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Biological Modulation by Lectins and Their Ligands in Tumor Progression and Metastasis

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Abstract

Lectins are a group of specific proteins that preferentially bind to carbohydrates inside and outside cells. To date, an increasing number of animal lectins have been found and categorized into several families in terms of the significant primary structural homology, while the classification is not always straightforward. These lectins can exert immense biological functions mainly through their specific carbohydrate-protein interactions in a variety of situations. In cancer biology, aberrant glycosylation changes on many glycoproteins and glycolipids are often observed and numerous experimental evidences have revealed that these structural changes are related to tumor malignancy. Galectins, which are broadly expressed animal lectins, can play crucial biological roles in tumor cell-cell or cell-matrix interactions through their binding activities to the tumor cell surface carbohydrate determinants. Certain galectin family proteins have also shown to affect tumor cell survival, signal transduction, and proliferation mainly inside the cell. Selectins, which are one of the C-type lectins and expressed leukocytes and/or vascular endothelium, can also play an immense role in tumor cell adhesion and invasion. In addition, certain annexin family proteins, which are originally known as phospholipid binding proteins, have been revealed to possess the carbohydrate binding activity, and these novel functions in tumors are being unveiled. Understanding how carbohydrate-protein interactions function in tumor cells will be one of the important goals in cancer research. This review focuses on the role of these lectins and their ligands in cancer progression and metastasis.

Keywords

Carbohydrate-binding proteins; lectin; galectin; selectin; annexin

1. INTRODUCTION

Malignant transformation from normal cells is normally caused by a disruption of homeostasis, e.g., cell cycle dysfunctional regulation, resistance to apoptotic stimuli, signal transduction abnormalities, and escape from immune surveillance. Abnormal glycosylation, resulting in expression of altered carbohydrate determinants inside and outside the cell, has been known as one of the most important changes during malignant transformation [1–3]. Carbohydrates can be conjugated with specific proteins or lipids through ER-Golgi apparatus and form glycoproteins, glycolipids or glycosaminoglycans. These glycoconjugates exist both inside and outside cells and have an important role in encoding

biological information due to their diversities [1–3]. In particular, it has been revealed that carbohydrate determinants of proteins and lipids on the cell surface can play an immense role in the cell-cell interactions and/or interactions of cells with extracellular matrix [3, 4], and their expression patterns are often varied during embryonic development or cell differentiation [3, 5]. It has been also reported that various alterations in the expression of cell surface carbohydrates are shown during malignant changes, indicating that a cell surface carbohydrates might play a critical role in forming distant metastasis associated with cell-cell and/or cell-matrix interactions [3, 5].

It has been revealed that some of these significant interactions related to carbohydrates are mediated through binding to specific proteins interacting with carbohydrate determinants. Specific proteins selectively binding to the carbohydrate determinants with no enzymatic activity are generally known as lectins. First discovered lectin was extracted from the plant (*Ricinus communis*) as a hemagglutinin possessing the ability to agglutinate erythrocytes [6], and the term lectin was invented to describe nonimmunoglobulin agglutinins with the capacity to distinguish between erythrocytes of different blood groups that are characterized by the specific sugar determinants [6, 7]. Thereafter, this term was generalized to all sugar-specific agglutinins of nonimmune origin [8]. Subsequently, a variety of animal lectins have been found and characterized, but categorized randomly among researchers because of their numerous numbers and diversities. A categorical name was firstly proposed in 1988, in which most animal lectins could be divided into two major families: the C-type lectins and the S-type lectins [9]. Although this comprehensive method was useful, it is no longer accurate concerning its definition. For example, S-type lectins were originally designated as such since these lectins were believed to require a thiolreducing reagent for the maintenance of their activity (“S” means SH-requiring) [10, 11]. However, it has been proved that this concept is no longer correct [10, 11]. Similarly, C-type lectins were designated as such because these lectins require Ca^{2+} for their activities (“C” means Ca-requiring) [9], whereas large diversity has been found in C-type lectins [12]. In addition, several lectins, which cannot belong to any categorized groups such as annexin family, have been revealed [13]. Therefore, classification of lectins under the concept of gene families is currently thought to be most appropriate [6, 12]. In this review, animal lectins are categorized as shown in Table 1.

Since most of lectins have no apparent catalytic activity, their exact biological functions had not been fully understood at the time of their discovery. However, for a few decades, cDNA cloning techniques followed by transfection of those cDNAs into mammalian cells have been developed and many studies used with these techniques have revealed the biological functions of many animal lectins, including cell-cell or cell-matrix interactions in cancer cells as well as normal cells (Fig. (1)). In addition, the creation and analyses of specific gene-targeting animals have brought us more accurately defined functions of lectins.

In cancer biology, well-studied lectins are S-type and C-type lectins [14, 15]. S-type lectins are now known as “galectins” and studied in particular as important factors for mediation of cell adhesion and/or of cell-cell interactions [14, 15]. The possible involvement of galectins in tumor metastasis formation could be an outcome of their interactions with cell surface glycoconjugates. In addition, it has been revealed that several galectins play an important role in malignant changes inside the cell, resulting in promotion of tumor progression. Meanwhile, the C-type animal lectins form an extremely large family, composed of members having diverse structures and functions [12], (Table 1). In these C-type lectins, the selectin family was initially found to play a specific role in leukocyte adhesion to endothelial cells through carbohydrate recognition [16], and is the most studied in cancer biology, in particular, cancer metastasis through their cell-cell interactions. Recently, there is increasing evidence that several annexins, a group of proteins having affinity to

phospholipids in a Ca^{2+} dependent manner, have a binding activity to glycosaminoglycans and are recognized as lectins [13]. Therefore, much attention is now paid to their functions as lectins. In this review, the role of annexins in cancer biology will be introduced and recent finding about annexins as a lectin will be also shown.

We would like to summarize the most recent advances in determining roles of these three lectin groups in cancer biology. Studies of endogenous lectins are multidirectional and show their immense importance in various biological functions. Therefore, we attempted to summarize the accumulated data related to the structure of three lectins (galectins, selectins, and annexins), their specific oligosaccharide binding activity, and their significant roles, particularly in regulation of tumor progression and metastasis. In addition, we will discuss future directions to elucidate more accurate function and role of carbohydrate and carbohydrate-binding proteins (lectins) in tumor progression and metastasis.

2. GALECTINS

Galectins, which were initially called S-type lectin as mentioned above, are defined as lectins having both galactose-binding capacity and conserved amino acid sequences, which characterize the galectin family [10, 17]. The first galectin was discovered in 1975 from extracts of electric eel tissues, which had the function of lactose-sensitive hemagglutinin activity [18]. After this discovery, a number of similar lectins were identified in other tissues from various species. In general, galectin proteins are soluble, and metal-independent in their activity. They also have many typical features of cytoplasmic proteins, i.e., no disulfide bridges, no glycosylation, no signal sequences, and in most cases their N-terminal amino acids are acetylated [19]. However, their expressions are localized not only in the cytoplasm but also in the nucleus, on cell surfaces and even the extracellular space, depending on each galectin protein [20]. Galectins outside the cell are precisely secreted from cells *via* non-classical secretory pathways [21]. These galectins' histological distributions have been studied in relation to a wide variety of biological phenomena including development, differentiation, morphogenesis, apoptosis, RNA splicing and so on [20, 21]. The first report of the involvement of galectins in cancer described the phenomenon that cancer cells aggregate in the presence of glycoproteins such as asialofetuin, and that protein extracts from these cells can induce hemagglutination activity [22]. In addition, a monoclonal antibody directed against B16 mouse melanoma cells could inhibit asialofetuin-induced homotypic cell aggregation and adhesion of melanoma cells [22]. However, relatively little is known about the mechanism by which galectins exert their functions, particularly in terms of carbohydrate recognition.

Many researchers have indicated much evidence that galectins have multiple, important functions in each step from normal cell to tumor development, so we will show the specific role of galectins in discrete steps during tumor progression and the metastasis process. But first of all, we will discuss general features of the galectin family and its carbohydrate recognition structures.

2.1. Galectin Family and Its Expression in Cancer

Galectins are a rapidly growing family of animal lectins that are defined by their ability to recognize the α -galactoside carbohydrate structure. So far, 15 members of this family have been identified and all of them share a consensus amino acid sequence of about 130 amino acids specific for carbohydrate recognition [15]. The galectin family can be classified into three groups on the basis of the content and organization of domains as follows: (i) the Prototype group (galectin-1, -2, -5, -7, -10, -11, -13, -14 and -15); (ii) the Chimera type (galectin-3); (iii) the Tandem Repeat type (galectin-4, 6, -8, -9, and -12) [10, 15]. It has been suggested that these multivalent members of the galectin family may play different

roles in various biological responses. In addition, analyses of three-dimensional structures of galectin-1, -2, -3, -7 and -10 using X-ray crystallography have revealed those differences more clearly. The CRD of those galectins show a highly tight folding structure, with two antiparallel β -sheets forming a sandwich-like structure [23].

