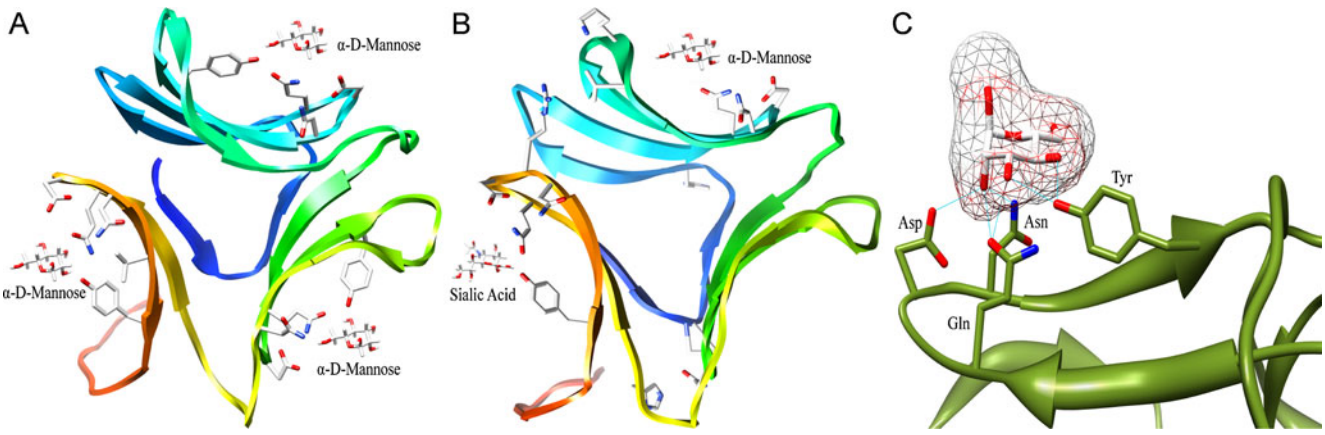

Fig. 1 Molecular structures of *Galanthus nivalis* agglutinin (GNA)-related lectins. **a** The three dimensional structure of GNA: Three binding sites are all active to bind α-D-mannose. **b** The three dimensional structure of *Polygonatum cyrtonema* lectin: one active mannose-binding site and two inactive mannose-binding sites. **c** Mannose binds to conserved motif "QXDXNXVXY" through a network of hydrogen bonds

that GNA-related lectins could possibly be applied in anti-tumor therapeutics and in anti-virus as well as crop protections [12, 25].

GNA-related lectins induce cancer cell death targeting apoptosis or autophagy

Plant lectins have been well-known to possess antitumor activities *via* targeting programmed cell death, which is a cell-intrinsic mechanism for maintaining homeostasis, including apoptosis and autophagy [31]. Apoptosis is a cell-intrinsic mechanism for suicide that can be regulated by numerous cellular signaling pathways and autophagy refers to an evolutionarily conserved, multi-step lysosomal degradation process in which a cell degrades long-lived proteins and damaged organelles [32, 33]. Several major lectin families, such as legume lectins, GNA related lectins and type II ribosome-inactivating proteins have been reported to induce apoptosis in various cancer cells for several years. Moreover, legume lectins and GNA related lectins have also been reported to induce autophagy by several autophagic related pathways (Table 1) [19, 24, 25, 34–37].

Targeting the mitochondria-mediated ROS–p38–p53 apoptotic or autophagic pathway

Previously, Garlic lectin, isolated from garlic (*Allium sativum* L) bulbs, one of the typical GNA-related lectins, was firstly reported to possess cytotoxic effects in human tumor cells. This lectin was able to strongly inhibit DNA synthesis in human U937 and HL60 cells and induced apoptosis at a low concentration [35].

