Recent advances in the use of legume lectins for the diagnosis and treatment of breast cancer

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Abstract

Poor lifestyle choices and genetic predisposition are factors that increase the number of cancer cases, one example being breast cancer, the third most diagnosed type of malignancy. Currently, there is a demand for the development of new strategies to ensure early detection and treatment options that could contribute to the complete remission of breast tumors, which could lead to increased overall survival rates. In this context, the glycans observed at the surface of cancer cells are presented as efficient tumor cell markers. These carbohydrate structures can be recognized by lectins which can act as decoders of the glycocode. The application of plant lectins as tools for diagnosis/treatment of breast cancer encompasses the detection and sorting of glycans found in healthy and malignant cells. Here, we present an overview of the most recent studies in this field, demonstrating the potential of lectins as: mapping agents to detect differentially expressed glycans in breast cancer, as histochemistry/cytochemistry analysis agents, in lectin arrays, immobilized in chromatographic matrices, in drug delivery, and as biosensing agents. In addition, we describe lectins that present antiproliferative effects by themselves and/or in conjunction with other drugs in a synergistic effect.

Keywords: Plant lectins; Breast cancer; Glycans

1. Introduction

Cancer is the most prevalent cause of death in a total of 112 countries worldwide and the cases are showing an upward trend with cases increasing every year. To put in perspective, about 10,000,000 deaths were a consequence of this disease in 2020 and this number is increasing year by year, which is putting severe pressure on healthcare systems around the globe. From the literature data, it is possible to observe that the most prevalent cancer type differ by sex, with the most prevalent being prostate cancer for men and breast cancer for women [1,2]. Considering this data, Breast Cancer (BCa) affects 27-97 women for every 100,000 individuals distributed among the American, Asian and African continents, with about 1/3 of cancers in women being diagnosed as BCa with a mortality/incidence rate of 15% [2,3]. The mortality induced by BCa depends on several factors, one of them being the early detection and the quality of the treatment. These factors make it so that different regions of the globe have different mortality rates for this disease [4,5]. BCa is the leading cause of death in young women. Particularly, in Brazil, Silva et al. conducted an epidemiological study from 1996 to 2017 and reported an increased mortality among young women with BCa and highlighted the importance of early detection [6]. About the disease itself, the malignancy starts to manifest predominantly at the mammary duct epithelium level with rare cases starting in the breast lobes. During the first stages, the disease is not symptomatic, but the data suggest very little metastasis rates. Early resection during this stage (*in situ*) is curative without the need for chemotherapy, thus avoiding its collateral effects and high treatment costs [7]. Over time, however, the metastasis potential increases and the malign cells invade other regions in the breast, lymph nodes and other organs, resulting in the need for more aggressive treatment strategies, such as surgery, chemotherapy, or radiotherapy. These steps preclude the chance of cure and create a very grim scenario characterized by escalating treatment costs and personal distress [7]. When a woman dies from BCa, it usually results from widespread metastasis [8,9].

Hereditary genetic factors play a big role in the development of BCa with mutations in specific genes, namely BRCA1 and BRCA2 resulting in an average cumulative risk of about 70%, with overall genetic predisposition responding to about 10% of all BCa cases worldwide [10–12]. Increased predisposition for BCa also occurs as a result of factors such as genetic syndromes, pregnancy, hormone therapy and lifestyle factors, e.g. high BMI, sedentary lifestyle, high alcohol intake, smoking, etc (Figure 1). Lifestyle changes appear to be one of the most effective methods to prevent BCa [13–15].

The treatment of BCa has been improved in the last decade. However, a wide disparity in survival exist in a dependency of early detection combined with access to properly treatment, which is performed by standalone or combinations of surgery, radiotherapy, chemotherapy and hormonal therapy to reduce the risk of spreading [16, 17]. Radiotherapy and chemotherapy can cure most cases, but these treatments, especially anthracyclines, a type of chemotherapeutic, induce serious DNA lesions mainly in chromosome 5 (deletion 5q) and chromosome 7 (monosomy 7). These abnormalities may, in turn, lead to secondary bone marrow cancers, such as acute myeloid leukemia and myelodysplastic syndrome, following prolonged treatment. When the disease is identified at an early stage, chemotherapy could be avoided, emphasizing the necessity of new techniques/methodology able to detect BCa in its initial forms. When considering late stages of BCa, the treatment options become more restricted, but the most successful approaches encompass combinations of chemotherapy, hormone-based therapy, and immunotherapy. For cases that need surgical intervention, partial mastectomy is used along with radiation therapy to minimize the chances of recurrence. The biological subtyping of BCa will determine how the medical treatment will be done before and after surgery [17,18].

To help detect and/or treat cancer natural compounds are interesting candidates [19]. Lectins are among these compounds. Lectins are proteins that bind to carbohydrates in a specific fashion. Although this group of proteins can be found in all organisms, plant lectins are the most extensively investigated[20]. Plant lectins are well known as protective molecules, acting against potential predators or pathogens in plants, while in animals, lectins most often aid in cellular interactions [21]. However, independent of their origin, all lectins share properties associated with physiological and pathological processes. Based on their defensive properties and ability to induce apoptosis in cancer cells, plant lectins have been examined as a prospective treatment option against cancer (Figure 2) [22]. The lectin-carbohydrate reversible and specific binding enables their use as tools in several fields including the biomedical, as anticancer agents, even in agriculture, as insecticidal agents [21,23]. In cancer cells, glycans are the main targets of lectins. It is well known that cancer cells have a different glycosylation pattern at the cell surface when compared to healthy cells. Thus, plant lectins can be used to detect these differences, allowing application in diagnosis or in treatment by direct and specific cytotoxic activity [19,23,24]. It is known that the structure of glycans in malign cells play a critical role in immune escape and immunomodulation [25–28] in a process similar to that of a pathogen that expresses glycans which hides them from the immune system or hijack its functions for its own benefit. This modification of glycans can also increase immune escape by modifying the functions of antigen-presenting cells, M2

macrophages, T lymphocytes and natural killer cells. Understanding how these endogenous lectin-glycan processes lead to immunosuppression in the tumor environment is fundamental to cancer research [25–29].

The current literature reports on the application of lectins of several origins in cancer models, namely of animal lectins [30–32] , microorganisms lectins [33,34], and, most of all, plant lectins. This review aims to highlight these studies with particular focus on plant lectins applied on the recognition of BCa glycans, as well as their cytotoxic/antiproliferative effects.

2. Glycosylation in BCa

Glycosylation is post-translational processing that occurs in proteins and is one of the main forms of modification at the molecular level that can occur in living beings. The addition of glycans to a target protein can influence its folding and, consequently, its biological function. *N*- and *O*-glycosylations are the two most main types of glycosylation (Figure 3) [35]. In the *N*-glycosylation, an oligosaccharide starting with a *N*-acetylglucosamine residue is covalently linked to the polypeptide chain by an *N*-glycosidic bond with the amide nitrogen of an asparagine residue. This asparagine residue is found at one or more points called glycosylation sites, usually in an Asn-X-Ser/Thr sequence (X is any amino acid, except proline). All *N*-glycan structures have a common core, Manα1–6(Manα1–3)Manβ1–4GlcNAcβ1–4GlcNAcβ1-Asn-X-Ser/Thr (MAN3), linked to the asparagine of the anchored protein, see Figure 3(1) [25,35]. In the rough endoplasmic reticulum, one or more units of the precursor oligosaccharide (Glc3Man9GlcNAc2) are covalently linked to the protein in their glycosylation sites and in the Golgi Complex (GC), this precursor is modified by addition or removal of units or groups of monosaccharides. Mannose residues can be cleaved and units of *N*-acetylglucosamine, *N*-acetylgalactosamine (GalNAc), galactose, fucose and sialic acid can be added at the end of the glycan structure. These modifications generate the three main classes of *N*-glycans: high mannose, complex or hybrid. High-mannose *N*-glycans are generated by modifying the oligosaccharide precursor to generate the MAN3 core, and more mannose residues may be added to generate larger structures. Complex-type *N*-glycans have branches, starting with *N*-acetylglucosamine and followed by β1–4-linked D-galactose residues, forming *N*-acetyllactosamine, which is bound to the MAN3 core. Hybrid *N*-glycans have branches composed of oligomannosides (similar to the high-mannose type), as well as branches composed of *N*-acetyllactosamine and other residues (similar to the complex type) [35,36].

In regards to the *O*-glycosylation, it is a process that involves the attachment of an GalNAc to a serine or threonine residue in proteins. This process occurs in the GC and can be mediated by 20 types of polypeptide transferases to generate different glycosidic patterns, such as the formation of Tn antigen (GalNAc-Ser/Thr), the simplest *O*-glycosylation which can be modified to generate other types of glycosylation [37,38]. Other common *O*-glycans include th TF antigen (Thomsen-Friedenreich, Gal-β(1→3)GalNAc-O), and other additional expanded structures that include the sT antigen (sialylated T antigen, NeuAcα2-3-Gal-β(1→3)GalNAc-O), sTn antigen (sialyl Tn antigen, NeuAcα2-6-GalNAcα1-O-Ser/Thr), sLeX (sialylated Lewis, Neu5Acα2-3Galβ1–4(Fucα1–3)GlcNAc-O), LeX (Lewis X, Galβ1–4(Fucα1–3)GlcNAc-O) and sulfo-sLeX, see Figure 3(2) [39,40].