Among these galectin families, expression of galectin-1, -3, and -8 in cancer cell lines from different origins and various tumors *in vivo* has been studied by many researchers [14, 15]. Many epithelial tumors, such as colon, thyroid and breast carcinomas, frequently express both galectin-1 and -3 [20, 24, 25]. High expression of galectin-1 in tumor cells is positively correlated with a metastatic phenotype and poorly differentiated morphology, but the expression of galectin-3 is highly variable in various stages of many tumors. Numerous reports supported a direct correlation of galectin-3 expression with colon, breast, gastric, endometrial, tongue and thyroid carcinomas, but some reports on colon and breast carcinoma presented opposite results [14, 25]. Concerning with other galectins, the presence of mRNAs for human galectin-1, -2, -3, -4, -7, -8, and -9 was monitored by the RT-PCR approach in a panel of 61 human tumor cell lines of different origin [26]. In this study, it was demonstrated that human tumor cells express more mRNA species for galectins than those for galectins-1 and -3. In particular, galectin-8, that has homology to PCTA-1, a prostate cancer antigen, was expressed in almost all of the cell lines the researchers tested [25, 26]. There are an increasing number of reports concerned with the expression of galectin families in many different tumors.

2.2. Carbohydrate Structures for Galectins

Galectin was identified as a protein that has the binding affinity to β -galactosides as mentioned above, but each galectin protein has distinct sugar binding specificity due to its different biochemical properties [27, 28]. To understand *in vivo* functions of diverse galectins, it is essential to understand both basic features that are conserved in each galectin and specialized features that each individual galectin has acquired under given condition. For this purpose, elucidation of their detailed carbohydrate-binding specificity and identification of their endogenous receptors' glycans are necessary.

Basically, all galectins have common properties, i.e. to bind lactose and *N*-acetyllactosamine [28], (Fig. (2)), while the interaction between a galectin CRD and the monosaccharide ligand, galactose is much weaker [27]. Interestingly, larger oligosaccharides, e.g., poly-*N*-acetyllactosamine exhibit even higher affinity than lactose [28], and this increased affinity for poly-*N*-acetyllactosamine is believed to be increased avidity to the multivalent ligands due to the binding and cross-linking properties of galectins [29–31]. More detailed biochemical properties about the specific structure recognized by each galectin family protein were analyzed by some researchers [27, 28, 32, 33]. Hirabayashi *et al.* reported that all galectins commonly require three OH groups (i.e., 4-OH and 6-OH of Gal, and 3-OH of GlcNAc) of *N*-acetyllactosamine, and galectin could not bind to any glycolipid-type glycans, complex-type *N*-glycans, of which both 6-OH groups are sialylated, or Lewis-related oligosaccharide structures [28].

Among galectin family proteins, galectin-3 showed a remarkably high affinity to almost all oligosaccharide structures compared to other galectins [28]. For example, A antigen is a 32-fold better ligand than *N*-acetyllactosamine for galectin-3, but a 3-fold worse ligand for galectin-1 [27, 28]. It is also considerable that galectin-3 could bind to the Le-containing oligosaccharides with relatively high affinity unlike galectin-1 [28]. Remarkable features about the differences between galectin-1 and galectin-3 binding specificity are depicted in Fig. (2). In addition, binding of galectin-3 to these oligosaccharides is increased by substitution of the terminal galactose with NeuAc 3-, SO₃-3-, Fuc 2-, and GalNAc 3(Fuc 2)-moieties, whereas binding is abolished by substitution with NeuAc 6-,

SO₃-6-, or Gal 4- residues [34]. Binding is maintained if the penultimate GlcNAc is substituted with fucose (Le^a or Le^x), but decreased by substitution of the penultimate GlcNAc with neuraminic acid. Galectin-3 binds weakly to mucin-type core 1 (TF antigen) and to NeuAc 3Gal 3GalNAc -, but binds strongly to 3-O-sulfate containing core 1 (SO₃-3Gal 3GalNAc -) and sulfated core 2 (SO₃-3Gal 3[GlcNAc 6]GalNAc -) [35].

However, many researchers have been reported an increasing number of ligands for the galectin family, some of which do not possess the carbohydrate structures [15, 20]. Therefore, we show the relationship between galectin proteins and their ligand proteins in cancer progression and metastasis mainly from the point of their interactions *via* protein-protein, rather than lectin-glycoconjugate, interactions. In addition, we will divide the phenomena of cancer initiation to metastasis formation to clearly show the interactions and functions in each tumor progression step. We aim to focus on a number of potential roles of galectins in the regulation of tumor development and growth, including tumor transformation, apoptosis, cell proliferation, and the tumor immune escape system. We also discuss the possible roles of several galectins in various steps of tumor metastasis, including their functions in tumor cell adhesion, migration and angiogenesis.

2.3. Role of Galectins in Tumor Progression

Cancer is initiated by multiple steps including malignant transformation, dysfunctional regulation of cell proliferation, defective response to apoptotic stimuli and/or defensive activity against immune surveillance. Interestingly, several galectins are likely to play a key role in these initial processes for tumor progression (Table 2).

2.3.1. Galectins and Tumor Transformation—Cellular transformation is an important initial step for tumorigenesis [36, 37]. Overexpression of oncogenes such as *src*, *c-myc* and/or suppression of tumor suppression genes such as *p53*, *Rb* is generally known to be related to the malignant transformation [38]. Ras proteins (H-Ras, K-Ras, and N-Ras) are among the major on-cogenic proteins, contributing to various aspects of malignant transformation and require anchorage to the plasma membrane for their activities [39]. So far, it is revealed that both galectin-1 and -3 can directly interact with these Ras proteins in certain cancer cells [40, 41]. Firstly, galectin-1 was found to become a binding partner of H-ras protein [40]. Overexpression of galectin-1 in tumor cells increased membrane association with Ras, Ras-GTP, and active ERK, resulting in cell transformation. Conversely, introduction of galectin-1 antisense RNA inhibited transformation by H-Ras and abolished the membrane anchorage of this protein. These results indicate galectin-1 and H-Ras interactions can be associated with cellular transformation in several human malignant tumors. Subsequently, galectin-3 was also found to bind preferentially to K-Ras protein [41]. As a result of interaction with K-ras, galectin-3 can promote the activation of RAF1 and phosphatidylinositol 3-kinase (PI3K) followed by the selective activation of signaling cascades and the regulation of gene expression at the transcriptional level. In a more recent study, it was shown that galectin-3 could regulate a molecular switch from N-Ras to K-ras through its different binding activity in BT549, human breast carcinoma cells [42]. Overexpression of galectin-3 in this cell induced a significant increase in K-Ras activation and decrease in N-Ras activation. These interactions resulted in functional alterations in Ras-related signaling cascade, whereby the activation of Akt and Ral signaling was suppressed and converted to the activation of extracellular signal regulated kinase (ERK). In addition to breast carcinoma cells, galectin-3 can also be involved in malignant transformation of thyroid cells *in vitro* [43, 44], whereas its mechanism associated with the interaction between galectin-3 and Ras proteins is not yet understood. NPA, human thyroid papillary carcinoma cells, lost their characteristic transformed phenotypes following down-regulation of galectin-3 expression by anti-sense cDNA transfection [43]. Conversely, the

introduction of galectin-3 into a normal thyroid follicular cell line, TAD-2, can induce malignant transformation in culture conditions [44]. Taken together, several galectins might be partially involved in tumor transformation of specific cell types.

Signal transduction abnormality might be related to cellular transformation. Ras-associated cell signaling can be changed with galectin-1 and/or galectin-3 expression as described above. In a more recent study, it was shown that several cytokine receptors on the cell surface were regulated by galectin-3, resulting in abnormal cell signaling [45]. Generally, cancer cells display increased specific carbohydrate structure, 1-6GlcNAc-branching, which is produced by β -1, 6-*N*-acetylglucosaminyltransferase V (Mgat5, GnT-V) and the preferred acceptor for poly *N*-acetylglucosamine extensions. Galectin-3 can crosslink poly-*N*-acetylglucosamine moieties on EGF and TGF- β receptors presumably through its multivalent binding properties, forming a “lattice” that reduces the rate of receptor removal by constitutive endocytosis [45]. It remains to be determined whether regulation of cytokine receptors by galectins is sufficient for tumorigenesis, but specific carbohydrate-mediated complex like this seems to affect malignant changes in several cancers.

2.3.2. Galectins and Cell Cycle (Cell Proliferation)—Cell cycle is strictly regulated in normal cells for proper cell proliferation. Disorder of this cell cycle regulation followed by abnormal cell proliferation might cause tumorigenesis in certain cell types [46]. Some galectins have been found to be associated with this cell cycle control. In particular, galectin-3 can affect cell cycle regulators such as cyclins and/or cyclin inhibitors in breast cancer cells [47]. Galectin-3 can play a role in the upregulation of the cell-cycle inhibitors p21 (WAF1) and p27 (KIP1), resulting in the high expression of cyclin D1 and the low expression of both cyclin E and cyclin A. In addition, this regulation is enhanced under anoikis (apoptosis induced by the loss of cell anchorage to extracellular matrix) [47]. Upregulation of cyclin D1 can also be induced by stabilization of nuclear protein-DNA complex at the SP1 and CRE (cAMP-responsive element) site of the cyclin D1 promoter with galectin-3 binding [48]. Oppositely, galectin-1 can interact with the α 5 β 1 integrin to restrict carcinoma cell growth *via* induction of p21 and stability of p27, resulting in inhibition of cyclin-dependent kinase 2 activity and G1 arrest [49]. Similarly, ectopic expression of galectin-12 in cancer cells induces cell-cycle arrest during G1 phase and cell growth suppression, although the molecular mechanisms of this have not been determined [50].