Recently, *Polygonatum cyrtonema* lectin has drawn many interests for its anti-tumor activities toward HeLa, MCF-7, A375 and L929 cells, but with concomitant low toxicity to the normal cells [3]. PCL was firstly reported to possess remarkable anti-proliferative and apoptosis-inducing activities toward HeLa cells in a dose-dependent manner and PCL could induce MCF-7 cell apoptosis with caspase participation [14, 19]. Moreover, PCL was shown to induce apoptosis, accompanied with caspase-9, caspase-8 and caspase-3 activation in L929 cells, suggesting that the apoptotic pathway is caspase-dependent [18]. Recent reports have shown that PCL could induce apoptosis in human melanoma A375 cells. The mechanism of apoptosis interfered by PCL involves in the regulation of Bax, Bcl-X_L and Bcl-2 proteins, which subsequently cause the collapse of mitochondrial membrane potential (MMP). Subsequently, cytochrome *c* is released, making up apoptosome with Apaf-1 and pro-caspase-9. After conjugating apoptosome, procaspase-3 turns into active caspase-3 that eventually triggering apoptosis of cancer cells [38]. The treatment with PCL also abrogated the glutathione antioxidant system, and induced mitochondria to generate massive reactive oxygen species (ROS) accumulation, which subsequently resulted in p38 and p53 activation [16, 39]. Accordingly, these findings indicate that PCL induces apoptosis or autophagy through a

Table 1 Inhibitory effects of *Galanthus nivalis* agglutinin (GNA)-related lectins on tumor cells

Lectin names	Abbreviations	Carbohydrate specificity	Cancer cells	Effects	References
Aspidistra elatior Blume lectin	AEL	Man	Human breast cancer cell(Bre-04), Human lung cancer cell(Lu-04), Human hepatocellular liver carcinoma cell (HepG2)	Antiproliferative activity and cytotoxicity	[34]
Garlic lectin	ASL	Man	Human Myeloleukemic (U937) cell, Human leukemic (HL60) cell, Chinese hamster ovary (CHO) cell	Apoptosis, Cytotoxicity/ tumor inhibition	[35–37]
Liparis noversa lectin	LNL	Man	Human Breast Adenocarcinoma(MCF-7) cell, Human cervical cancer (HeLa) cell	Apoptosis	[19]
Narcissus Pseudonarcissus agglutinin	NPA	Manα(1-6)	Human hepatoma (H3B) cell	Cytotoxicity/tumor inhibition	[36]
Ophiopogon japonicus lectin	OJL	Manα(1-3), Manα(1-6)	Human Breast Adenocarcinoma(MCF-7) cell, Murine fibrosarcoma (L929) cell, Human cervical cancer (HeLa) cell	Apoptosis	[19]
Polygonatum cyrtonema lectin	PCL	Man, sialic acid	Human melanoma (A375) cell, Human Breast Adenocarcinoma(MCF-7) cell, Human cervical cancer (HeLa) cell, Murine fibrosarcoma (L929) cell	Apoptosis and autophagy	[3, 17, 18]
Polygonatum odoratum lectin	POL	Man	Murine fibrosarcoma cell (L929) cell, Human melanoma (A375) cell	Apoptosis	[20]
Typhonium Divaricatum lectin	TDL	Man	Human prostatic cancer (Pro-01), Human breast cancer cell(Bre-04), Human lung cancer cell(Lu-04)	Antiproliferative activity and cytotoxicity	[23]

mitochondria-mediated ROS–p38–p53 pathway in cancer cells.

Targeting Ras–Raf and PI3K–Akt anti-apoptotic or anti-autophagic pathways

It is well known that Ras–Raf and class I phosphatidylinositol 3-kinase (PI3K)–Akt pathways are two significant anti-apoptotic/survival signaling pathways in cancer cells [40]. Recent reports have shown that PCL can induce apoptosis in murine fibrosarcoma L929 cells and inhibition of Ras could promote L929 cell death, suggesting that Ras–Raf signaling pathway plays the key negative regulator in PCL-induced apoptosis. Furthermore, class I PI3K–Akt signaling pathway is further shown to play the negative regulator in PCL-induced apoptosis. PCL can induce apoptosis or autophagy in murine fibrosarcoma L929 cells by inhibition of Ras signal pathways, suggesting that Ras–Raf signaling pathway plays the key negative regulator in PCL-induced apoptic or autophagic cell death [17]. Furthermore, Phosphorylated Akt has several effects, both in the cytoplasm and in the nucleus, which include the inhibition of pro-apoptotic factors such as BAD, caspase-9, and FOXO [41]. Akt-mediated activation of mammalian target of rapamycin (mTOR) is also important in stimulating cell proliferation [42]. Moreover, activated Ras binds to Raf, which, in turn, triggers the phosphorylation of mitogen-activated protein kinase 1/2 (MEK1/2) and extracellular signal-regulated kinase 1/2 (ERK1/2) [43]. Taken together, PCL induces cancer cell apoptosis and autophagy through activating mitochondrial ROS–p38–p53 pathway, as well as blocking Ras–Raf and PI3K–Akt pathways.