Cancer cells exhibit genomic, proteomic and transcriptomic abnormalities, but their patterns of *N*- and *O*-glycosylation can be significantly altered in composition, structure and abundance compared to those of normal cells. Glycosylation plays key roles in several biological processes fundamental to tumor survival, such as inter- and intracellular signaling processes, cell adhesion, interaction between cell and extracellular matrix, cellular metabolism and immune surveillance. Therefore, glycan imbalance, as well as the presence of aberrant glycosylation, can influence all these processes by modulating protein activity and interactions [25]. These alterations are caused by alterations in glycosyltransferases expression, the localization of these enzymes in the GC and the availability of molecular resources for biosynthesis. These factors can lead to incomplete synthesis of glycans with formation of truncated glycan structures, high expression of specific glycans and/or the synthesis of new glycosidic structures [38,41,42]. These phenomena can occur simultaneously, but incomplete synthesis of glycans is more often observed in the early stages of cancer, and both overexpression and formation of new structures are more frequently observed in later stages [26,42].

In BCa, a deregulation in the process of glycan synthesis that leads to aberrant glycosylation has been reported [26,43]. Among the types of aberrant glycosylation, the most frequent are extension and branching of *N*-glycans, *O*-glycan truncation, both with increased rates of fucosylation and sialylation and increased rated of *O*-GlcNAc glycans [44,45].

One of the most common changes in BCa, occurs due to an overexpression of the *N*-acetylglucosaminyltransferase V (GnT-V) which induces an increase in poly-*N*-acetyllactosamine groups (poly-Galβ1,4GlcNAcβ1,3- and poly-LacNAc) in *N*-glycans branches, these changes being associated with the progression of mammary carcinomas and metastasis in mouse models [46,47]. Besides increased extension and branching,

additional sialylation and fucosylation of complex *N*-glycans is a common change in BCa and has been detected in at least 5 BCa cell lines and samples from patients [48–50]. Increased sialylation on integrins are correlated with the clustering of these proteins and their subsequent signaling pathways, which, in turn, elevates the migratory and metastatic potential of cancer cells [51–53]. Experiments involving BCa cells overexpressing the transferase responsible for this high sialylation demonstrated abnormal levels of cell adhesion to collagen, fibronectin and laminin substrates [54,55].

As prevalent in BCa as increased sialylation, is the increase in fucosylation levels. Central fucosylation refers to the addition of a fucose residue to the innermost GlcNAc residue of the MAN3 core via an α1-6 glycosidic bond. Terminal fucosylation can occur at *N*-glycans and *O*-glycans, generating patterns corresponding to those of Lewis antigens [56]. Many glycosylated BCa receptors show central fucosylation in their *N*-glycans, one example being the increased content of α1-6-fucosylation in EGFR which correlates with tumor growth and malignancy [57]. In BCa cell lines, the same pattern of glycosylation occurs in the Transforming Growth Factor beta (TGF-ß) that induces epithelial-to-mesenchymal transition (EMT) [58]. Mucins and CD44 proteins are membrane glycoproteins rich in sLeX and sLea antigens (sialyl Lewis A; Neu5Acα2-3Galβ1-3[Fucα1-4]GlcNAcβ-O) in BCa cells. These antigens are recognized by *P*- and *E*-selectins present on the surface of endothelial cells of blood vessels, and this interaction is responsible for the extravasation of tumor cells present in the bloodstream, facilitating metastasis [41,42,59]. Balance between LeX and sLeX present in breast tumor cells is important to modulate angiogenesis and metastasis of these cells, as well as ensure greater tumor resistance to drugs [60–62].

One of the most studied proteins studied by cancer researchers is MUC1, a large extracellular, heavily glycosylated protein which expression and glycans are deeply altered in cancer conditions. In healthy breast epithelial cells, *O*-glycosylation of MUC1 occurs via GalNAc-transferases that catalyze the reaction of GalNAc addition to threonine and serine residues of the protein. An extension reaction of this glycan then occurs by the addition of galactose to form T antigen (core 1) mediated by T synthase. T antigens are further extended by the addition of GlcNAc to GalNAc, forming a branch-like structure called core 2. Moreover, core 2 is extended to form branched polylactosamine structures, which can further be fucosylated or sialylated [42,63,64]. However, in BCa cells, truncated structures of these *O*-glycans result in a greater frequency of Tn, sTn, T and ST antigens. Tn, T and ST antigens are present in most breast cancers, whereas sTn is found in 20 to 25% [65–67]. The aberrant glycans of MUC1 interfere in signaling processes that protect the protein from being degraded. This means that MUC1 creates a favorable

microenvironment for tumor growth in the breast region [68,69]. Aberrant glycans in serum immunoglobulin A1 (IgA1), including increased sialylation and T antigen rates, in *N*- and *O*-glycans, respectively, have been reported in patients with metastatic and nonmetastatic BCa [50].

The presence of *O*-GlcNAc glycans is intensified in BCa and is quite important for breast tumor progression, tumor growth, invasion and metastasis [70,71]. *O*-GlcNAc modifications may impact molecular events required for tumor progression, such as cell proliferation, or, more specifically, the expression levels of the forkhead box protein M1 (FoxM1), and cyclin D1, a cell cycle protein and transcription co-regulator, both involved in the progression of cell cycle [72–74]. Furthermore, this type of glycosylation is related to a role in cancer survival by creating a tumor-friendly microenvironment, acting on signaling processes involved in angiogenesis, upregulation of vascular endothelial growth factor A (VEGFA), matrix metalloproteinases (MMPs), in addition to participating in the processes of invasion and metastasis of cancer cells. [75–77].

The knowledge about the glycosylation patterns found in BCa helps tremendously in research about the application of lectins in this area. The recognition of glycan structure in the cell surface is widely accepted as the first step for lectins to elicit their biological activities. Not surprisingly, the application of lectins in BCa research depends on the protein specificity, with the most promising one apparently being mannose-specific lectins, lectins that recognize poly-LacNAc structures, and those that can recognize fucose and Neu5Ac-specific.

3. Lectins as diagnostic tools against BCa

Understanding the human glycome comprises a crucial portion of cancer research since targeting cancer-related glycan structures is quickly emerging as an effective diagnostic method [78–80]. In this context, several studies have reported on the use of lectins to map changes in the glycosylation patterns of malignant cells, making it possible to follow and predict the progression of the disease and the degree of malignancy [55,81–86]. To accomplish this, several well-known techniques can be used, including, for example, Enzyme-Linked Lectin Assay (ELLA) [87,88], immunohistochemistry [89–91], lectin nanoparticle assays [92], glyco-functionalized quantum dots (QDs) with lectins [93], lectin blot analysis [58,94], lectins conjugated with fluoro-microbeads and antibody–lectin sandwich assay [87,95], SDS-PAGE/Western Blotting [96], and fluorescent labeling techniques [97,98], among others [99–101]. However, in this review, we highlight the most widely applied techniques, such as lectin arrays, affinity chromatography and histochemistry/cytochemistry analysis. These techniques allow for a broad-spectrum study of the

interaction of lectins with different ligands, and they do not require glycan detachment and derivatization before the measurements.

3.1. Lectin arrays

The lectin microarray technique, developed in 2005, has become widely known for its high-throughput glycan analysis [102–104]. Its main advantages, when compared to traditional methods based on Mass Spectrometry (MS), include its relative simplicity and high sensitivity for the analysis of global glycomic profile, relatively low necessity of sample purity, and straightforward sample preparation without protein digestion or glycan release steps [105]. Even though this technique does allow for the complete structural characterization of the glycan, lectin microarray enables for a comparative analysis to differentiate the different glycosylation profiles in biological samples. Currently, this technique has been shown to be promising in the characterization of whole-cell glycans [106,107], expanding the perspective of cancer researchers on the implications of glycosylation in tumorigenesis [104,107–112]. With around 100 lectins in different arrangements, this highly multiplexed characterization allows for the detection of subtle differences between cells and the identification of specific lectins responsible for recognition of these differences, which can then be exploited in diagnostic applications [106,113,114].

Limited data have found their way into the literature of BCa research. Arndt and colleagues applied a glycan matrix with 18 lectins, predominantly of plant origin, and showed that BCa cells MDA-MB435 interacted with ConA and ECA, lectins from *Canavalia ensiformis* and *Erythrina cristagalli*, respectively. The glycans of this cell line recognized by ECA lectins (Gal(1,4)GlcNAc > Gal(1,4)Glc > GalNAc > Gal) and ConA (Branched Man > Glc > GlcNAc) were also found in cell lines from other types of cancer and also recognized with virtually the same intensity with the same lectins [115]. Similarly, a microarray screening of 91 lectins was performed by Zhou and colleagues. The study revealed an increase in binding of *Ricinus communis* lectin (RCA-I) with triple-negative breast cancer (TNBC) cells in proportion to the advancing degree of metastasis. In addition, the lectin inhibited cell invasion, migration and adhesion [116].

Another study by Fry et al. [117] showed that the lectins PNA, GSL-II, GSL-I-B4, PWM, PTL-I, and jacalin (JAC) are potential biomarkers of metastatic primary breast tumors. A microarray test was performed using forty-five lectins with binding specificity to *N*- and *O*-glycans, together with evanescent field-activated fluorescent detection. A single 50 µm section of a primary breast tumor or <1 µL of serum or urine from a BCa patient was

sufficient to detect glycosylation changes associated with metastatic Bca, as inferred from lectin binding patterns. In serum samples, four lectins exhibited statistically significant differences in binding between metastatic and non-metastatic sera. When compared to with metastasis-negative BCa patients' sera, the metastasis-positive sera showed a higher binding to AOL and *Galanthus nivalis* agglutinin (GNA) with a concomitant decrease in binding to RCA120 and *Phaseolus vulgaris* erythroagglutinin (PHA-E). In urine samples of patients with metastasis-positive BCa, three lectins, namely *Trichosanthes japonica* agglutinin I (TJA-I), RCA120 and *Bauhinia purpurea* (BPL), showed significant increase in binding compared to the group without metastasis [117,118]. The authors also verified that significant increases in binding only took place in metastasis-negative urine samples. According to the authors, the tested lectins could cover most of the known human oligosaccharides and, therefore, have the potential to detect subtle differences, but is suggested that further research is needed to apply these molecules to detect BCa metastasis.