On the other hand, several galectins are concerned with cell proliferation inside and outside cells. For example, nuclear galectin-3 can interact with the TTF-1 (thyroid-specific transcription factor) in papillary thyroid carcinoma cells and this binding complex stimulates the transcriptional activity of TTF-1 contributing to the proliferation of the thyroid cells [51]. Inhibition of galectin-1 expression in human glioma cells suppresses the anchorage-independent cell growth [52], while overexpression of galectin-7 in a colon carcinoma cell line showed slower cell growth compared to control cells [53]. Meanwhile, exogenous addition of both galectin-1 and -7 recombinant proteins can inhibit neuroblastoma cells' growth [54], whereas exogenous recombinant galectin-3 can promote cell proliferation of human fibroblast [55]. However, detailed molecular mechanisms of these cell proliferation in regard to the interaction between these galectins and their ligands are still not understood.

2.3.3. Galectins and Apoptosis—Apoptosis is a natural biological phenomenon observed during cell differentiation or against immune response [56]. The acquisition of resistance to apoptotic stimuli in tumor cells enables to avoid tumor cell death induced by anti-tumor drugs or natural effector cells like natural killer (NK) or killer T-cells, resulting in an increased potential in malignancy and tumor progression. Many groups have indicated

during this past decade that many galectins are associated with tumor apoptosis mechanisms in various cell types and conditions.

Within the galectin family, only galectin-3 seems to have anti-apoptotic activity in a variety of cells. The first report about anti-apoptotic activity of galectin-3 included evidence that galectin-3 cDNA transfected human T lymphoma cells were more resistant to apoptosis induced by anti-Fas antibody or staurosporine (protein kinase inhibitor) compared to control cells [57]. Subsequently, breast carcinoma cells, in which galectin-3 was overexpressed, were more resistant to apoptosis induced by anti-cancer drugs compared to controls [58]. However, the molecular mechanisms by which galectin-3 can inhibit apoptosis induced by various stimuli remains to be determined. Firstly, there is evidence that galectin-3 has sequence homology with Bcl-2, a major anti-apoptotic protein [57, 58]. Both proteins are rich in proline, glycine, and alanine in the N-terminal domain and contain the Asp-Trp-Gly-Arg (NWGR) motif in the C-terminal domain. It was reported that single amino acid substitution in the NWGR motif of galectin-3 resulted in no resistance to apoptosis induced by cisplatin in breast carcinoma cells [58], suggesting that this NWGR motif is critical for its anti-apoptotic activity by galectin-3. Moreover, galectin-3 can bind to Bcl-2 protein *in vitro* and might mimic its activity by forming heterodimers [57]. Thus, galectin-3 might be a mitochondrial-associated apoptotic regulator through interaction with Bcl-2 in several tumor cells. Secondly, it was reported that synexin (annexin A7), a Ca^{2+} - and phospholipid-binding protein (also see later section), is a direct binding partner of galectin-3 for prevention of mitochondrial damage and cytochrome *c* release following the treatment of anticancer drug to breast carcinoma cells [59]. Galectin-3 failed to translocate to the perinuclear membranes when expression of synexin was down regulated by antisense oligonucleotide introduction, resulting in no effect of anti-apoptotic activity. This study indicates that synexin is another important cytoplasmic ligand of galectin-3 associated with its anti-apoptotic activity. It might be possible that the interaction with K-Ras protein also affects galectin-3 related anti-apoptotic activity, since galectin-3 modified Ras signal can increase PI3K activity that it related to anti-apoptotic activity. A recent study indicated that overexpression of galectin-3 in bladder carcinoma cells acquired resistance to TRAIL induced apoptosis through up-regulation of Akt phosphorylation, which is activated by PI3K activity [60], whereas there is no direct evidence that this Akt activation is due to the K-Ras/galectin-3 complex in this cell. However, galectin-3 is not the implicit anti-apoptotic factor as are Bcl family proteins, so it should be recognized that galectin-3 could act as an anti-apoptotic activity with different binding partner(s) in various cell types against distinct apoptotic stimulations.

Other galectins can also regulate apoptosis, but they seem to induce apoptosis, unlike galectin-3. It was reported that overexpression of galectin-7 in several tumor cells is apt to undergo apoptosis following a number of different apoptotic stimuli that trigger distinct signaling pathways [61]. Galectin-12 also promoted apoptosis when it is overexpressed in fibroblast cells [62]. However, detailed mechanisms by which these galectins exert pro-apoptotic activity, i.e. the binding partner, are still unknown. There are also important facts that many galectins can induce apoptosis of T-cells including leukemia cells, but this normally has the effect that many tumor cells escape immune system attack, resulting in tumor progression, therefore, we will discuss about this in next chapter.

2.3.4. Galectins and Tumor Immune Escape—Natural effector cells like natural killer (NK) or killer T-cells can detect the molecules immunologically foreign to the host and digest those to protect the self. Tumor cells also express unusual proteins on the cell surface, which could be recognized by specific immune effectors, resulting in breakdown of tumor cells, therefore the immune system might play a critical role in inhibiting initial tumor formation. However, more malignant tumors have the ability to escape and survive from this

immune surveillance by, in part, secretion of various cytokines or chemokines, which can influence the immune and inflammatory response surrounding the tumors [63].

Many galectins are also likely to associate with this tumor immune escape mechanism. There is ample evidence that many galectin proteins are expressed in many different immune and inflammatory cells and also galectins are generally secreted from normal and/or tumor cells, presumably through a non-classical secretion pathway [21]. Therefore, it is reasonable that several galectins can modulate a variety of inflammatory responses and help tumor cells to escape from immune surveillance. Within the galectin family, galectin-1 was firstly found to be able to induce apoptosis of activated T-cells [64]. This activity has been demonstrated by adding exogenous recombinant galectin-1 to T-cells *in vitro*. Since galectin-1 is known to exist normally as a dimeric formation and bind to multiple glycoconjugates, pro-apoptotic activity to T-cells of galectin-1 is likely to be the results of binding and cross-linking to several cell-surface glycoproteins on T-cells [65]. Actually, galectin-1 binds to several cell-surface glycoproteins, e.g., CD2, CD3, CD7, CD43 and CD45 in a carbohydrate-dependent manner, and can cause a redistribution of some of these proteins into segregated microdomains within the plasma membrane [66]. In addition, galectin-1 can also modulate T-cell receptor complex (TCR) signaling, which in part enhances TCR apoptosis [67]. With the fact that galectin-1 can induce T-cell apoptosis, one might predict that several tumor cells can impair the function of infiltrating effector T-cells, such as NK cells or killer T-cells, by secretion of galectin-1 from them, resulting in an immunosuppressive environment around the tumor. In fact, galectin-1 mediated tumor immune escape phenomena were observed *in vitro* and *in vivo* experiments [68]. Therefore, galectin-1 can administer the immune response toward tumor cells through carbohydrate determinants on the T-cell surface, resulting in apoptosis induction of cancer-infiltrating T-cells for tumor immune escape and progression.

Similarly, a recent study indicates that extracellular galectin-3 can also induce apoptosis of some kinds of T-cells [69]. Unlike galectin-1, CD29 and/or CD7 seem to be involved in galectin-3 induced apoptotic activity since antibodies to those molecules inhibited its apoptotic activity. Moreover, some glycoprotein receptors on the T-cell surface have been also identified that can bind to galectin-3 and these interactions might regulate T-cell activation to some extent. First of all, extracellular galectin-3 can bind to poly-lactosamine structure on TCR and form clustering followed by downregulation of TCR activity [70]. Secondly, CD98, which is associated with the regulation of cell adhesion, growth and apoptosis, is also a galectin-3 binding partner found on the surface of T-cell [71]. It suggests that galectin-3 interaction with this CD98 could modify specific integrin activation, since the cytoplasmic domain of CD98 could bind to the cytoplasmic tail of integrin β subunit inside the cell followed by integrin activation. However, the problem of this pro-apoptotic activity to T-cells by galectin-3 as well as galectin-1 is that relatively high protein concentration is required for those activities compared to the one in sera from clinical cancer patients. This might be explained with the fact that a large amount of galectin can be presented in the extracellular matrix spaces as binding forms, so these concentrated galectins might induce apoptosis more effectively in T-cell apoptosis than simple exogenous application from the point of binding specificities. Additional evidence indicates that other galectins can also regulate T-cell activity. Galectin-2 and galectin-9 can induce T-cell apoptosis [72, 73], and galectin-4 activates CD4⁺ T cells [74].

Taken together, many galectins have the potential ability to suppress the effector immune responses against tumor progression. However, further studies are required in order to understand the combined and/or redundant effects of galectins *in vivo*.

2.4. Galectins in Tumor Metastasis

Tumor metastasis is a major fatal complication in cancer. The process of tumor metastasis is thought to be very complicated, involving the following steps; angiogenesis at the primary tumor site, detachment from the primary tumor, invasion in the extracellular matrix, dissemination through the blood flow or lymph ducts, tumor embolization in capillary vessels and growth at the secondary tumor site. Many changes in cell-cell and/or cell-extracellular matrix interactions are associated with these processes and several galectins seem to be required in above multiple steps during tumor metastasis (Table 2).