The apoptotic activities of other GNA-related lectins

Polygonatum odoratum lectin, a typical GNA-related lectin, has been reported to possess remarkable anti-proliferative activities toward murine fiborsarcoma L929 cells by inducing apoptosis in a caspase-dependent manner [20]. And, POL can also induce cell death through the extrinsic apoptotic pathway by increasing the levels of FasL and Fas-Associated protein with Death Domain (FADD) proteins and results in caspase-8 activation. Moreover, POL treatment leads to mitochondrial transmembrane potential collapse and cytochrome *c* release and subsequent activations of caspase-9 and caspase-3. Interestingly, POL can also amplify the apoptotic effect of tumor necrosis factor-α (TNF-α) at low concentrations in L929 cells [25]. Additionally, another mannose-binding lectin (named CML), a possibly GNA-related lectin, was isolated from *Clematis montana* Buch.-Ham stem (*Ranunculaceae*) and reported to induce L929 apoptosis through the caspase-dependent and death-receptor pathways [44].

To sum up, GNA-related lectins can promote mitochondria-mediated ROS–p38–p53 pathway as well as block Ras-Raf and PI3K-Akt apoptotic or autophagic pathways, thereby, ultimately culminating in cancer cell death (Fig. 2). Taken together, these finding may illuminate the intricate relationships between the carbohydrate-binding specificities and complex molecular mechanisms by which GNA-related lectins induce cancer cell death. With the complex molecular mechanisms of GNA family induced PCD becoming better elucidated, there is no doubt that new therapeutic strategies would be developed into targeting apoptotic and autophagic cell death pathways for cancer therapeutics.

Anti-virus mechanisms of GNA-related lectins

Antiviral activities of a number of lectins that bind high-mannose or complex carbohydrates have been described in the past. Examples of such lectins include GNA, HHA, jacalin [45], Concanavalin A (Con A) [46], *Urtica diocia* agglutinin (UDA) [47]. Plant lectins probably are the most diverse group of Carbohydrate binding agents (CBAs) with a wide variety of carbohydrate-binding properties depending on the plant species and even the anatomic site of isolation. Strikingly, the vast majority of plant lectins that are active against HIV are endowed with a carbohydrate specificity directed against mannose oligomers. So far, only one plant lectin that shows specificity for GlcNAc found in the rhizomes of the stinging nettle *Urtica dioica* has shown pronounced anti-HIV activity [48]. Most HIV-inhibitory plant lectins are derived from the GNA related families [47]. Several lectins of GNA-related lectin family, including GNA, PML, PCL, LRA, SCA, HHA, NPA, LOA, EHA, CHA, APA and AUA, have been reported to effectively inhibit the infection of HIV *in vitro* (Table 2) [48–53].

Blocking the entry (or fusion) of the virus into its target cells

Mannose-binding lectins could bind to some mannose-containing envelope proteins of virus (in particular HIV) and thus block virus entry into target cells. The human immunodeficiency virus (HIV) has two envelope glycoproteins that form trimeric complexes on the viral surface namely surface protein gp120 and transmembrane protein gp41. Infection of target cells by HIV is a complex, multi-stage process involving attachment to host cells and CD4 binding, coreceptor binding, and membrane fusion [54]. The initial interaction between HIV and a target cell involves the high-affinity attachment of the CD4-binding domains of gp120 to CD4, a receptor present on certain T cells, macrophages, dendritic cells, and microglial cells [55]. Binding of HIV to the CD4 receptor induces a conformational change that brings gp120 into proximity with a cellular coreceptor