Guo and colleagues [119] mapped the *N*-glycans pattern of normal mammary epithelial cells (MCF10A) after contact with a conditioned medium of malignant cells (MDA-MB-231) using the technique of MS and lectin array. As a result, seven lectins had a higher affinity for MCF10A cells treated with MDA-MB-231 medium; among them, the *Lycopersicon esculentum* lectin (LEL), that recognizes poly-LacNAc structures and GlcNAc, the *Solanum tuberosum* lectin (STL), that recognizes the GlcNAc oligomers and the *Psophocarpus tetragonolobus* lectin (PTL-II), that recognizes Gal-containing structures. However, sixteen other lectins had a lower affinity, among them the *Sophora japonica* lectin (SJA), which recognizes GalNAc and Gal terminal structures and the *Aleuria aurantia* lectin (AAL) that recognizes fucosylated structures. Furthermore, the MALDI-TOF/TOF-MS results showed that proportions of complex, hybrid and multi-antennary *N*-glycans were higher in MCF10A treated with MDA-MB-231 medium in comparison with the untreated cells. In addition, high-mannose-type glycans were less expressed in cells treated with MDA-MB-231 medium than in the control group. Following the same methodology, Tan et al. verified aberrant glycosylation patterns of MCF7 and MDA-MB-231 cell lines in a hypoxia-induced EMT model. The authors observed a reduction in levels of GlcNAc structures bisection and deregulation of the corresponding enzyme expression (MGAT3) [120].

To evaluate the glycopatterns of human saliva to find possible biomarkers for diagnosis of early-stage BCa, Liu et al. [121] applied the lectin microarray and blotting analysis in samples from 337 patients previously diagnosed with stage I/II BCa or benign BCa and compared to samples from 110 healthy people. As a final result, they built a diagnostic model based on nine lectins from a battery of 37 with different binding preferences. According to

the double-blind test results, the stage I BCa diagnostic model had a high accuracy of 0.902. Thus, this study shows that saliva glycosylation patterns revealed by lectin microarray can be used to screen patients with early-stage BCa based on the precise changes in those patterns.

3.2. Affinity chromatography

Based on their ability to bind carbohydrates, lectins can be immobilized on chromatographic matrices for glycoprotein enrichment. In the Lectin Affinity Chromatography (LAC), a lectin is covalently linked to a polymeric matrix (e.g., agarose or Sepharose). Similarly, in a Multi-Lectin Affinity Chomatography (MLAC), several lectins with different specificities can be immobilized on the same matrix and bind to several different targets. The steps for using these matrices involve (1) binding of glycoproteins through their glycans to the carbohydrate-recognition domain (CRD) of immobilized lectin(s), (2) washing the matrix to elute unbound material, and (3) elution of glycoproteins retained by competition through the use of the specific carbohydrate of each lectin, or changing the conditions of the elution solution, such as the pH or the ionic strength [101,122].

This strategy has been used for some time to detect glycosylated biomarkers in BCa. A frutalin-sepharose matrix was employed in a proteomics approach which involved the enrichment of plasma glycoproteins from BCa patients. The plasma proteome profile of three different stages of the disease was compared with the profile of healthy patients. After the fraction's enrichment, samples were analyzed by mass spectrometry. Retinol-binding protein, apolipoprotein A-II, apolipoprotein C-III, serum paraoxonase/arylesterase, alpha-1-acid glycoprotein, clusterin and protein C4b_B were differentially expressed and potentially associated with BCa progression [123]. ConA-immobilized LAC in combination with 2D-PAGE were used to compare the secretome glycoprotein profiles from MCF-7 cancer cells with and healthy HMEpC cells and differences were noted in levels of HSC70, haptoglobin, α-1-antitrypsin and carboxypeptidase CPA4 in the medium of MCF-7 cells [124,125].

The development of MLAC to study the glycoproteome of target cells is facilitating the search for biomarkers for various types of cancer [126,127]. A study using the MLAC platform containing wheat germ agglutinin (WGA), JAC and concanavalin A (ConA), lectins with specificity for NeuNAc/GlcNAc, Gal and Man/Glc, respectively, identified several proteins as potential biomarkers in the serum from BCa patients, including tenascin-X, serum amyloid P component, alpha-1B-glycoprotein, thrombospondin-1 and 5 [128]. ConA and WGA were also used in the search for biomarkers among non-tumor mammary cell lines, such as MCF-10A, and tumor cell lines such as

MDA-MB-231 and MCF-7. As a result, 82 proteins retained by MLAC showed higher expression in MDA-MB-231 and MCF-7 in comparison with MCF-10A [129].

Another type of MLAC containing ConA, WGA and *Sambucus nigra* agglutinin (SNA) resulted in the identification of 45 different proteins in the serum of sick patients only [130]. ConA and WGA were applied in another study to assess BCa progression in which the glycoproteomic profile of premalignant and malignant cells with metastatic potential was evaluated. Lectins were covalently linked to agarose and packed into spin columns. The membrane fraction was submitted to chromatography and the retained fraction analyzed by MS. Consequently, 27 membrane glycoproteins with different expression levels between the two strains were identified and, in special, γ-glutamyl hydrolase, CD44, syndecan-1 and galectin-3 binding protein, which were considered biomarkers of malignant cells [131].

AAL and SNA lectins were used in a LAC-MS approach to determine the glycoproteome of 10 BCa cell lines, 5 luminal cell lines and 5 triple-negative cell lines, in which 1011 glycosylation sites for 533 glycoproteins were identified, 100 of which were specific for triple-negative cancer [132]. With a more optimized approach, another study used MLAC, but with three columns containing a different lectin immobilized in each and then used in sequence for glycoprotein enrichment of serum from BCa patients and healthy patients. ConA, WGA and RCA-1 were used in various settings to evaluate the sequence with the best yield. The arrangement in which WGA was used first, then ConA and RCA-I, proved to be the best configuration. This effort resulted in the detection of 23 proteins, with different degrees of expression in the serum of sick and healthy patients [133].

3.3. Histochemistry/cytochemistry

Lectins have long been considered useful tools for the study of glycomics and glycoproteomics and can be used alone or in conjunction with other methods based on their wide availability, affinity and relative specificity [127]. Lectin-based approaches focused on histochemistry/cytochemistry are applied for the specific labeling of glycans, to increase the understanding of aberrant glycosylation and carcinogenesis, as well as aiding in biomarker recognition and quantitative detection [81]. Some BCa-specific studies are reported in the literature, citing lectins as useful tools in glycobiology during carcinogenesis.

Carvalho et al. [134], applied conjugates of QDs and Cramoll, a lectin from the plant *Cratylia mollis*, in healthy breast tissues, tissues with fibroadenoma (FB) and tissues with invasive ductal carcinoma (IDC). Using this

technique, the researchers targeted and evaluated the differences in labeling in normal and transformed tissues. QDs-Cramoll conjugates revealed differences in labeling patterns between FB and IDC with differences of 200% and 300% in the fluorescence signal in comparison to the control group (without labeling). Previous studies with ConA and *Ulex europaeus* agglutinin-I (UEA I) conjugated with QDs already reported this difference in binding between FB and IDC tissues. The results of both conjugates showed that QDs-ConA had greater specificity for the stroma and QDs-UEA I for the ductal cells. The authors pointed out that the distribution of D-glucose/mannose and L-fucose in tissues relates to the possible expression of glycosyltransferases and glucosidases [135]. L-fucose has been previously described as predominant in cases of malignant phenotype in BCa, where it is associated with several glycosylated biomolecules involved in various stages of cancer progression and a target of several experimental therapies useful in the treatment. Furthermore, it has been reported to be fundamental in the construction of the malignant and metastatic phenotype of several human BCa [56]. Further studies involving histochemical analysis in BCa with ConA and Cramoll conjugated with peroxidase and ConA and peanut agglutinin (PNA) conjugated with acridinium ester resulted in significant differences between the tissues of IDC and FB tissues [135–137].

Pally and collaborators [146] investigated the sialic acid expression on the cancer cells surface within growing tumors through a combination of lectin-based flow cytometry technique, lectin cytochemistry, histochemistry and glycomic analysis using mass spectrometry. Histochemical tests with FITC-conjugated SNA and TRITC-conjugated *Maackia amurensis* lectin (MAA) probed α2,6- and α2,3-Sial, respectively. The results showed heterogeneity in the α2,6-Sia and uniform levels of α2,3-Sia in tissue samples from invasive human BCa and MDA-MB-23 cells. The authors also showed that average levels of α2,6-Sia in cells are related to a better adhesion to the collagenous and laminin-rich extracellular matrix (ECM) and, consequently, a higher migration velocity in comparison to cells with high levels of 2,6-Sia. Cells with excellent adhesion to the ECM can better invade a fibrillar environment similar to the surrounding stroma, facilitating the efficient migration of a malignant BCa [138].

Several studies report on the use of plant lectins as biomarkers for benign and malignant BCa. For example, Rego et al. [139] applied ConA, UEA-I, WGA and PNA to evaluate differences in glycan expression of ductal carcinoma cells under hypoxic and non-hypoxic conditions. Dos Santos et al. [140] evaluated the relationship between the expression of tumor-associated carbohydrates and well-established prognostic factors in patients with invasive ductal carcinoma (IDC) of the breast using SNA, MAL-II, and PHL. With those same lectins, they compared it with ductal carcinoma in situ (DCIS) to test for differences between premalignant and malignant lesions. The results

proved to be significant and reproducible. Other studies applying fluorescein isothiocyanate (FITC)-labelled lectins show significant differences in the glycosylation pattern between MCF-7 and MDA-MB-231 cell lines [141]. Also, Zhou et al. [116] showed that RCA-I lectin specifically binds to cell surface glycans associated with metastasis in triple-negative BCa. In these studies, all lectin-labeling in cancer tissues were reversed upon the immobilization of lectins with their respective specific ligands, demonstrating that the carbohydrate-binding is essential to the effect. In addition, research involving lectin binding to tissues or cell lines in studies of antiproliferative/cytotoxic effects were carried out through the labeling of lectins [81].