2.4.1. Galectins and Angiogenesis—Angiogenesis is required for the process of inflammation, wound healing, invasive tumor growth, and metastasis [75]. The formation of new vessels is required for tumor growth at the primary site and provides a gateway for the dissemination through the blood flow. Carbohydrate interactions may have a pivotal role in angiogenesis because many angiogenesis related factors such as FGF (fibroblast growth factor) family and VEGF (vascular endothelial growth factors) initially exist on the proteoglycans in extracellular matrix before binding to their original receptors, and some of the adhesion molecules bind to glycoconjugates on the endothelial cell surface [76]. Among galectin family proteins, galectin-3 is partially involved in tumor angiogenesis [77]. It can stimulate chemotaxis and capillary tube formation of human umbilical endothelial cells (HUVEC) *in vitro*. This capillary tube formation was inhibited by lactose, inhibitor for CRD mediated binding of galectin, suggesting the involvement of carbohydrate-binding activity in angiogenesis induced by galectin-3. Scattered plot analysis revealed the presence of two galectin-3 ligands on the HUVEC cell surface, but the endothelial cell surface receptors for galectin-3 are not yet identified.

2.4.2. Galectins and Cell Matrix Interaction—Most of the cells are surrounded by extracellular matrix and anchored it through various cell-matrix adhesion molecules such as integrin family proteins. Many of cell adhesion molecules are the transmembrane proteins, which generally have extracellular, intracellular and transmembrane domain are highly glycosylated in the extracellular domain. On the other hand, the galectin family has no transmembrane domain and no typical signal sequence for secretion, but it is known to exist on the cell surface and extracellular spaces [20, 21]. Moreover, since both of extracellular matrix and cell-matrix adhesion molecules on the cell surface include glycoproteins, galectins outside cells could mediate the binding between cancer cells and extracellular matrix (Fig. (1)). These complicated interactions among extracellular matrix, cell-matrix adhesion molecules and galectins could play an important role in the process during the tumor cell detachment from the primary site. For example, exogenous addition of recombinant galectin-1 and galectin-3 can inhibit the adhesion of tumor cells to extracellular matrix proteins [78]. Similarly, soluble galectin-8 inhibits the adhesion of a number of different cell lines to culture plates [79]. After tumor cells detach from the primary tumor site, they have to bind to the extracellular matrix, in which glycoconjugates such as laminin, fibronectin and collagen IV are rich. These glycoconjugates in the matrix regulate various cellular adhesions through its binding to integrin-family proteins and/or non-integrin binding proteins. Several galectin family proteins belong to the non-integrin binding proteins for extracellular matrix and functions as a modulator of cell adhesion. For example, galectin-3 inhibits melanoma, breast cancer, fibrosarcoma and prostate cancer cells adhesion to laminin [80]. Exogenous galectin-3 promotes the attachment of neutrophils to fibronectin in a β 2-integrin-dependent manner and to laminin in a calcium-dependent manner [81]. Breast cancer cells expressing galectin-3 demonstrated adhesion to laminin and collagen IV, but not fibronectin [82]. Laminin and fibronectin have also been demonstrated to be the receptors for galectin-1 [83, 84]. Interestingly, galectins can also directly bind to some integrin-family proteins, which are major cell-matrix adhesion molecules to bind extracellular matrix, and

play an immense part in tumor metastasis through their adhesive interactions. For example, galectin-1 binds to $\alpha 5 \beta 1$ [49], $\alpha 7 \beta 3$ [83], galectin-3 binds to $\alpha 1 \beta 1$ [80], $\alpha 3 \beta 1$ [85], and galectin-8 binds to $\alpha 3 \beta 1$, $\alpha 6 \beta 1$, and αM [86, 87]. It is well known that integrins are highly glycosylated transmembrane proteins and all these binding characteristics are mediated through CRD domain. Taken together, galectins, on tumor cell adhesion to extracellular matrix, could mediate the interactions between these integrins and extracellular matrix proteins as a biological cross-linker due to their bivalent or multivalent properties. In addition, galectin-3 can upregulate integrin expression [82], suggesting that galectins might modulate tumor metastasis not only by binding to integrins, but also by regulating their expression.

2.4.3. Galectins and Tumor Embolization—Aggregation of tumor cells can lead to tumor embolization in microcapillaries and this circulating tumor embolus is followed by extravasation at the secondary tumor sites. There is the evidence that tumor cells injected as aggregated complex result in formation of more lung colonies in mice than those injected as single cell suspensions [88]. A positive correlation has been demonstrated between *in vitro* aggregation property and *in vivo* metastatic potential [89]. This homotypic cell aggregation or heterotypic cell aggregation are normally mediated by other cell adhesion transmembrane molecules such as cadherin family proteins, due to their cell-cell interacting properties. However, cell surface or soluble galectins can also induce homotypic cell adhesion by bridging with soluble complementary glycoconjugates. Firstly, galectin-3 was shown to be a functional mediator of homotypic cell aggregation in the presence of asialofetuin, which is strong binding partner of galectin-3 [90]. Subsequently, Mac-2 binding protein, originally identified as a ligand for galectin-3, is a heavily *N*-glycosylated secreted glycoprotein, and this complex can induce homotypic aggregation of melanoma cells [91]. This cell aggregation is inhibited by lactose or anti-galectin-3 antibody, suggesting that Mac-2 binding protein interacts with galectin-3 on tumor cell surfaces in a carbohydrate dependent manner. Moreover, immunofluorescent staining of aggregated cancer cells showed that galectin-3 is clustered at the sites of cell-cell contact portion [92]. We have also shown that pH-modified citrus pectin (MCP), a competitive inhibitor of galectin-3, inhibit asialofetuin induced homotypic aggregation of B16-F1 melanoma cells *in vitro* [93]. These results indicate that galectin-3 induces homotypic aggregation, resulting in tumor embolism, but it remains unknown how galectins can interact with the other cell-cell adhesion molecules.

3. SELECTINS

The selectin family, which is one of the C-type lectin families, is presumably the most-studied C-type lectin as an adhesion molecule related to cancer metastasis as well as leukocyte homing process. They were initially discovered in 1989 as a result of the production of monoclonal antibodies against cell surface molecules on cultured endothelial cells activated with inflammatory mediators, followed by molecular cloning and sequence analyses of the proteins recognized by those antibodies [94, 95]. Three adhesive proteins were unveiled and originally named as the murine lymph node homing receptor (MEL-14 antigen), the endothelial-leukocyte adhesion molecule-1 (ELAM-1 or LECAM-2) and the platelet granule membrane protein-140 (GMP-140, LECAM-3 or PADGEM), respectively [94]. These molecules share about 50% sequence identity in their C-type lectin domains and are now known as L-selectin (CD62L), E-selectin (CD62E) and P-selectin (CD62P), respectively [94]. Generally, selectin is a membrane protein with one transmembrane domain and recognized as a family of cell adhesion molecules on the cell surface mediating the carbohydrate-protein interaction with specific ligands. It has been indicated that selectin mediated cell adhesion is implicated in extravasation of leukocytes, homing of lymphocytes, and infiltration of malignant cells including leukemia and cancer cells. These cell adhesion interactions are controlled by the expression of specific carbohydrate ligands recognized by

the C-type lectin domain in the selectin molecules as well as expression level of selectin protein itself.

Many researchers have focused on the molecular mechanisms of cancer metastasis in which selectin family proteins are involved, therefore we tried to show the specific role of selectins in the process of tumor metastasis. But first of all, we will mention about the general features of selectin family and its recognition carbohydrate structures.

3.1. Selectin Family and Its Expression in Cancer

The selectin family is composed of L-, E- and P-selectins that are encoded by genes located on human and murine chromosome 1 [13]. Each selectin has a calcium-dependent CRD (carbohydratebinding domain) in its N-terminus, followed by an EGF-like domain, two to nine SCR (short consensus repeat) units homologous to complement binding proteins, a transmembrane domain and a short cytoplasmic domain. Three selectins are closely related having about 40 % identity in the SCR domains and up to 65% identity in the CRD and EGF domains [96].

L-selectins (also known as LAM-1, LECAM-1, gp90mel, or CD62L) are constitutively expressed on almost leukocytes [94]. L-selectin was firstly recognized as a lymph node receptor involved in the regulation of naïve T lymphocytes trafficking to peripheral lymph nodes [16]. High endothelial venules (HEV) are known to serve as an entrance for those lymphocytes homing into peripheral lymph nodes by their binding to specialized high endothelial venules (HEV).

E-selectin (also known as ELAM-1, or CD62E) is indubitably expressed on vascular endothelium upon stimulation with cytokines such as TNF- α , IL-1 α , or α -1 due to transcriptional activation of E-selectin mRNA, and thought to be involved in the recruitment of leukocytes during the inflammation process [97]. In other words, E-selectin is not constitutively expressed on resting endothelial cells, unlike L-selectin.

P-selectin (also known as PADGEM, GMP-140, or CD62P) was first detected on α -granules of activated platelets and Weibel-Palade granules in endothelial cells. It is rapidly induced on the surface of endothelial cells or platelets following their activation with some agonists such as histamine or thrombin, and reacts with the carbohydrate ligands expressed on leukocytes [94]. P-selectin can also bind to myeloid cells and some T-cells.