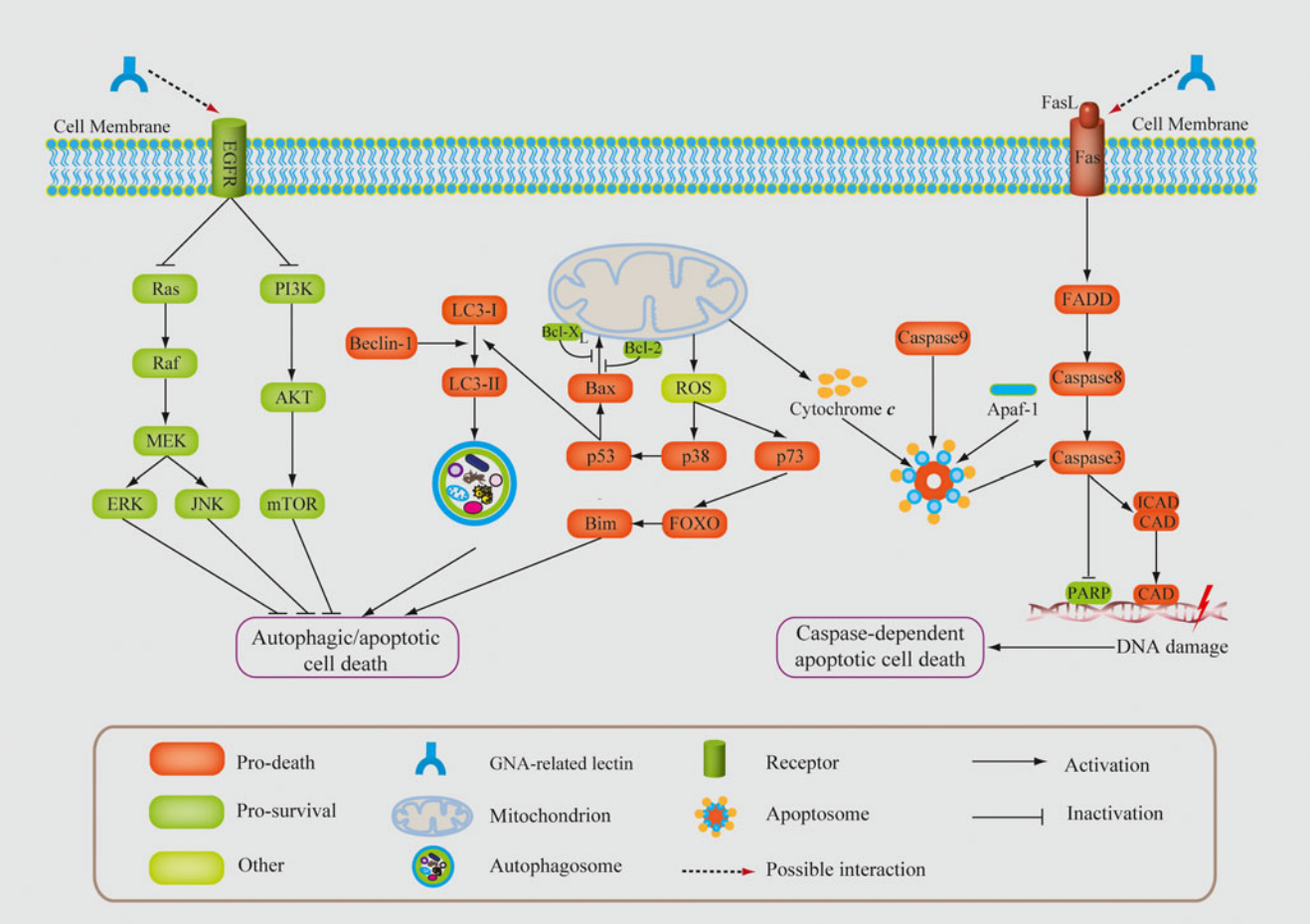

Fig. 2 GNA-related lectins induce cancer cell death *via* targeting apoptosis or autophagy signaling network

CCR5 or CXCR4. This binding triggers other conformational changes, resulting in the insertion of the fusion peptide of gp41 in the cellular membrane and eventual fusion of the virus with the target cell membranes [56].