Considering the data presented in sections 3.1, 3.2 and 3.3, one can conclude that, not surprisingly, the application of lectins in BCa studies depends on the protein specificity and CRD accessibility to substructures present on BCa-specific glycans. The most applied lectins in BCa studies are mannose-specific lectins of the ConA-like type, especially ConA and Cramoll, followed by lectins that recognize poly-LacNAc structures, which have an abundance of galactose residues, such as RCA-I, PNA, and jacalin. Additionally, lectins extracted from *Bauhinia* plants are promising considering their specificity towards Tn-related glycan structures, WGA and UEA-I which are able to recognize fucose residues in BCa glycosylations and SNA, a galactose/lactose specific lectin which also interacts with sialic acid linked to terminal galactose at α-2,6 bond. These lectins are the most promising for the recognition of glycosylated patterns present in BCa cells possessing several applications that were described in this review. However, new lectins are constantly being discovered, and even lectins with closely related specificities can demonstrate interesting outcomes due to their different binding properties caused by variations in their binding sites composition and fold.

4. Lectin-based BCa therapeutic prospects

In addition to the application of plant lectins in the diagnosis of BCa, robust studies show that lectins can act as an antitumor agent for some types of cancer [24,99,142,143]. Studies reporting on the antiproliferative effect of lectins against BCa are still preliminary, and further research is still needed, but the current data allows for a glimpse within the mechanism by which lectins can act as antiproliferative agents.

The mechanism of action by which plant lectins induce cell death is suggested to be partly due to induction of apoptosis by stimulation of caspases-dependent pathways whereby mitochondria secrete ROS thus activating the SEK/JNK caspase pathway [144,145]. A second possibility is a caspase-independent pathway by which

the lectin binds to a glycosylated receptor attached to the cancer cell membrane. Through this last route, there is a reduction in the cell growth by generating large amounts of ROS and triggering the activation of cancer suppressor proteins p38 and p53 [146,147].

As mentioned, in addition to recognizing the glycosylation patterns of tumor cells and determining the degrees of malignancy, lectins can also interfere with cancer physiology by inducing cell death [148]. For example, ML lectin, extracted from mistletoe, can induce apoptosis in different cancer strains (*in vitro*) in addition to considerably reducing the size of several tumors in animal models. ML is suggested to be a strong inhibitor of telomerase and Bcl-2 while also ML upregulating the pro-apoptotic protein Bax (p53- and p21-independent pathway) in hepatoma cells [149]. Similar processes were confirmed using ConA and the lectin from *Polygonatum cyrtonema*, both of which can induce mitochondrial autophagy after internalization in the tumor cells [150,151]. In BCa, ConA was also shown to induce apoptosis by causing mitochondrial transmembrane potential collapse, cytochrome c release, and caspase activation. ConA-based anticancer agents have already entered preclinical trials [152]. Other examples of antiproliferative effects achieved through necrosis, anti-angiogenesis and immunomodulation [24].

Not all studies of lectins with cytotoxic effects on BCa cells report direct involvement of the CRD. For different types of cancer, the CRD is reported to be involved in antiproliferative effects in the following lectins: *Abelmoschus esulentus* lectin (AEL), *Dioscorea opposita* lectin (DOL), *Liparis noversa* lectin (LNL), *Ophiopogon japonicus* lectin (OJL), *Polygonatum cyrtonema* lectin (PCL), *Polygonatum obtusifolium* lectin (GPL), *Remusatia vivipara* (RVL) and *Typhonium divaricatum* lectin (TDL). Among these, only DOL did not show reduction in the antiproliferative activity by previous incubation with the specific carbohydrate. The authors raised the hypothesis that other (non-lectin) domains of the protein interacted with tumor cells [143]. It is also worth considering that the specificity of lectins varies significantly with the type of carbohydrate structure (from simple to complex) in the millimolar to nanomolar range [153].

As previously described, studies of lectins in BCa are still preliminary. Most experiments have been carried out *in vitro*, using MCF7 cells. The MCF-7 cell line has been used for more than 40 years to perform BCa research [154]. For this same cell line, the IC_{50} of lectins, in part, ranged from 0.18 to 327 μg/ml / 24 h for BVL and WFL, respectively. This shows that the cytotoxic effects of lectins can be quite different. It should be noted that other measurement units were used with corresponding variation in measurement time (24 to 72 h) and methodology. Other BCa cell lines have been used to test the cytotoxicity of lectins (Table 1). Some review papers demonstrate the diversity

of plant lectins with their potential biological activities and the purification processes and structural characterization [155–159].

4.1 Proposed anticancer mechanism of lectins

Although a precise mechanism of action of lectin activity has not been completely elucidated experimentally, there is sufficient data to propose a pathway. We also describe a possible mode of action of plant lectins through interaction with glycosylated membrane receptors. As shown in Figure 4, the receptor-like protein, tyrosine phosphatase alpha (RPTPα or PTPα), a type I transmembrane glycoprotein with complex *N*-glycans, was identified as a substrate of GnT-V. It is interesting that the addition of b1,6 GlcNAc branches on PTPα enhanced its cytomembrane assembly in MCF-7 cells transfected with GnT-V. Thus, glycosylation appears to reduce the degradation of PTPα, promoting cell-surface retention and enhanced catalytic activity of PTPα, followed by Src activation via dephosphorylation of Src kinase at Tyr529. Taken collectively, this cascade of events could promote MCF-7 cell migration, suggesting PTPα as a factor regulating migration of BCa cells [204]. Moreover, PTPα may regulate membrane type 1-matrix metalloproteinase (MT1-MMP; MMP14) localization to plasma membrane protrusions, suggesting a role for PTPα in intracellular trafficking of MT1-MMP. Importantly, MDA-MB-231 tumors depleted in PTPα exhibit reduced ECM degradation with reduced invasion into the surrounding mammary fat pad [205]. In spite of all these indications, no direct studies show modulation of PTPα by plant lectins. However, in MDA-MB-231 human breast carcinoma cells, it is important to note that ConA induced MMP-2-activation by upregulation of MT1-MMP expression via transcriptional and nontranscriptional mechanisms [206], including MT1-MMP membrane localization [207]. Additionally, it was found that ConA-induced MT1-MMP expression and MMP2 activation are dependent on tyrosine phosphorylation and clustering effects of ConA [208]. In the same cells, it is notable that agents which increased cAMP levels also abrogated ConA induction of MT1-MMP mRNA and protein in parallel with MMP-2 activation [209], while increased intracellular Ca^{2+} caused a dose-dependent inhibition of ConA-induced MMP-2 activation with no effect on MT1-MMP mRNA levels [210]. When taken together, these results give a robust indication of the lectin's capacity to modulate the intricate pathways that orchestrate cell adhesion, matrix remodeling and BCa cell migration via glycan binding of target glycoproteins not yet well identified.

A further target that could be modulated by lectins involves matrix metalloproteinase 1 (MMP-1) and protease-activated receptor 1 (PAR1) signaling pathway. PAR1, a G protein–coupled receptor activated by proteolytic

cleavage of its extracellular domain is not expressed in normal breast epithelia, but is upregulated in invasive breast carcinomas [211,212]. PAR1 is both required and sufficient to promote growth and invasion of breast carcinoma cells, and MMP-1 functions as a protease agonist of PAR1, cleaving the receptor at the proper site to generate PAR1-dependent signals, such as Akt activation, which are involved in cell survival, proliferation, and migration [211,212]. Moreover, it was demonstrated that NF-κB signaling contributes to interstitial collagenase (MMP1) expression in MDA-MB231 breast cancer cells, causing PAR1 activation in adjacent lymph endothelial cells [213]. This signaling promotes formation of large cell-free areas in the lymph endothelium, so called "circular chemorepellent induced defects" (CCIDs), allowing tumoral cells to penetrate the vasculature and producing metastasis [213]. All these observations are reinforced by a recent study [214] indicating that MDA-MB-231-HM cells can secrete exosomes enriched with MMP1, which can be taken up and enhance invasion and migration activities of triple-negative breast cancer cells, such as MDA-MB-231, MDA-MB-468 and BT549. Noteworthy, after uptake of exosomes cells secrete more MMP-1, which via PAR1 activation promotes epithelial-mesenchymal transition (EMT) enhancing capability of migration and invasion. Clinically, the enrichment of MMP-1 has been detected in exosomes extracted from serum of patients with metastasis. Hence, MMP1 may be a key protein to mediate the transfer of metastatic ability between tumor cells through exosomes [214] and it has been suggested prioritizing this MMP as a candidate for development of therapeutic strategies [214,215]. MMP1 is a glycoprotein that displays differences in the glycan expressed in tumoral cells as compared to non-tumoral [216]. Notably, the interaction of plant lectins, such as ConBr and ConGF, with the MMP1 glycans has been demonstrated [217,218] and could be involved in the effects triggered by the lectins, such as inhibition of Akt signaling, induction of autophagy, as well as blockage of cell migration. Hence, plant lectins could be applied for identification or to counteract cell invasion via interaction with the MMP1 glycans expressed by BCa cells, see Figure 5.

Moreover, the cell fate in response to glycosylation and underlying signaling mechanisms may be target-specific, since overexpression of the glycosyltransferase MGAT3 in breast cancer MDA-MB-231 cells significantly enhanced the bisecting N-GlcNAc on EGFR which further resulted inhibition of malignant phenotype of BCa via downregulation of EGFR/Erk signaling [219].