In immunohistochemical studies on colon carcinoma, the small blood vessels surrounding cancer cell nests abundantly expressed E-selectin [98]. Judging from *in vitro* experiments testing the ability of culture supernatant of human cancer cells to induce E-selectin expression on cultured endothelial cells, cancer cells seem to be able to induce endothelial E-selectin expression partially by IL-1 α and/or α -1 [99]. Moreover, in cancer patients, the elevated levels of soluble E-selectin are often observed in their serum since this selectin is known to be cleaved from the cell surface after expression on the endothelial cells [100]. However, the expression levels of serum E-selectin seem to depend on various conditions in cancer patients [97].

Compared with the expression levels of selectin family proteins, it is known that the expression levels of carbohydrate determinants for selectin family are often increased in various cancer cells and this might be more significant in selectin family related tumor progression mechanism. Therefore, we will refer of specific carbohydrate structures recognized by selectin family.

3.2. Carbohydrate Structures for Selectins

Following the molecular cloning of three selectin family, a simultaneous study was performed in a number of laboratories to demonstrate the characteristics of the minimal carbohydrate epitope recognized by the selectins. Accordingly, different groups identified that sialyl Lewis^x and sialyl Lewis^a are the minimal carbohydrate structure for selectin-mediated binding by distinct approach [101, 102]. These carbohydrates are usually found at the non-reducing terminus of *N*- or *O*-linked oligosaccharides, or on glycosphingolipids [97]. Since the C-type lectin domains of the three selectin members are considerably homologous, it is reasonable that three selectin family proteins share similar ligand binding specificities and have functional redundancies. Commonly, the affinity levels of each selectin family protein to oligosaccharides are not relatively high, therefore, the interactions between multivalent carbohydrate determinants and repeated CRDs are important to exert their biological functions.

In cancer biology, it was reported that sialyl Lewis^x and sialyl Lewis^a determinants on epithelial cancer cell surface were highly expressed and found to serve as ligands for endothelial E-selectin [97]. Cancer cells expressing sialyl Lewis^x and/or sialyl Lewis^a strongly adhere to activated endothelial cells in the monolayer cell adhesion assay. Now, these sialyl Lewis^x and sialyl Lewis^a determinants expressed on cancer cells are thought to be involved in the process of hematogenous metastasis of cancers [103]. One might think about specific carbohydrate recognition properties among three selectin family proteins, and in fact, each member of the selectin family has distinct ligand specificity, which administers the complex processes of specific homing of various leukocytes and inflammatory distribution of activated leukocytes [94]. We will describe known different ligands for each selectin family in more details below.

L-selectin binds to several mucin-like glycoproteins, such as GlyCAM-1, CD34, MAdCAM-1, Sgp200, on HEV in lymph nodes. These molecules are proposed as L-selectin ligand-specific core proteins, which carry sialylated, fucosylated and sulfated carbohydrate-harboring molecules that can provide clusters of *O*-linked sugar chains to L-selectin. The detailed carbohydrate structures on these proteins was identified as sialyl 6-sulfo Lewis^x [104], while 6'-sulfated and 6,6'-disulfated structures were proposed to be predominant for HEV in murine peripheral lymph nodes [105]. Considerable difference in the carbohydrate ligands for L-selectin is found between humans and mice, but 6-sulfation seems to be essential in both species, as functional L-selectin ligands can be reconstituted by the transfection of co-transfection of a fucosyltransferase gene and a 6-sulfotransferase gene [106]. Subsequently, two genes for 6-sulfotransferase isoenzymes; *N*-acetylglucosamine-6-*O*-sulfotransferase 1 (GlcNAc6ST-1) and GlcNAc6ST-2, which are the candidate enzymes for the synthesis of sialyl 6-sulfo Lewis^x in HEV, were cloned [107], and the disruption of GlcNAc6ST-2 in mice was shown to reduce L-selectin-mediated lymphocyte homing to 50 % of that in wild-type mice [108]. More recent study demonstrates that both GlcNAc6ST-1 and -2 cooperatively can control lymphocyte homing through L-selectin ligand biosynthesis in HEV [109]. Mutant mice, in which both GlcNAc6ST-1 and -2 were disrupted, had approximately 75% less homing of lymphocytes to the peripheral lymph nodes than wild-type mice.

E-selectin binds to E-selectin ligand-1 (ESL-1), a unique 150 kDa core protein with *N*-linked carbohydrate chains, in mouse myeloid cells [110]. Initially, L-selectin was proposed to be one of the major core proteins that carry carbohydrate ligands for E-selectin [94]. PSGL-1, described as a specific core protein for P-selectin (see below), provides mutual carbohydrate determinants to E-selectin for binding as well. The carbohydrate determinants for E-selectin on leukocytes were firstly shown to be sialyl Lewis^x, and sialyl Lewis^a is also known as a carbohydrate ligand as well [94]. However, there is considerable evidence

showing that carbohydrate ligands for E-selectin are not limited to sialyl Lewis^x and sialyl Lewis^a. Some internally fucosylated structures are also able to bind to E-selectin, even when the determinant does not carry a terminal sialyl Lewis^{x/a} structure [111], suggesting that E-selectin ligands on the cell surface are a set of heterogeneous sialyl Lewis^{x/a}-like carbohydrate determinants. It is generally thought that E-selectin preferentially recognizes the carbohydrate portion of the ligand molecules, and does not require a specific core protein for its binding, whereas core proteins would indirectly affect the binding activity through optimizing the conformation of the carbohydrate ligands on cell surface. In cancer cells, sialyl Lewis^x and sialyl Lewis^a determinants are found to be carried by several glycolipids and highmolecular-weight mucin molecules, such as MUC-1.

P-selectin binds to a 220 kDa mucin-like P-selectin glycoprotein ligand-1 (PSGL-1) that was originally discovered on neutrophils, but is now known to be expressed on essentially all types of leukocytes. Earlier studies demonstrated that recombinant P-selectin binds to sialyl Lewis^x and sialyl Lewis^a [112]. PSGL-1 should carry a sialyl Lewis^x on its carbohydrate side chains, and it is also reported that L-selectin and E-selectin also bind to PSGL-1, as mentioned above. However, there are several significant differences between the E-selectin and P-selectin ligands. In the case of PSGL-1, both sulfated tyrosine and carbohydrate chains near the amino terminal region are reported to be required for optimum binding to P-selectin, whereas E-selectin requires only sialyl Lewis^x determinant [113]. In addition, recombinant P-selectin characteristically requires a 5~10-times higher concentration to obtain the binding level with E-selectin in binding to immobilized sialyl Lewis^x. P-selectin-mediated cell adhesion is known to be abrogated by inhibitors of sulfation such as NaClO₃, whereas E-selectin-mediated cell adhesion is not. The P-selectin ligands on leukocytes were known to be protease-sensitive, whereas those for E-selectin were shown to be quite resistant to protease treatment. It was also reported that L- and P-selectin, but not E-selectin, bound to sulfated proteoglycan 3-sulfated galactosyl ceramide (sulfatide), heparin, heparin sulfate proteoglycans, fucoidin and dextran sulfate [114].

Taken together, these results clearly established that the three members of the selectin family have relatively distinct requirements for ligand sulfation, as depicted in Fig. (3).

3.3. Selectins in Tumor Progression and Metastasis

Since selectin family proteins were originally found to mediate adhesion between leukocytes and the endothelial cells in blood vessels and also their expressions including their ligands are found on the surface of several tumor cells, it should be considered to play other important biological roles in which selectins are involved in the adhesion of circulating tumor cells to endothelium in several tumor metastasis process. A considerable amount of research has been carried out in regard to this possibility. Furthermore, the monoclonal antibody approach to cancer-associated antigens in the early 1980's accelerated research on these carbohydrate determinants, because not a few monoclonal antibodies in those studies showed preferential reactivity to several carbohydrate determinants such as sialyl Lewis^x and sialyl Lewis^a [97]. The monoclonal antibody recognizing sialyl Lewis^a, namely N19-9 (CA19-9), was first generated and, this antibody is now known to increase in sera of several cancer patients with pancreatic and biliary tract cancers. Some studies indicate that the level of expression of selectin ligand might have a prognostic value in cancer patients. Therefore, CA19-9 was used frequently as a tumor marker of, in particular, pancreatic and biliary duct carcinomas. Numerous studies have also shown that sialyl Lewis^x and sialyl Lewis^a are overexpressed in a majority of human carcinomas of the colon, bladder, breast and lungs.

Overexpression of sialyl Lewis^{x/a} is often associated with the advanced forms of malignancies [115]. Furthermore, a comparative analysis of sialyl Lewis^x and sialyl Lewis^a expression by tumor cells from primary and metastatic lesions indicated that the expression

of sialyl Lewis^x and/or sialyl Lewis^a was higher in metastatic lesions in most patients [116]. Thus, selectins are generally thought to play a critical role in tumor metastasis, in particular, the adhesion process of cancer cells to the endothelium cells.