The HIV-1 gp120 envelope glycoprotein is highly glycosylated. Approximately half of its molecular weight is contributed by its carbohydrate content [57]. The recombinant HIV-1(IIIB) gp120 expressed in Chinese hamster ovary (CHO) cells is occupied by 11 high-mannose- or hybrid-type glycans and 13 complex-type glycans [58]. Carbohydrate binding agents (CBAs), such as the plant lectins HHA, GNA, UDA and BanLec, or the prokaryotic cyanovirin-N (CV-N), Scytovirin (SVN), *Microcystis viridis* lectin (MVL), actinohivin (AH) and griffithsin can bind to multiple glycans that are presented on the envelope of HIV. The binding between lectins and glycans on HIV gp120 might result in steric hindrance, which would disturb the efficient interaction between gp120 and its receptors [48]. In addition, the conformational change of gp120 after CD4 binding include the movement of V1/V2 and V3 loop structures and expose the coreceptor binding site, Lectins may cross-link several glycans and freeze the conformation of gp120 consequently preventing further interaction with the coreceptor and block the entry/fusion of the virus into its target cells [59, 60].

Blocking the transmission of the virus

Recent studies have suggested that the high-mannose type oligosaccharides on gp120 can serve as the ligands of dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN) receptor [61, 62]. DC-SIGN is a C-type lectin receptor present on both macrophages and dendritic cells. DC-SIGN binds various microorganisms by recognizing high-mannose containing glycoproteins on their envelopes and especially functions as receptor for several viruses such as HIV and HCV [63, 64]. DC-SIGN does not function as a receptor for viral entry into DC but instead promotes efficient infection trans of cells that express CD4 and chemokine receptors. DC-SIGN efficiently captures HIV in the periphery and facilitates its transport to secondary lymphoid organs rich in T cells, to enhance infection trans of these target cells [65]. Thus binding to DC-SIGN is an essential process for HIV

Table 2 Inhibitory effects of *Galanthus nivalis* agglutinin (GNA)-related lectins against HIV

Lectin names	Abbreviations	Carbohydrate specificity	Cells	Effects	References
Galanthus nivalis agglutinin	GNA	Manα(1-3)	Human T lymphocytes (MT-4) cell, T lymphoid Leukemia (CEM) cell, Human cutaneous T-cell lymphoma (HUT-78) cell, Lymphoid (Sup-T1) cell	Anti-HIV-I, anti-HIV-II and Preventing HIV induced syncytium formation.	[4, 48]
Hippeastrum hybrid agglutinin	HHA	Manα(1-3), Manα(1-6)	Human T lymphocytes (MT-4 cells), T lymphoid Leukemia Cell (CEM) cell, Human cutaneous T-cell lymphoma (HUT-78) cell, lymphoid (Sup-T1) cell	Anti-HIV-I, anti-HIV- II and Preventing HIV induced syncytium formation.	[4, 48]
Narcissus pseudonarcissus agglutinin	NPA	Manα(1-6)	Human T lymphocytes (MT-4) cell, T lymphoid Leukemia (CEM) cell, Human cutaneous T-cell lymphoma (HUT-78) cell and lymphoid cell	Anti-HIV-I, anti-HIV-II and Preventing HIV induced syncytium formation.	[4, 48]
Listera ovata agglutinin	LOA	Manα(1-3)	Human T lymphocytes (MT-4) cell	Anti-HIV-I, anti-HIV-II and Preventing HIV induced syncytium formation.	[48, 49]
Epipactis helleborine agglutinin	EHA	Man	Human T lymphocytes (MT-4) cell	Anti-HIV-I, anti-HIV-II and Preventing HIV induced syncytium formation.	[48, 49]
Cymbidium hybrid agglutinin	CHA	Man	Human T lymphocytes (MT-4) cell	Anti-HIV-I, anti-HIV-II and Preventing HIV induced syncytium formation.	[48, 49]
Allium porrum agglutinin	APA	Man	Human T lymphocytes (MT-4) cell	Anti-HIV-I and anti-HIV-II	[48, 49]
Allium ursinum agglutinin	AUA	Man	Human T lymphocytes (MT-4) cell	Anti-HIV-I and anti-HIV-II	[48, 50]
Polygonatum cyrtonema Hua lectin	PCL	Man, Sialic acid	Human T lymphocytes (MT-4) cell, T lymphoid Leukemia (CEM) cell	Anti-HIV-I, anti-HIV- II and Preventing HIV induced syncytium formation.	[11, 13]
Polygonatum multiflorum lectin	PML	Man	T lymphoid Leukemia Cell (CEM) cell	Anti-HIV-I, anti-HIV-II and Preventing HIV induced syncytium formation.	[51]
Scilla campanulata agglutinin	SCA	Manα(1-3), Manα(1-6)	Human T lymphocytes (MT-4) cell and T lymphoid Leukemia (CEM) cell	Anti-HIV-I, anti-HIV-II and Preventing HIV induced syncytium formation.	[52]
Lycoris radiate agglutinin	LRA	Man	Human T lymphocytes (MT-4) cell and T lymphoid Leukemia (CEM) cell	Anti-HIV-I, anti HIV- II and Preventing HIV-induced syncytium formation.	[53]