4.2 Synergistic effect of lectins applied to BCa treatment

One of the ways to treat BCa is through chemotherapy, and multidrug resistance is one of the main obstacles to its success [220]. In an attempt to reverse this process, lectins also play a role in BCa treatment by their association with other drugs. For example, anti-daunorubicin-resistant liposomes associated with WGA have been developed to bypass multidrug resistance of MCF-7 and MCF-7/ADR cells. *In vitro* tests demonstrated good intracellular uptake of the liposomes and reduction of resistance through P-gp inhibition, as both WGA-modified daunorubicin liposomes and WGA-modified anti-daunorubicin liposomes strongly induced apoptosis of MCF-7/ADR cells compared to free daunorubicin. Furthermore, *in vivo* results revealed prolonged residence of the liposomes on the circulatory system that could remarkably accumulate at the tumor site. The anticancer efficacy was proven to be caused by activation of the pro-apoptotic proteins Bax and Bok and caspases 8, 9 and 3 [221].

Studies by Hong and collaborators [222] showed the synergistic effects of the *Viscum album var. coloratum* agglutinin (VCA) with doxorubicin (DOX) on MCF-7 and MDA-MB23. The combination of the lectin and the drug was more effective in apoptosis-induction than VCA and DOX separately. Quantitative flow cytometry analysis showed increased expression of pro-apoptotic proteins in both cell lines and inhibition of Bcl-2 in MCF-7. There was also a marked difference in cell cycle progression.

Knowing that the effectiveness of drugs in the treatment of BCa is related to a good bioavailability, studies carried out by Liu et al. [223] through the use of WGA coated with pHPMA, (oral nanocarrier) together with silibinin and cryptotanshinone demonstrated a strong toxic activity in 4T1 cells, with inhibition of invasion and migration of these cells, making these oral drug nanocarriers promising in inhibiting lung metastasis of BCa. More studies on the synergistic effects of plant lectins with new or classically used drugs in the treatment of BCa are needed, considering the high potential of this approach.

5. New applications and perspectives

In view of the latest discoveries in the studies of lectins as tools in the fight against different types of cancer, diagnostic technologies based on biosensors for early detection of various types of cancer have been gaining considerable attention due to their advantages over existing diagnostic techniques, which include noninvasive nature, cost-effectiveness, easy interpretation of results, and multiplexing capability [84]. Lectin biosensors can act by recognizing antigens produced by cancer cells and specific glycans present on the cell surface. For example, a biosensor

for pancreatic cancer involves the conjugation of SNA, ConA and AAL lectins with janus nanoparticles (JNP) [224]. To detect BCa, PNA and SNA were conjugated with fluorine microspheres to form a sandwich between antibody and lectin. This assay involves immobilizing the specific antibody CA15-3 on the detection surface. As a result, even when applied in small amounts, PNA and SNA showed significant sensitivity, speed of response and reproducibility, thus characterizing these lectins as important tools for quantitative analysis of CA 15-3 and glycomarkers used in clinical applications in BCa [95].

Another application of lectins in early cancer detection involves targeted drug delivery [82]. Assays with fluorescein-labeled *Triticum vulgare* and UEA I lectins showed a stronger interaction on the membrane surface in regions with large amounts of *N*-acetyl-D-glucosamine, sialic acid and α-L-fucose residues. These findings have provided an important resource for studies involving the targeted delivery of drugs to bladder cancer cells. The development of drug delivery methods leads to decreased toxicity, increased exposure time and greater efficacy [225]. In recent experiments, it was observed that two H-type lectins, *Helix pomatia* agglutinin (HPA) and *H. aspersa* agglutinin (HAA), could specifically identify non-standard glycosylated forms of proteins expressed by cancer contain GalNAc. Likewise, these two lectins present themselves as two possible tools to aid in the diagnosis of carcinogenesis and as components of drug delivery systems [226].

The use of therapeutic nanoparticles associated with biomolecules capable of identifying cancer may allow the selective isolation of tumors and targeted treatment. As shown in the studies by Obaid et al. [227], where water-soluble gold nanoparticles (GNP) were stabilized with a mixed monolayer of a hydrophobic zinc phthalocyanine photosensitizer (C11Pc) and hydrophilic polyethylene glycol (PEG) to obtain C11Pc-PEG-GNP. To examine the effects of the conjugate, the group analyzed its interaction with jacalin, a lectin with specificity for the TF-antigen. In additional experiments, the same GNP was conjugated to a specific human epidermal growth factor receptor-2 (HER-2). It was observed that both forms of conjugated GNP showed phototoxicity against colorectal adenocarcinoma HT29 cells and mammary adenocarcinoma SK-BR-3 cells. Showing a greater effect against mammary adenocarcinoma (> 99%) than colorectal adenocarcinoma (80-90%). In addition, GNP-based treatment strategies have been developed to ensure better quality of life and survival time for BCa patients [228].

Conclusion

As shown in the current work, plant lectins can represent an important tool against BCa. The literature reports on the potential of these biomolecules for diagnosis, biomarker search and drug delivery through specific recognition of glycans found in malignant cells. The glycosylation analysis is emerging as an important factor for understanding important processes such as tumor progression and metastasis. In addition, some lectins demonstrate a direct antiproliferative activity essentially by inducing programmed cell death processes. Overall, the potential of plant lectins is undeniable, the application of lectins in BCa research depends on the protein specificity, with the most promising one apparently being mannose-specific lectins, lectins that recognize poly-LacNAc structures, and those that can recognize fucose and Neu5Ac-specific. Although lots of data has been generated about the application of lectin in breast cancer, the current state of research is lacking and needs to be advanced further to validate their effects and generate valuable biotechnological tools.

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Figure legend

Figure 1. Causes and factors associated with breast cancer (BCa) and its stages. BCa starts in the breast lobules and ducts progressing with the increase of malign cells to the most advanced and invasive stage (metastasis). A high percentage of BCa cases result from environmental and lifestyle factors.

Figure 2. Importance of plant lectins to biomedical research. Several lectins have diverse activities against breast cancer; peanut agglutinin (PNA) is one of them.

Figure 3. Representation of glycosylation patterns in animal cells. These patterns are altered in breast cancer cells. The process is carried out by glycosyltransferases, starting at the rough endoplasmic reticulum (RER) (1) and ending at the golgi complex (GC) (2), and then directed to different locations in the cell.

Figure 4. Mechanism of Modulation of membrane type 1-matrix metalloproteinase (MT1-MMP) in breast cancer (BCa) Cells. (1) tyrosine phosphatase alpha (PTPα) acts as a substrate of N-acetylglucosaminyltransferase V (GnT-V). Addition of b1,6 GlcNAc branches on PTPα promotes MCF-7 cell migration, suggesting that PTPα is a factor regulating migration of BCa cells. (2) PTPα may also regulate membrane type 1-matrix metalloproteinase (MT1-MMP; MMP14), suggesting a role for PTPα in intracellular trafficking of MT1-MMP. MDA-MB-231 tumors depleted in PTPα exhibit reduced extracellular matrix (ECM) degradation with reduced invasion into the surrounding mammary fat pad. (3) ConA induced MMP-2-activation by upregulation of MT1-MMP expression via transcriptional and nontranscriptional mechanisms, including MT1-MMP membrane localization. Taken together, the capability of lectins

to modulate the intricate pathways that orchestrate cell adhesion is robustly indicated, as well as matrix remodeling and breast cancer cell migration via glycan binding of target glycoproteins not yet well identified.

Figure 5. Scheme depicting how interstitial collagenase (MMP1) can trigger cell proliferation and invasion via protease activated receptor 1 (PAR1) signaling and how plant lectins could counteract this pathway. Increased expression of MMP1 and PAR1 in tumor tissue are related with malignancy of breast cancer. PAR1, a G-protein coupled receptors, is activated by MMP1 and its downstream signaling pathway triggers tumor cell proliferation, invasion, and migration. Noteworthy, plant lectins via interaction with the glycan expressed on MMP1 could be applied to counteract this effect.

Table 1. Main lectins with cytotoxic activity in cell lines several of BCa.

Species/Lectin	Specificity	Cells lines	IC_{50}	Action	Method(s)	Ref.
Abelmoschus esulentus (AEL)[c]	Lactose	MCF7	100 µg/ml / 72 h	Antiproliferative: apoptosis.	• Antiproliferative activity (MTT); • Gene expression profile; • Flow cytometric analysis.	[160,161]
Abrus precatorius (AGG)	Galactose	MDA-MB-231, MDA-MB-157, MCF-7 e T47D	10 - 100 µg/ml / 72 h	Antiproliferative: apoptosis and antiangiogenic.	• Immunohistochemical analysis; • In vitro wound-healing, Endothelial cell invasion, Capillary-like tube formation, Caspase assays and Chorioallantoic membrane (CAM) assays; • Human angiogenesis protein array; • Western blotting; • ELISA for IGFBP-2.	[162–164]
Bauhinia forficata (BfL)	GalNAc	MCF7	10 µg/ml / 72 h	Antiproliferative: apoptosis and necrosis (caspase-9 inhibition).	• Antiproliferative activity (MTT); • Cell adhesion and Cell death assay; • Cell cycle and DNA fragmentation; • Determination of caspase-9 activation; • Western blotting.	[165,166]
Bauhinia purpurea (BPL)	GalNAc, Lactose, Melibiose and Galactose	MCF7	446 µg/ml / 24 h	Antiproliferative: loss in cell viability, increased LDH release and ROS, Cell cycle arrest and apoptosis	• Antiproliferative activity (MTT); • Lactate Dehydrogenase Leakage Assay; • Reactive Oxygen Species Assay (ROS Assay); • Assay of Caspase-3 Activity; • Cell Cycle Analysis.	[167,168]
Bauhinia variegata (BVL)	Melibiose, Galactose, GlcA and Lactose	MCF7	0.18 µg/ml / 24 h	Antiproliferative	• Antiproliferative activity (methyl-3H) thymidine labeling and radiometric quantification	[169]
Canavalia brasiliensis	Mannose,	MCF7	1146 nM /	Antiproliferative:	• Antiproliferative activity	[170,171]