3.3.1. Selectins and Tumor Cell Adhesion—The carbohydrate determinants, sialyl Lewis^x and/or sialyl Lewis^a have been utilized for initial diagnosis of cancers, detection of recurrence after surgical operation, and monitoring the therapeutic effect of radio- and chemotherapy [97]. However, the pathophysiological significance of these determinants in cancer progression was unclear when they were first applied for serum diagnosis. It was not until the early 1990's that sialyl Lewis^x and sialyl Lewis^a on the surface of cancer cells were found to play a principal role in the adhesion to the endothelium of cancer cells derived from the lower digestive organs, such as the colon and rectum, as well as from the pancreas and biliary tract, while the sialyl Lewis^x determinant was found to associate with the adhesion of breast, ovarian and pulmonary cancer cells [97]. The experimental results also support the importance of selectin-ligand interactions in tumor cell adhesion and metastasis formation. For example, human colon carcinoma cells were divided into the several groups with differently expressed sialyl Lewis^x, and cells with high expression of sialyl Lewis^x showed increased ability to bind to endothelial cells and metastasize into liver [117]. Conversely, selection of colon carcinoma cells for high ability to metastasize into liver was revealed that the metastatic variants expressed a higher level of sialyl Lewis^x than parental cells [118]. In addition, blocking of sialyl Lewis^a with specific antibody resulted in an inhibition of metastasis formation by human pancreatic adenocarcinoma cells in nude mice. Since the adhesion of cancer cells to endothelium requires the presence of endothelial E-selectin as well as carbohydrate ligands on cancer cells [97], the expression levels of E-selectin on the surface of vascular vessels is a secondary rate-limiting factor for this adhesion. In patients with non-small cell lung cancers, a high serum concentration of E-selectin was found to correlate strongly with patients' prognosis [119]. Similarly, elevated levels of serum E-selectin were shown to be indicative of a poor prognosis in patients with colon cancer, when the cancer cells express sialyl Lewis^{x/a} [115]. Interestingly, vascular E-selectin expression is enhanced even in the patients at early stages of cancer having no apparent metastasis [97]. There are many other factors that lead to an induction of vascular E-selectin expression, such as surgical operation, chemo- or radiotherapy.

L-selectin mediated adhesion mechanisms are also demonstrated using L-selectin-deficient mice, in which cancer metastasis is impaired [120]. Recently a sulfated carbohydrate determinant, sialyl 6-sulfo Lewis^x was shown to be the specific L-selectin ligand as mentioned above, and sialyl Lewis^x determinant without 6-sulfation has much weaker binding activity to L-selectin compared to the sulfated ligand. The sialyl 6-sulfo Lewis^x determinant is preferentially expressed on non-malignant epithelial cells, and its expression tends to decrease upon malignant transformation [121]. Therefore, the significance of L-selectin in tumor progression and metastasis process in epithelial carcinomas would be less than the one of E-selectin. On the other hand, some malignant cells of leukocyte origin, such as leukemia or malignant lymphoma, are known to express L-selectin, mediating adhesion of malignant cells to endothelial cells when the latter cells express an appropriate ligand for L-selectin. Actually, some blood vessels in malignant lymphoma lesions are known to express carbohydrate ligands for L-selectin, and this may be involved in the blood-borne tissue infiltration of the malignant lymphoma/leukemia cells.

Cancer metastasis is also impaired in P-selectin-deficient mice, which may point to a role for P-selectin [120]. It has been known that adhesion of platelets to cancer cells plays a significant role in cancer metastasis, therefore, the decrease in cancer metastasis in P-selectin-deficient animals might be due to impaired platelet function in these animals rather than to impairment of cancer cell adhesion to endothelium. Contrary to the evidence, there

are also several reports suggesting possible participation of P-selectin in the adhesion of cancer cells to endothelial cells. However, most epithelial cancer cells lack PSGL-1, which might be the reason why P-selectin plays a less prominent role in cancer cell adhesion compared to E-selectin. Some core proteins, such as CD24, expressed on cancer cells are reportedly capable of replacing the role of PSGL-1 [122], and this can affect P-selectin mediated tumor cell adhesion.

3.3.2. Selectins and Angiogenesis—There is now enough evidence that the interactions between cancer cells and endothelial cells through mediation by selectins might have biological function not only in cancer metastasis, but also in tumor angiogenesis. Among selectin family proteins, both E-selectin and P-selectin seem to be involved in angiogenesis. First of all, E-selectin is involved in capillary-like tube formation and chemotaxis of bovine capillary endothelial cells, and specific antibodies against E-selectin could inhibit the tube formation [123]. Also recombinant human soluble E-selectin was found to stimulate chemotaxis of human umbilical vein endothelial cells (HUVEC) and human dermal microvascular endothelial cells (HMEC) [124]. Moreover, soluble E-selectin has been found to be preferentially synthesized from angiogenic or proliferating endothelial cells, which are presented in human hemangioma, newborn foreskin and placenta tumors [125], and sialyl Lewis^{x/a} determinants or glycolipid ligands of E-selectin and P-selectin were also found to be preferentially synthesized by endothelial cells undergoing differentiation. In addition, antibodies directed against sialyl Lewis^{x/a} were shown to reduce capillary tube formation in endothelial cells. P-selectin was also shown to promote endothelial cell migration, which was blocked by specific anti-P-selectin antibodies, whereas the role of P-selectin is likely to be less important than that of E-selectin [126]. Irrespective of a large amount of evidence, the detailed role of E and P-selectin in angiogenesis *in vivo* is not so clear, since deficient mice develop normally and are able to form new corneal blood vessels in experimentally induced angiogenesis [120], suggesting that there is alternative redundant mechanism, which compensates for the lack of E- and P-selectin. Collectively, these observations served to corroborate the idea that cell adhesion mediated by selectins and sialyl Lewis^{x/a} determinants was significantly involved in tumor angiogenesis.

4. ANNEXINS

The annexin family, which is now recognized as a new lectin family, is originally defined as a family of membrane phospholipid-binding proteins in a Ca^{+2} dependent manner [127]. Ca^{+2} signaling has a pivotal role in the regulation of many cellular processes in all eukaryotic organisms, in particular, through a link between Ca^{+2} signaling and membrane functions. Therefore, annexins are considered to play important roles in the regulation of vesicular transport, membrane-membrane interactions, membrane-cytoskeleton interactions and ion channels by forming networks on the membrane surface [128]. Accordingly, it has also been reported that they are implicated in a wide variety of general cellular processes, including mitogenic signal transduction, apoptosis, and cellular transformation [127, 128]. Generally, they are ubiquitous proteins and share a similar structure characterized by a conserved C-terminal domain with Ca^{+2} binding sites and a variable N-terminal domain. Annexin proteins were initially discovered in the late 1970s, but they were called with diverse and unrelated names referring to their biochemical properties at the time of finding. Subsequent biochemical work such as protein and cDNA sequencing led to the realization that all such proteins shared similar biochemical properties, thus, the common name “annexin” was introduced [127], which was defined as a protein to fulfill two major criteria. First, it must be capable of binding in a Ca^{+2} dependent manner to negatively charged phospholipids. Second, it has to contain as a conserved structural element, “the annexin repeat”, a segment of about 70 amino acid residues.

The first annexin family member discovered with carbohydrate-binding activity was annexin A4 [13]. Accordingly, annexin A5 and A6 have also been found to have a carbohydrate-binding activity as well (see below). This concept that the annexin family can serve as a lectin is relatively new in animal lectin history, therefore little is known about the biological functions of the annexin family in cancer regarding the interactions between annexins and their carbohydrate determinants. However, many annexin family proteins have been found to play several critical roles in tumor progression and metastasis without detailed molecular mechanisms, so these functions might be explained with several specific annexins' lectin activities.

4.1. Annexin Family and Its Expression in Cancer

The annexin family is composed of 12 annexin proteins (annexin A1-A11, A13) in humans, which have molecular weights ranging between 30 and 40 kDa (only annexin A6 is 66 kDa), and each annexin protein often shows a tissue- and cell type-specific expression pattern [128, 129]. Structurally, an annexin protein can be divided into two distinct parts, a highly conserved protein core comprising the C-terminal portion of the molecule and a variable N-terminal region. The N-terminal domains of annexins are diverse in sequence and length (ranging from 11 to 196) on each annexin member. In contrast, the C-terminal regions consisting of four (eight only for annexin A6) α -helical domains of about 70 amino acid residues are well conserved among annexin family proteins. The Ca^{2+} - and phospholipid-binding sites are located in the C-terminal domains.

At least one of the members can be found to express in almost all eukaryotic cell types and the expression levels are rather high. This fact may be helpful in determining whether the task of annexins is unique or have to interact with various ligands both in outside and inside cells to act in multiple roles. In cancer cells, it has been reported that annexin A1 is strongly upregulated in a variety of cancers such as a prostate cancer cell line, esophageal cancer, a stomach cancer cell line, mammary adenocarcinoma, and hepato-carcinoma [130–132]. Moreover, the latter study also showed that annexin A1 is upregulated during normal hepatocyte proliferation after partial hepatectomy and that the proliferative rate of both normal and malignant hepatocytes was attenuated with introduction of antisense oligonucleotides to annexin A1. In addition, overexpression of annexin A2 is observed in a majority of cancers including those of the pancreas, the breast, and the brain [133]. Annexin A4 was reported to be upregulated in renal cell carcinoma [133]. Conversely, it was reported that annexin A1, A2, A4-7, and A11, have been shown to be downregulated in several cancer cells. In particular, the correlation between expression of annexin A6 and growth suppression extends to melanoma, where a genetic screen identified annexin A6 as a protein downregulated in the transition from a non-metastatic to a metastatic phenotype [134]. Similarly, annexin A2 appears to be down-regulated through DNA-methylation in prostate cancer [135], and loss of annexin A5 was identified as a marker for cutaneous squamous cell carcinoma [133]. Annexin A7 is expressed at low levels in the most metastatic malignant melanomas [136], and analysis of annexin A7 expression in prostate tumor microarrays revealed low levels of expression in metastatic and local recurrences of hormone refractory prostate cancer compared with primary tumors. In cancer pathology, an inverse correlation has been observed in the level of expression of annexin A1 and histological grade of head and neck squamous cell carcinoma and in esophageal squamous cell carcinoma [137, 138]. In addition, the advent of global gene expression analysis using *pro*Originally, annexins were known to bind to phospholipids such as phosphatidylserine, phosphatidylethanolamine, teomics and DNA chip technology has also revealed changes in annexin gene expression in numerous cancers and other diseased or stressed cell status [127]. Some of the most striking findings here include upregulation of annexin A5 in

melanomas and annexin A9 in prostate and colon cancers and downregulation of annexin A5 in leukemias and annexin A1 in prostate cancers.