infection. It has recently been demonstrated that several mannose or GlcNAc specify lectins such as GNA, HHA, UDA have a pronounced inhibitory effect on virus capture by cells that express DC-SIGN and on the subsequent transmission of the virus to uninfected T cells [48]. Additionally, GNA related lectins, such as GNA, HHA and NPA, also markedly prevent syncytium formation between persistently HIV-infected cells and uninfected T-lymphocytes, and thus inhibit dendritic cell HIV infection and dendritic cell-directed HIV transfer [26, 60].

Forcing virus to delete glycans in its envelope protein

The exposure of HIV to any antiviral drug will eventually result in the appearance of phenotypic resistance, both in cell culture and in HIV-infected individuals [66]. *In vivo*, HIV-1 continuously changes its glycan shield to escape the immune system in virus-infected individuals to avoid neutralization. Long-term exposure of HIV to CBAs in cell culture results in the progressive deletion of N-glycans of HIV gp120 in an attempt of the virus to escape drug pressure [47, 66]. In this respect, the CBAs are endowed with a

high genetic barrier: multiple mutations (at least four to five) at N-glycosylation sites are required before pronounced phenotypic drug resistance development becomes evident [26]. Moreover, it has been assumed that such glycan deletions will result in creating 'holes' in the protective glycan shield of the HIV envelope, whereby the immune system may become triggered to produce neutralizing antibodies against previously hidden immunogenic epitopes of gp120 [47]. In addition, deletion of one or more N-glycans in the dense glycan shield of the gp120 may allow better availability of other CBAs interaction with previously hidden glycans, making the mutant virus now vulnerable to inactivation by an extensive neutralizing antibody (Nab) response [67]. Taken together, treatment of HIV with CBAs may consist of a novel chemotherapeutic concept with dual mechanisms of antiviral action: a direct antiviral activity by preventing HIV entry and transmission to its target cells, and an indirect antiviral activity by forcing HIV to delete glycans in its gp120 envelope and triggering Nab production upon the selection of mutant virus strains (Fig. 3).

Comparison between GNA-related lectins and other carbohydrate-binding agents

In recently years, some lectins derived from marine organisms, such as CV-N, SVN, MVL and GRFT, exhibit the highest activity among the lectins that have been investigated so far, because they inhibit HIV replication with half-maximal effective concentration (EC_{50}) values in the low nanomolar to picomolar ranges [68]. Most of those marine derived lectins recognize the reducing or nonreducing end mannoses of Man-9, the predominant oligosaccharide on the HIV viral surface glycoprotein gp120 [69]. For example, Cyanovirin-N specifically recognizes Manα(1-2)Man linked mannose substructures in the D1 and D3 arms of Man-9, MVL specifically interacts with the Manα(1-6)Manβ(1-4)GlcNAcβ(1-4)GlcNAc tetrasaccharide, Scytovirin revealed specificities for Manα(1-2)Manα(1-6)Manα(1-6)Man, Griffithsin contained three single mannose units and specifically interacts with reducing end mannose of the triantennary Man-9 [70, 71]. In contrast, GNA-related