(ConBr)	glucose, and fructose		24 h	apoptosis	(sulforhodamine B or SRB).	
Canavalia ensiformis (ConA)	Mannose and glucose	MCF7	15 µg/mL / 24 h	Antiproliferative: apoptosis without affecting control cells.	• WST-1 and cell counting kit-8 (CCK-8) assays; • Cell morphology changes; • Cell cycle; • Caspase assay; • Western blot analysis.	[172,173]
Canavalia maritima (ConM)	Mannose, glucose, maltose and threalose	MCF7	1382 nM / 24 h	Antiproliferative: apoptosis	• Antiproliferative activity (sulforhodamine B or SRB).	[170,174]
Clematis montana (CML)	Mannose	MCF7	1 - 10 pM / 24 h	Antiproliferative: apoptosis and necrosis.	• Antiproliferative activity (MTT); • Flow Cytometry Analysis.	[175]
Dioclea lasiocarpa (DLasiL)	Glucose and mannose	MCF7	275 nM / 24 h	Antiproliferative: apoptosis	• Antiproliferative activity (sulforhodamine B or SRB).	[170,176]
Dioclea sclerocarpa (DSclerL)	Galactose and glucose	MCF7	1250 nM / 24 h	Antiproliferative: apoptosis	• Antiproliferative activity (sulforhodamine B or SRB).	[170,177]
Dioscorea opposita (DOL)	Galactose	MCF7	3,71 μM / 24 h	Antiproliferative: through the externalization of phosphatidylserine and consequently apoptosis.	• Antiproliferative activity (MTT); • Flow Cytometry Analysis.	[178]
Euphorbia tirucalli (ETL)	Lactose and Galactose	MDA-MD-231 and MCF-7	>100 µg/mL / 18 h	Antiproliferative: apoptosis. #	• Antiproliferative activity (MTT)	[179,180]
Glycine max (SBA)	β-N-acetylgalactosamine and galactopyranosyl	MCF7 and MDA-MD-231	2,6 - 40 μM / 72 h	Antiproliferative: The action of the lectins on tumor	• Antiproliferative activity (MTT) and (methyl-3H) thymidine labeling and radiometric quantification;	[181,182]

				cell membranes; the reduction of tumor cell proliferation, the induction of tumor-specific cytotoxicity of macrophages, and apoptosis.	• Reactive Oxygen Species Assay (ROS Assay); • Fluorescence microscopy; • Caspases (3/7, 8 and 9) and apoptosis assay.	
		BT 20	>100 μg / mL / 48 h	Antiproliferative: apoptosis. #	• Colorimetric XTT-based assay kit	[183]
Liparis noversa (LNL)[c]	Mannose, N-acetyl glucosamine and thyroglobulin	MCF7	14 μg/mL / 24 h	Antiproliferative: apoptosis.	• Antiproliferative activity (MTT); • Electron microscopy.	[184,185]
Morus alba (MLL)	Galactose, galactosamine and N-acetyl galactosamine	MCF7	8.5 μg/mL / 24 h	Antiproliferative: apoptosis caspase-dependent ; morphological changes and DNA fragmentation.	• Antiproliferative activity (MTT); • Phase-contrast microscopy; • Apoptosis assay by acridine orange/ethidium bromide staining; • Annexin V Cy3 staining; • Cell cycle and caspase 3 analysis.	[186,187]
Musa acuminata (MAL)	Fructose and glucosamine	MCF7	16.5 μg/mL / 24 h	Antiproliferative: apoptosis and antiangiogenic.	• Antiproliferative activity (MTT); • In-vivo peritoneal angiogenesis and MVD evaluation in the peritoneum; • In-ovo Chorio-Allantoic Membrane (CAM) assay; • In-vitro and in-vivo apoptosis cell morphological studies.	[188,189]
Ophiopogon japonicus (OJL)[c]	Mannose and fructose	MCF7	22 μg/mL / 24 h	Antiproliferative: apoptosis.	• Antiproliferative activity (MTT); • Electron microscopy.	[184,190]

Phaseolus vulgaris (PHA)	N-acetylglucosamine and mannose	MCF7	2 μM / 48 h	Antiproliferative: disruption of the mitochondrial transmembrane potential and disorganization of the inner mitochondrial membrane were induced.	• Antiproliferative activity (MTT); • Annexin V and propidium iodide staining; • Annexin V and propidium iodide staining of Z-IETD-FMK; • Measurement of mitochondrial transmembrane potential by JC-1 staining; • Cell cycle analysis; • DNA fragmentation detection; • Western-blot analysis.	[191–193]
Phaseolus vulgaris (EAPL)	Galactose	MCF7	172 μM / 48 h	Antiproliferative: apoptosis.	• Antiproliferative activity (MTT); • Microscopy; • Reactive Oxygen Species Assay (ROS Assay).	[194]
Polygonatum cyrtonema (PCL)[c]	Mannose	MCF7	~39 μg/mL / 24 h	Antiproliferative: apoptosis.	• Antiproliferative activity (MTT); • Electron microscopy.	[184]
Polygonatum obtusifolium (GPL)[c]	Mannose	-	100 μg / mL / 48 h	Antiproliferative	• MTT assay	[195]
Polygonatum odoratum (POL)	Mannose	MCF7	10 μg/mL / 24 h	Antiproliferative: apoptosis and autophagy.	• Antiproliferative activity (MTT); • Electron microscopy; • Flow cytometry; • Western blot analysis.	[196,197]
Remusatia vivipara (RVL)[c]	Mannose	MDA-MB-468 and MCF-7	7.2 - 40 μg/mL / 24 - 48 h	Antiproliferative: apoptosis; motility, and invasiveness reduced; antiangiogenic	• Binding of RVL to cells; • MTT assay; • Cells morphology; • Analysis of Cell cycle, ROS and mitochondrial membrane potential; • Chorioallantoic membrane (CAM) assay.	[198]

Sophora flavescens (SFL)	Mannose	MCF7	20 µg/mL / 24 h	Antiproliferative: apoptosis without affecting control cells.	• WST-1 and cell counting kit-8 (CCK-8) assays; • Cell morphology changes; • Cell cycle; • Caspase assay; • Western blot analysis.	[172]
Typhonium divaricatum (TDL)[c]	Mannose	Bre-04	100 µg / mL / 48 h	Antiproliferative: apoptosis.	• Antiproliferative activity (MTT); • Electron microscopy.	[199]
Triticum aestivum (WGA)	N-acetylglucosamine	MCF-7, T47D, BT 20	2 - 70 µg / mL / 48 h	Antiproliferative: apoptosis. #	• Colorimetric XTT-based assay kit	[183,200]
Viscum album (ML-I)[b]	Galactose	MAXF 401NL	0.6 - 0.03 mg/mL / 48 h	Antiproliferative: apoptosis, necrosis, immunomodulatory and anti-angiogenic properties. #	• Antiproliferative activity (MTT)	[201,202]
Wisteria floribunda (WFL)	N-acetylgalactosamine	MCF7	327 µg/ml / 24 h	Antiproliferative: loss in cell viability, increased LDH release and ROS, Cell cycle arrest and apoptosis	• Antiproliferative activity (MTT); • Lactate Dehydrogenase Leakage Assay; • Reactive Oxygen Species Assay (ROS Assay); • Assay of Caspase-3 Activity; • Cell Cycle Analysis.	[167,203]

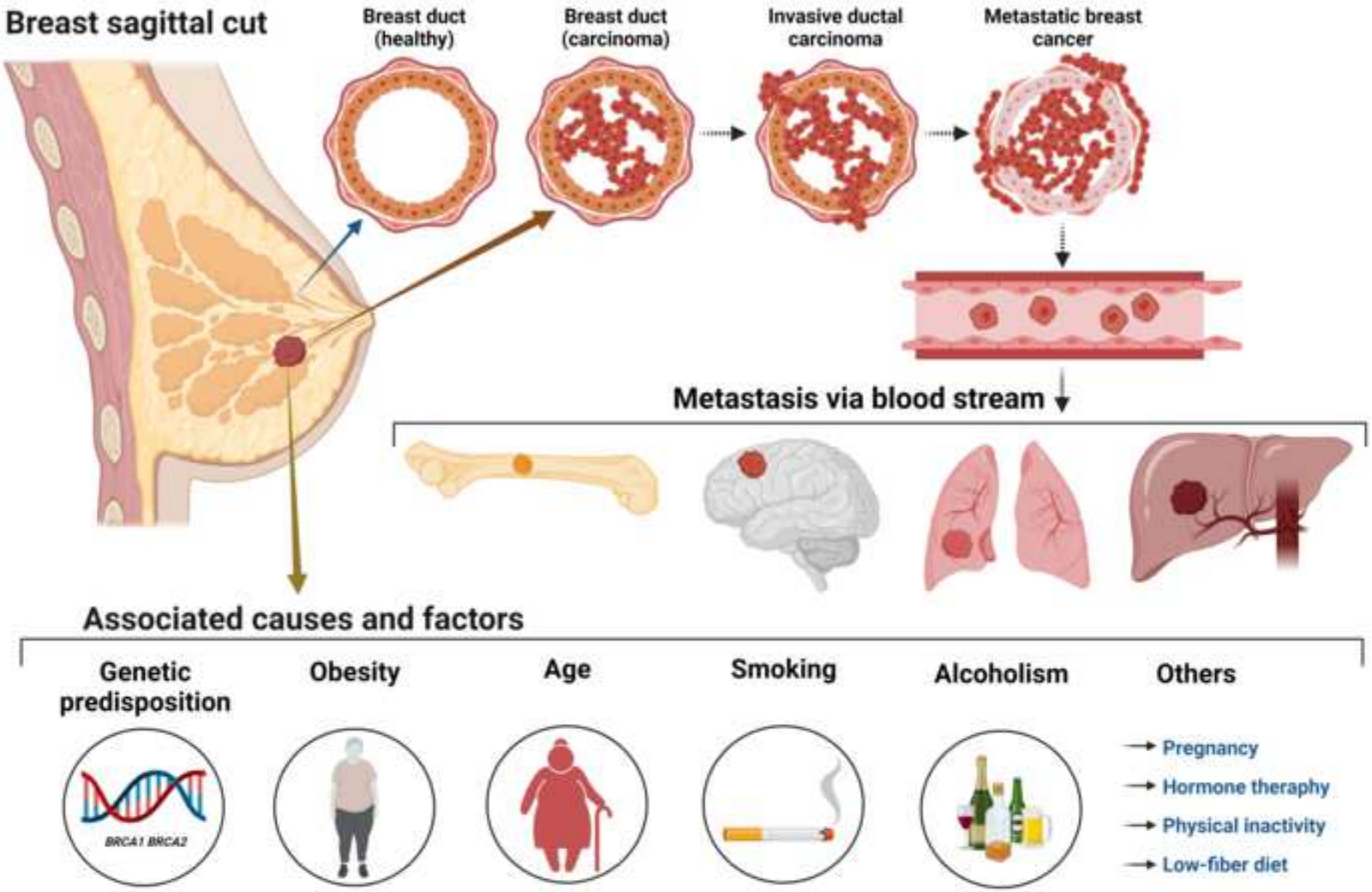
[a]In the studies by Lam and Ng (2010) the concentration of 5 µg/ml was not toxic for MCF7.