4.2. Carbohydrate Structure for Annexins

Originally, annexins were known to bind to phospholipids such as phosphatidylserine, phosphatidylethanolamine, and phosphatidylinositol, known to be rich in the inner leaflet of the plasma membrane. Now, an increasing number of studies have revealed that several annexin family proteins can also bind to specific carbohydrate structures (Fig. (4)). Among annexin family proteins, at least, annexin A4, A5, and A6 have been found to have specific lectin binding activity. As mentioned above, annexin A4 was firstly found to have lectin activity in the annexin family proteins with the fact that Ca^{2+} -dependent carbohydrate-binding proteins (p33/41) purified from bovine kidney extracts turned out to be identical to annexin A4 [13]. The amino acid sequence of this protein showed neither conserved sequences corresponding neither to known CRDs of animal lectins nor to motifs reported for heparin-binding proteins. Subsequently, annexin A4 has been found to bind to a wide variety of sialoglycoproteins and glycosaminoglycans, including heparin, heparin sulfate, and chondroitin sulfates A, B, C in the presence of Ca^{2+} [139]. Annexin A4 protein is highly expressed in the tissues of kidney and pancreas, and the result of *in vitro* binding assay of several glycoconjugates suggest that annexin A4 is involved in the formation of apical sorting (secretory) vesicles due to the interaction with some GPI-anchored glycoproteins and proteoglycans such as GP-2 [140]. It was suggested that GP-2 is an endogenous ligand of annexin A4 through protein-carbohydrate recognition. Annexin A5 also binds to glycosaminoglycan structures of heparin and heparin sulfate, but not to any chondroitin sulfate structure. [139]. In addition, this protein can bind to a sulfatide, one of the glycosphingolipids of the platelet cell membrane [141]. Specifically, annexin A5 has also been documented to bind a “bisecting GlcNAc structure”, a unique structure found in *N*-linked oligosaccharide [142]. This oligosaccharide structure is formed by the attachment of a 1-4GlcNAc residue to a core β -mannose, which is catalyzed by β -1, 4-*N*-acetylglucosaminyltransferase (GnT-III), referred to a bisecting GlcNAc. On the other hand, annexin A6 was bound to glycosaminoglycan carbohydrate structures, but its binding manners seem to be different; heparin and heparan sulfate in a Ca^{2+} -independent manner, and chondroitin and chondroitin sulfate in a Ca^{2+} -dependent manner [139]. Nevertheless, evidence in support of crucial roles for any annexins in the development of cancer or in cancer cell metastasis regarding to carbohydrate-protein interactions is still mainly circumstantial.

4.3. Annexins in Tumor Progression

It is perhaps reasonable that the annexin family can affect tumor formation process through the dynamic, significant gene expression changes consistently associated with cancer progression status, since almost every solid-state, gene-chip or differential display study seems to pull out the annexin family proteins among the genes differentially regulated in neoplasia [133]. There is also the evidence that cell types at different developmental stages often have very variable annexin expression profiles [127].

In cancer biology, annexins A1 and A2 were first discovered as major cellular substrates for phosphorylation on tyrosine by the EGF receptor and the transforming gene product of the Rous sarcoma virus [127], respectively, implicating these proteins in signaling pathways known to be involved in tumor transformation. In fact, it was reported a correlation between the level of annexin A1 expression in RAW 264.7 macrophages and the cellular responsiveness to lipopolysaccharide (LPS) of components of the mitogen-activated protein (MAP) kinase pathway [143]. A similar investigation reported a correlation between annexin A1 expression and mobilization of intracellular Ca^{2+} in MCF-7 breast carcinoma

cells [144]. Another study provided evidence that annexin A1 overexpression in rat 2 fibroblasts leads to direct inhibition of cytosolic PLA₂, which in turn depresses the serum response element of c-fos [145]. Collectively, these studies all imply a growth-suppressive role for annexin A1 despite the apparent mechanistic diversity underlying each case. Other annexin family proteins have also been linked with cell growth and transformation, frequently in studies using similar experimental designs to those described above for annexin A1. For example, overexpression of annexin A5 in MCF-7 cells leads to inhibition of phorbol ester-mediated activation of the MAP kinase pathway [146], and heterologous expression of annexin A6 at physiological levels in human A431 carcinoma cells leads to inhibition of growth factor-mediated Ca²⁺ influx and impaired tumor formation in nude mice [147]. Annexin A6 was also shown to be downregulated in murine metastatic melanoma B16-F10 cells compared to a melan-a-immortalized melanocyte cell line [134]. These results indicate a potential role of annexin A6 as tumor suppressor molecules in malignant progression. Moreover, annexin A7 has also been implicated as a tumor-suppressor gene, in a study of micro-dissected tumor cells derived from prostate tumors [148]. The authors showed significant loss of heterozygosity in a region around the annexin A7 gene. Using micro-arrays the same group showed a higher rate of loss of annexin A7 expression in metastatic and local recurrences of hormone refractory prostate cancer as compared with primary tumors [148]. More recently, they suggest that elevated levels of annexin A7 are also associated with metastatic disease and decreases in survival time in breast cancer [149]. These investigators also demonstrated that an annexin A7 hemizygous mouse demonstrated genomic instability, frequent loss of other tumor-suppressor genes, and was prone to cancer [150]. In contrast, an independently derived annexin A7 null mutant mouse generated in a different laboratory was found to be normal in this regard and indeed had virtually no overt phenotype [151]. Recently, another group has also implicated annexin A7 in prostate cancer in an independent study [152]. The expression of annexin A1 and A2 was significantly reduced as tumors progressed from benign prostatic epithelium to androgen stimulated prostate cancer. At this stage, levels of annexin A7 remained constant, but the protein was lost as the cells progressed further to recurrent prostate carcinoma.

Several studies have been suggested that changes in annexin expression during tumorigenesis may result in resistance to chemotherapeutic agents. For example, overexpression of annexin A1 correlates with the multiple drug resistance of numerous cancer cell lines. Overexpression of this protein in drug-sensitive MCF-7 cells rendered them 2~5-times less sensitive to treatment with adriamycin, melphalan, and etoposide; whereas anti-sense down-regulation of annexin A1 in drug-resistant MDA-MB-231 cells resulted in 3~8-times more sensitive to these drugs [153]. In various carcinoma cell lines upregulation or overexpression of annexin A4 was shown to lead to drug resistance to paclitaxel [154] and in a chicken pre-B lymphocyte cell line (DT40) targeted disruption of annexin A5 resulted in resistance to a range of pro-apoptotic stimuli [155].

4.4. Annexins in Tumor Metastasis

It is not also difficult to imagine that the acquisition of a metastatic tumor cell phenotype could be mediated in part by changes in expression of annexin family proteins, since they seem to play a pivotal role in linking the plasma membrane to the cytoskeleton, the maintenance of cell-cell junctions, and in the turnover of membrane components during endocytosis [128]. Furthermore, annexin proteins are exported from cytosol to the outside of cells across the plasma membrane [127]. Although annexins lack hydrophobic signal peptides, secretion and expression on cell surface experienced by some annexins are evident in some cell types. After being exported from the cells, some annexins have been shown to function as receptors for extracellular proteins. For example, annexin A5 binds to collagen and annexin A2 binds to tissue plasminogen activator and tenascin [156]. Moreover,

involvement of some annexins in cell-adhesion (or inhibition of cell-adhesion) has been found.

Annexin A2 is relatively known among annexin family proteins to play several roles in cellular adhesion. Annexin A2 and its binding partner S100A10 have both been shown to directly interact with a splice variant of carcinoembryonic antigen cell adhesion molecule-1 (CEACAM1). This epithelial cell adhesion molecule is down-regulated in colon, prostate, breast, and liver cancers [157]. In cell lines derived from gastric carcinoma, higher levels of expression of annexin A2 correlated with c-erbB-2 overexpression, differentiation, metastasis, and poorer prognosis [158]. Annexin A6 could play some roles in cell adhesion of tumor cells through its carbohydrate binding activity. Chondroitin sulfate proteoglycans, which are rich in the extracellular matrix and one of the annexin A6 ligands, inhibit cell-substratum adhesion through their chondroitin sulfate chains, whereas these chains did not prevent cell attachment presumably due to specific receptor for this glycosaminoglycan. A431 cells, which express no annexin A6 expression, were unable to attach to the chondroitin sulfate substratum, and conversely, the stable transfectants expressing annexin A6 conferred the ability of this attachment, suggesting that annexin A6 is a candidate receptor for chondroitin sulfate chains [159]. In addition, annexin A5 may have some effects on cell adhesion, tumor metastasis through its interaction with its ligand glycoproteins since the bisecting GlcNAc structure, carbohydrate determinants for annexin A5, can be a negative sorting signal for cell surface glycoproteins [160]. In more recent study, heat shock protein 47 (Hsp47), a stress related glycoprotein in the ER that binds specifically to various types of collagens and procollagens, is a novel binding partner of annexin A5 mediated by a bisecting GlcNAc oligosaccharide structure [161], whereas the detailed biological functions are still unknown.