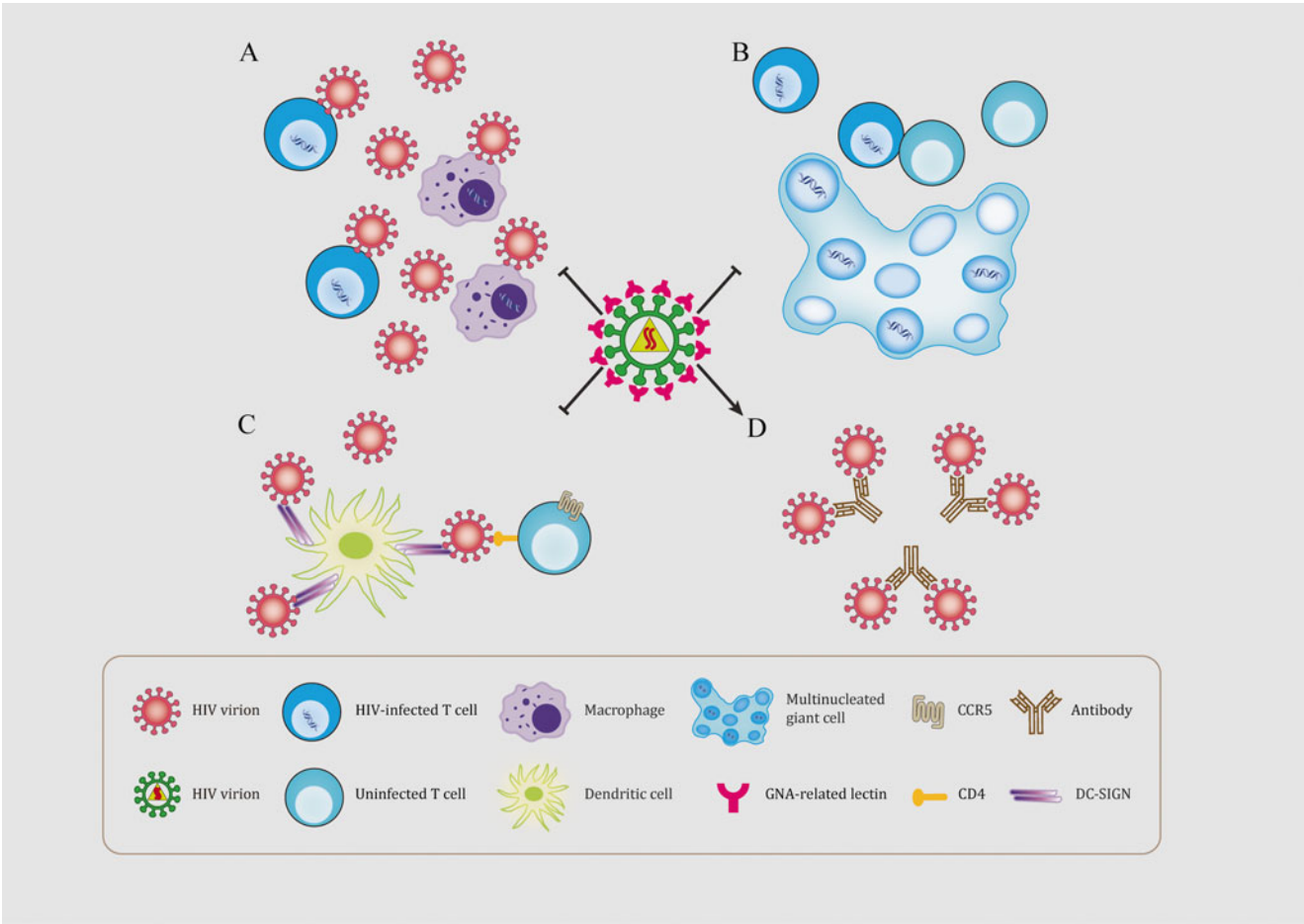

Fig. 3 Overview of the anti-HIV activities of GNA-related lectins. **a** GNA-related lectins inhibit the infection of T cells and macrophages by cell-free HIV particles. **b** GNA-related lectins inhibit syncytia formation between HIV-infected cells and uninfected T cells. **c** GNA-related lectins inhibit DC-SIGN captured HIV transmission to T cells. **d** GNA-related lectins force the virus to delete part of the protective glycan and trigger an enhanced neutralizing antibody response