[b]*Viscum album* preparations (VAPs) obtained from mistletoe growing on oak (*Quercus robur* and *Q. petraea*, VAP-Qu), apple tree (*Malus domestica*, VAP-M), pine (*Pinus sylvestris*, VAP-P) or white fir (*Abies pectinata*, VAP-A), on the in vitro growth of breast and bladder carcinoma cell lines.

[c]Biological effect related to the CRD of the lectin.

(-) Data not reported.

Figure 1


Breast sagittal cut
Breast duct (healthy)
Breast duct (carcinoma)
Invasive ductal carcinoma
Metastatic breast cancer
Metastasis via blood stream
Associated causes and factors
Genetic predisposition
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Obesity
Age
Smoking
Alcoholism
Others
Pregnancy
Hormone theraphy
Physical inactivity
Low-fiber diet

Figure 2

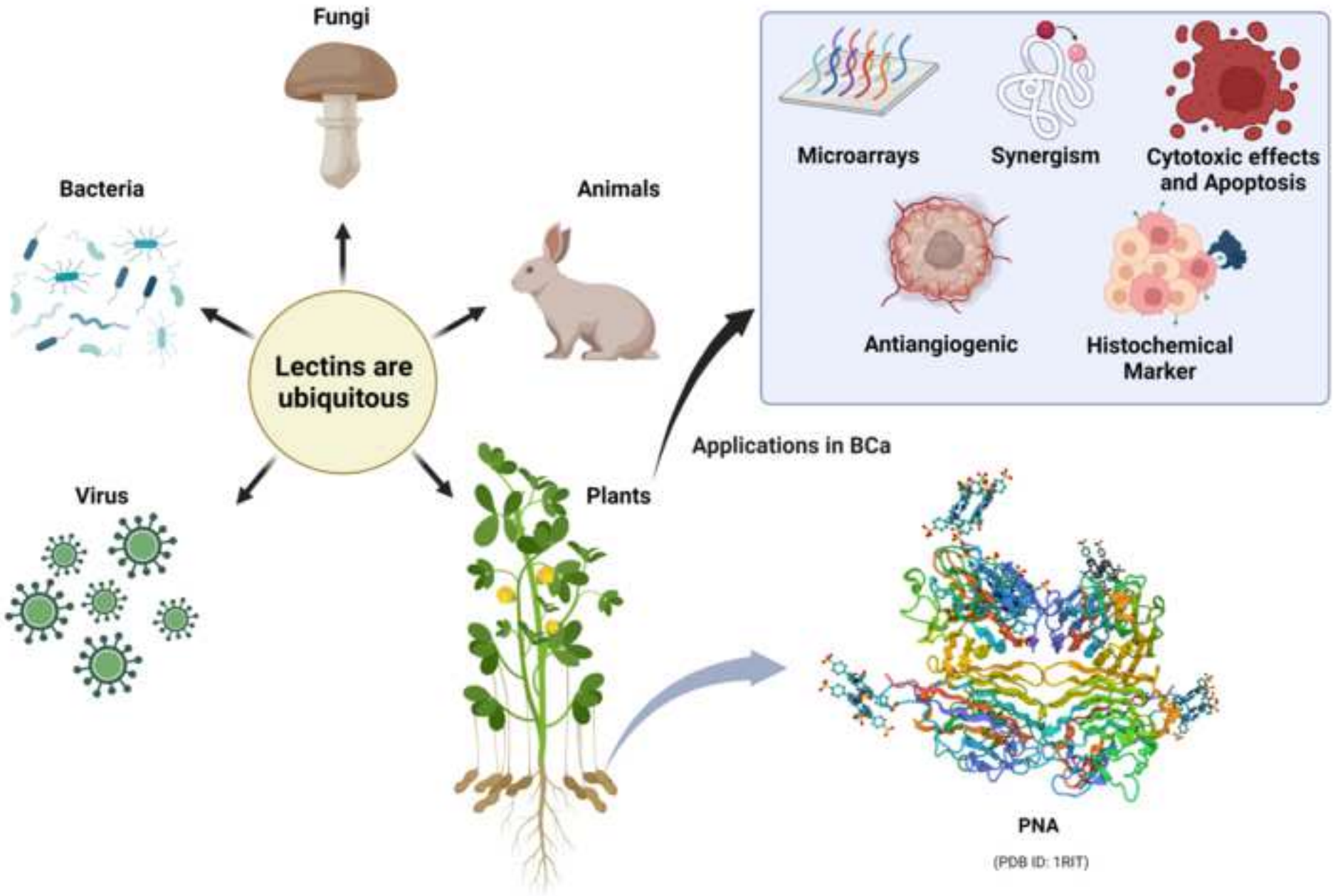
Fungi
Bacteria
Animals
Lectins are ubiquitous
Virus
Plants
Applications in BCa
Microarrays
Synergism
Cytotoxic effects and Apoptosis
Antiangiogenic
Histochemical Marker
PNA
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Figure 3

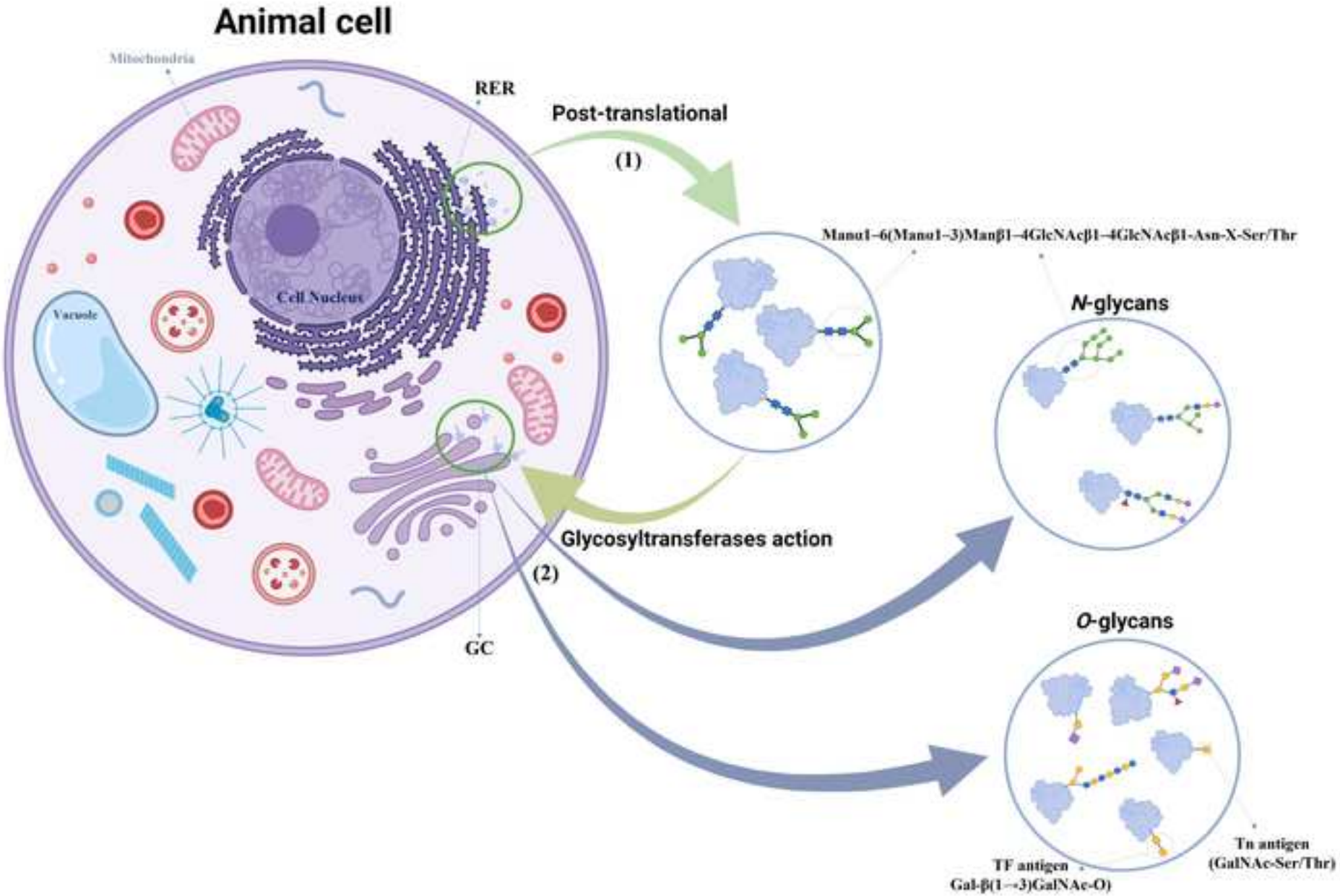

Animal cell
Mitochondria
RER
Post-translational
(1)
Cell Nucleus
Vacuole
Manα1–6(Manα1–3)Manβ1–4GlcNAcβ1–4GlcNAcβ1-Asn-X-Ser/Thr
N-glycans
Glycosyltransferases action
(2)
GC
O-glycans
TF antigen
Gal-β(1→3)GalNAc-O)
Tn antigen
(GalNAc-Ser/Thr)