Future studies should aim at identifying endogenous glycoconjugate ligands recognized by annexins in a variety of cases and investigating the carbohydrate recognition mechanism.

5. CONCLUSIONS

The studies reviewed in this article have documented the functional roles of carbohydrate-binding proteins (lectins) and their ligands in the process of tumor progression and metastasis. We selected three animal lectin family; galectins, selectins, and annexins for that purpose, but the most extensive work in regard to cancer biology has been carried out on galectin family proteins. Galectins are α -galactoside carbohydrate structure binding proteins, and these proteins play various roles in this process inside and outside cells. In particular, there are an increasing number of direct evidences that galectin-1 and/or galectin-3 are associated with many stages from malignant cellular transformation to cancer metastasis. Selectins are sialyl Lewis ^a carbohydrate structure binding proteins, and these proteins mainly play a role in tumor cell adhesion and metastasis step. Annexins are novel carbohydrate structure binding proteins, and these proteins seem to play various roles in multiple steps presumably similar to the galectins' functions.

As everyone understands, there have been many distinct, complicated, assorted biological processes during tumor progression *in vivo*. Moreover, it is no doubt that those processes should be much variable among several tumor groups or even in individual. Presumably, aberrant glycosylation on specific glycoproteins or glycolipids is deeply related to this diversity of tumor behavior, therefore, understanding the biological functions and significances of glycosylation including carbohydrate determinants and lectin interactions in tumor will reach the goal of cancer research. So far, it has been very difficult to find the detailed carbohydrate structure on certain glycoproteins or glycolipids as well as the binding partner for several lectins. However, the development of mass spectrometry analyses to

detect the accurate carbohydrate structure with small amount of glycoproteins has been observed, and this technique will probably become the main tool to search each tumor related carbohydrate structure on glycoproteins or glycolipids. Meanwhile, one might argue with the usage of the gene transfection approach, i.e., overexpression of several lectins, to prove the exact biological functions including the carbohydrate-lectin interactions, since the experimental results from this approach do not reflect *de facto* phenomenon *in vivo* and might show artificial development. In this regard, more restricted studies, e.g., using small interfering RNA (siRNA) approach or cancer cells derived from specific lectin deficient animal, will be useful or rather essential for understanding accurate biological functions of specific lectins in cancer biology.

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ABBREVIATIONS

GlcNAc	<i>N</i> -acetylglucosamine
GnT	<i>N</i> -acetylglucosaminyltransferase
NeuAc	<i>N</i> -acetylneuraminic acid
Fuc	Fucose
GalNAc	<i>N</i> -acetylgalactosamine
Glc	Glucose
Gal	Galactose
Ido	<i>L</i> -iduronic acid
GlcA	<i>D</i> -glucuronic acid
Cer	Ceramide
Man	Mannose

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Fig. (1).
Cell-Cell and Cell-Matrix interactions by lectins.

Structure		Binding activity	
		Gal-1	Gal-3
Gal β 1-4Glc (Lactose)	→	+	+
Gal β 1-4GlcNAc (<i>N</i> -acetylglucosamine)	→	++	++
NeuAc α 2-3Gal β 1-4GlcNAc	→	±	+
Gal α 1-3Gal β 1-4GlcNAc	→	—	++
Gal β 1-4Glc Fuca1 ²	→	±	+
GalNAc α 1-3Gal β 1-4Glc Fuca1 ²	→	±	++++
Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc	→	++	++++++
Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc Fuca1 ³	→	±	+++
Fuca1 ⁴ -3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc	→	±	+++

Fig. (2). Carbohydrate structures for galectin family proteins

Relative carbohydrate binding activities of galectin-1 (Gal-1) and galectin-3 (Gal-3) are depicted compared to the lactose binding activity (+), respectively. Relative activities are estimated with the combined results from Ref. [27 and 28].

	Structure	Binding activity			Ref.
		L-S	E-S	P-S	
Sialyl Lewis ^x		+	+	+	(101) (102)
Sialyl Lewis ^a		+	+	+	
Sialyl 6-sulfoLewis ^a		+	—	—	(104)
Heparin Heparan sulfate Sulfatide		—	+	—	(111)
	(see Figure 4)	+	—	+	(114)

Fig. (3). Carbohydrate structures for selectin family proteins

Specific carbohydrate binding activities of L-selectin (L-S), E-selectin (E-S), and P-selectin (P-S) are depicted.

	Structure		Binding activity			Ref.
			A4	A5	A6	
Heparin	-4IdoA α 1-4GlcNAc β 1-	➡	+	+	+	(139)
Heparan sulfate	$ \begin{array}{c} \text{SO}_3^- \\ \\ \text{6} \\ \\ -4\text{GlcA}\beta 1-4\text{GlcNH}_2\beta 1- \\ \quad \\ \text{3} \quad 2 \\ \quad \\ \text{SO}_3^- \quad \text{SO}_3^- \end{array} $	➡	+	+	+	
Chondroitin sulfate	$ \begin{array}{c} \text{SO}_3^- \\ \\ \text{6} \\ \\ -4\text{GlcA}\beta 1-3\text{GalNAc}\beta 1- \end{array} $	➡	+	—	+	
Sulfatide	$ \begin{array}{c} \text{Gal}\beta 1-1'\text{Cer} \\ \\ \text{3} \\ \\ \text{SO}_3^- \end{array} $	➡	—	+	—	(141)
Bisecting GlcNAc	$ \begin{array}{c} \text{GlcNAc}\beta 1-2\text{Man}\alpha 1 \\ \\ \text{6} \\ \\ \text{GlcNAc}\beta 1-4\text{Man}\beta 1- \\ \\ \text{3} \\ \\ \text{GlcNAc}\beta 1-2\text{Man}\alpha 1 \end{array} $	➡	—	+	—	(142)

Fig. (4). Carbohydrate structures for annexin family proteins

Specific carbohydrate binding activities of annexin A4-A6 (A4-A6) are depicted.

Table 1

Classification of Major Animal Lectins

Lectin	Soluble Type	Transmembrane Type	Ref.
C-type lectin	Lecticans Collectins, The Reg group, The tetranectin group, EMBP, SEEC, CBCP/Frem1/QBrl, CK	The ASGR group (asialoglycoprotein receptor subgroup, DC-SIGN subgroup, macrophage receptors, Langerin and Kupffer cell receptors, scavenger receptor) Selectins (L-, P-, E-selectin), NK receptors, Multi-CTLD endocytic receptors, The chondrolectin group Polycystin 1 Attractin DGCR2, The thrombomodulin group Bimlec	[12]
S-type lectin	Galectin family (Galectin-1-15)	(Galectin-4)	[15] [20] [162]
I-type lectin		Siglecs (Siglecs-1-13) Non-siglec proteins (NCAM, P0, ICAM-1, CD83, CD2, Hemolin)	[163] [164]
L-type lectin		ERGIC-53 VIP-36	[95]
P-type lectin		CD-MPR IGF-II/MPR	[165]
Pentraxins	CRP SAP PTX3 PTX4 NP1 NP2	NPR	[166]
Annexins	Annexin family (Annexin-A4, 5, 6)		[20] [95]
Calnexin group	Calreticulin	Calnexin Calmeglin	[95] [167]

Table 2

Galectin Family Proteins and Their Ligands in Tumor Progression and Metastasis

Biological Function	Galectin Family	Ligand(s)	Ref.
Intracellular			
Tumor transformation	Galectin-1 Galectin-3	H-Ras K-Ras	[40, 41]
Signal transduction changes	Galectin-3	EGF-R, TGF- β -R	[45]
Cell cycle regulation (Cell proliferation)	Galectin-3	SP1-CRE site TTF-1	[48, 51]
Anti-apoptosis	Galectin-3	Bcl-2 Synexin	[57, 59]
Extracellular			
Cell cycle regulation	Galectin-1	$\alpha_5 \beta_1$ integrin	[49]
T-cell apoptosis	Galectin-1 Galectin-3	CD2, 3, 7, 43, 45 CD7, 29	[66, 69]
T-cell signaling changes	Galectin-1 Galectin-3	TCR, CD98	[67,70, 71]
Cell-matrix interactin	Galectin-1 Galectin-3	Laminin, Fibronectin, Collagen IV	[80–84]
Cell adhesion	Galectin-1 Galectin-3 Galectin-8	$\alpha_7 \beta_1$ integrin $\alpha_1 \beta_1$, $\alpha_3 \beta_1$ integrin $\alpha_3 \beta_1$, $\alpha_1 \beta_1$, M integrin	[80, 83, 85–87]
Cell-cell interaction	Galectin-3	Mac-2 binding protein	[91]