lectin recognizes a cluster of high-mannose glycans on the viral envelope glycoprotein gp120, GNA has specificity for terminal α(1-3)-linked mannose residues, HHA recognizes both terminal and internal α(1-3) and α(1-6)-linked mannose residues, their interaction with Man-9 maybe similar with above mentioned lectins. Importantly, some of these high mannose-binding plant lectins exhibit a strong anti-HIV activity, but others are weak or completely inactive, such different biological activities virtually depend on the diverse carbohydrate specificities of these plant lectins [68]. In addition, many lectins have been reported to induce undesirable side effects including secretion of inflammatory cytokines and activation of host T-cells. For example, CV-N has been studied in great detail for its development as a microbicide, but a recently study has shown that CV-N induce cytokines/chemokines in peripheral blood mononuclear cell cultures, the potential side effects can compromise the application of these lectins as an efficient microbicide [72, 73]. Griffithsin (GRFT), the other mannose-specific CBAs originally isolated from the red algae *Griffithsia* sp. shows a very potent and broad-spectrum anti-HIV-1 activity in the picomolar range, as well as an outstanding safety and efficacy profile [74, 75]. However, the chemical and physical stability of GRFT remain to be evaluated in transmission animal models.

Some GNA related lectins such as GNA and HHA are not cytotoxic, anti-metabolically active, or mitogenic to human primary T lymphocytes at concentrations that exceed their anti-viral active concentrations by 2 to 3 orders of magnitude [4]. Moreover, a beneficial property of these plant lectins to be used as potential microbicides is their resistance to denaturation at low pH (≥2) and at high temperatures (≥50°C for prolonged time periods). HHA and GNA are also perfectly fitted to microbicide guidelines in the way they are odorless, tasteless, colorless, not mitogenic and not anti-metabolically active at anti-viral concentrations [22]. So, further investigations of the GNA-related lectins in the preclinical and clinical stages are imperative before these lectins could be applied as potential anti-HIV microbicides.

Summary

Nowadays, plant lectins are routinely used in cell biology and immunology, for diagnostic, immunomodulatory as well as therapeutic purposes, especially in oncology and infection. Recent studies have demonstrated that plant lectins also possess an inhibitory effect on the development of cancer due to the cytotoxic-, apoptosis- and autophagy-inducing effects and some of them such as Con A and mistletoe lectins have been applied in pre-clinical and clinical therapies in fighting human cancers. However, the pre-clinical animal studies and exact tumor selectivity about GNA-related lectins have not been proposed, and some of the aforementioned findings of GNA-related lectins are still rudimentary. Recent research about GNA-related lectins has provided us more convincing evidence to understand the anti-cancer molecular mechanisms implicated in apoptosis and autophagy, which would lead to further investigation about clinical application of GNA-related lectins.

Besides, virus adsorption and fusion inhibitors, lectins may be ideal candidates as microbicides, since they do not require uptake by the cells. CBAs such as GNA-related lectins can effectively bind to high-density clustering oligomannose structures of envelope proteins and exerting anti-viral activities by blocking the entry of the virus into its target cells, preventing transmission of the virus as well as forcing virus to delete glycan in its envelope protein and triggering neutralizing antibody. Moreover, plant lectins did not measurably affect the anti-viral activity of other clinically approved anti-HIV drugs used in the clinic when combined with other drugs such as reverse transcriptase inhibitors, protease inhibitors and chemokine inhibitors. Combination of those drugs with CBAs can behave synergistically against HIV-1, HIV-2, and CBA-resistant HIV-1 replication.

In conclusion, GNA related plant lectins, subject of the present study, are endowed with a number of favorable and interesting properties that make them primary candidate drugs to be considered for further (pre)clinical investigations as potential anti-tumor agents and microbicides. Additional clinical trials and the mechanisms of action research would help scientists and clinicians to further understand the therapeutic effects and toxicity of GNA-related lectins and thus it may be utilized as promising drugs against cancer and HIV in the near future.

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