Figure 4

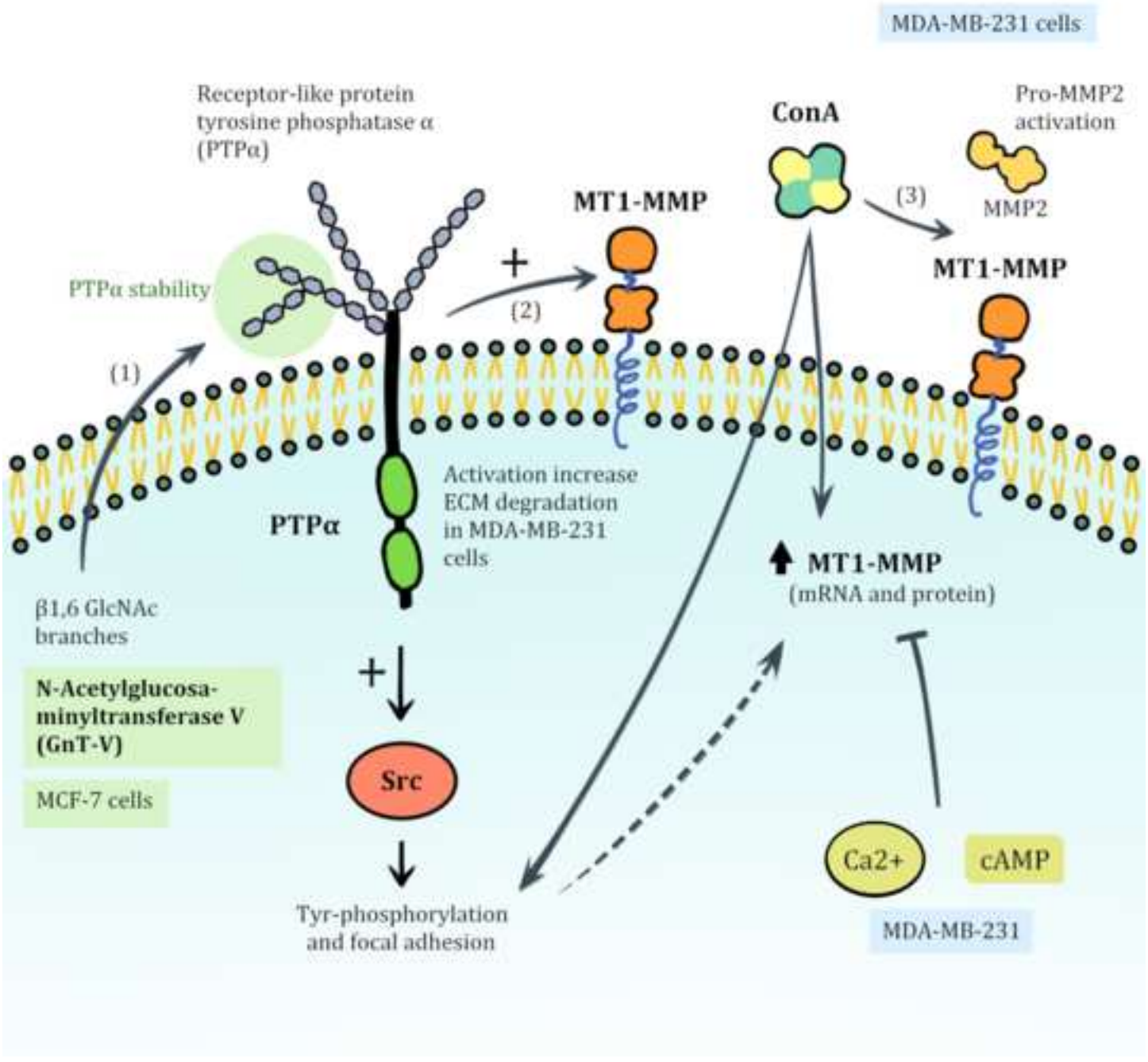
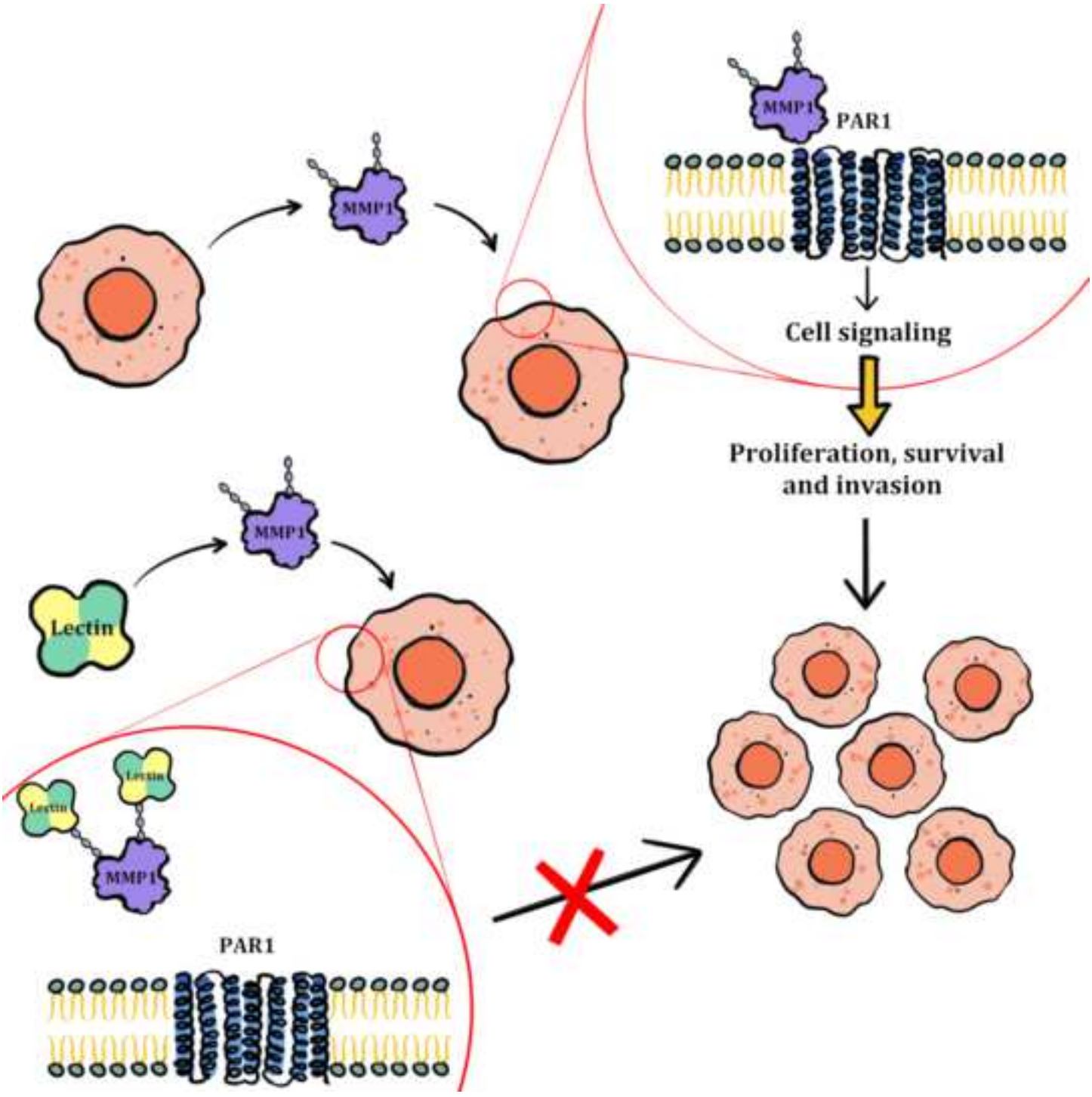

MDA-MB-231 cells
Receptor-like protein tyrosine phosphatase α (PTPα)
ConA
Pro-MMP2 activation
MT1-MMP
(3)
MMP2
MT1-MMP
PTPα stability
+
(2)
(1)
Activation increase ECM degradation in MDA-MB-231 cells
PTPα
MT1-MMP
(mRNA and protein)
β1,6 GlcNAc branches
N-Acetylglucosa-minyltransferase V (GnT-V)
MCF-7 cells
+
Src
Ca2+
cAMP
Tyr-phosphorylation and focal adhesion
MDA-MB-231

Figure 5


MMP1
PAR1
Cell signaling
Proliferation, survival
and invasion
MMP1
Lectin
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