Glycoconjugate Journal 19, 543–549, 2004

Galectin-3 and metastasis

Yukinori Takenaka[1], Tomoharu Fukumori[1] and Avraham Raz[1,2]

[1] *Tumor Progression and Metastasis Program, Karmanos Cancer Institute, Wayne State University, Detroit, Michigan 48201, USA,*
[2] *Department of Pathology, Wayne State University, School of Medicine, Detroit, Michigan 48201, USA*

Galectin-3, a 31 kDa member of the β-galactoside-binding proteins, is an intracellular and extracellular lectin which interacts with intracellular glycoproteins, cell surface molecules and extracellular matrix proteins. Galectin-3 is expressed widely in epithelial and immune cells and its expression is correlated with cancer aggressiveness and metastasis. Galectin-3 is involved in various biological phenomena including cell growth, adhesion, differentiation, angiogenesis and apoptosis. Recent research revealed that galectin-3 is associated with several steps of invasion and metastasis, like angiogenesis, cell-matrix interaction, dissemination through blood flow and extravasation. Recently, we and others have shown that galectin-3 can be a reliable diagnostic marker in certain cancers and one of the target proteins of cancer treatment. In this review, we describe the involvement of galectin-3 in each steps of metastasis and clinical significance of galectin-3.
Published in 2004.

Keywords: **galectin-3, metastasis, cancer**

Abbreviations: **CP, citrus pectin; CRD, carbohydrate recognition domain; ECM, extracellular matrix; HUVEC, human umbilical endothelial cells; K_d, dissociation constant; LAMP, lysosome associated membrane protein; MCP, modified citrus pectin; MMP, matrix metalloproteinase; NWGR, asparagine-tryptophan-glycine-arginine; PIN, prostatic intraepithelial neoplasia.**

Introduction

Galectin-3, previously described as IgE binding-protein, CBP35, CBP30, Mac-2, L-29, L-31 and L-34, is one of the β-galactoside-binding proteins, that bind to the carbohydrate portion of cell surface glycoproteins or glycolipids. Recent studies have revealed that galectin-3 is expressed broadly in normal and neoplastic cells, and regulates cell growth, cell adhesion, differentiation and cell death. It has been shown that galectin-3 expression correlates with neoplastic transformation in some types of cells. More recently, we and other investigators have demonstrated that the expression of galectin-3 is correlated with metastatic potential in certain malignancies. Therefore, the detection of galectin-3 may be of diagnostic value. Here, we describe the evidence for galectin-3 to play an important role in tumor progression and metastasis.

Structure and distribution of galectin-3

Galectin-3 is an intracellular and extracellular lectin which is presumed to interact with glycoproteins of the cell surface matrix. It consists of three domains: a NH2-terminal domain; a repetitive collagen-like sequence rich in glycine, proline, and tyrosine; and a COOH-terminal carbohydrate recognition domain (CRD, lectin domain). The NH2-terminal domain contains only 12 amino acids that control its cellular targeting. The COOH-terminal domain, cleavable with trypsin, contains the carbohydrate recognition domain consisting of 140 amino acid residues, which define the molecule as a galectin [1–3].

Galectin-3 is expressed widely in epithelial and immune cells (Table 1). The expression of galectin-3 is associated with tumor invasion and metastatic potential in human head and neck, thyroid, gastric, and colon cancers (Table 2). In mice, we previously showed that metastatic potential of mouse melanoma and fibrosarcoma cells correlates with the extent of galectin-3 expression on the cell surface [18]. In contrast, for some tumors such as breast, ovarian and prostate cancer, the expression of galectin-3 is inversely correlated with metastatic potential (Table 2). Thus, the levels of galectin-3 expression depend on the organ or tissue, suggesting that tumor or tissue specific factors may modulate the galectin-3 expression.

Galectin-3 and metastasis

Metastasis is a major fatal complication associated with malignancies. Understanding of molecular mechanism of metastasis

To whom correspondence should be addressed: Avraham Raz, Ph.D., Karmanos Cancer Institute, 110 E. Warren Ave., Detroit, MI 48201, USA. Tel: (313) 833-0960; Fax: (313) 831-7518; E-mail: raza@karmanos.org; mailto:raza@karmanos.org

Table 1. Distribution of galectin-3 in human cells

Tissue	Reference
Normal tissue	
Monocytes and macrophage	[4]
Gastric mucosa	[5]
Colon	[6,7]
Mammary epithelium	[8]
Prostate	[9]
Neoplastic tissue	
Anaplastic large cell lymphoma	[10]
HTLV-1 infected T cells	[11]
Thyroid carcinoma	[12]
Breast carcinoma	[8]
Gastric carcinoma	[5]
Colon carcinoma	[6,7]
Ovarian carcinoma	[13]
Prostate carcinoma	[9]
Melanoma	[14]

Table 2. The correlation in neoplastic cells between galectin-3 expression and tumor progression

Tissue	Reference
Positive correlation	
Anaplastic large cell lymphoma	[10]
Head and neck squamous cell carcinoma	[15]
Thyroid carcinoma	[12]
Gastric carcinoma	[5]
Negative correlation	
Breast carcinoma	[8]
Ovarian carcinoma	[13]
Prostate carcinoma	[9]
Conflicting data	
Colon carcinoma	[6,7,16,17]

Table 3. Metastasis related functions

Function	Reference
Angiogenesis	
Stimulates capillary tube formation *in vitro* and neovasularization *in vitro*	[21]
Adhesion to ECM	
Promotes adhesion of neutrophils to laminin	[22]
Promotes adhesion of breast cancer cell line Evsa-T to laminin, fibronectin, vitronectin	[23]
Promotes adhesion of breast cancer cell line BT549 to laminin, collagen IV	[24]
Inhibits melanoma, fibrosarcoma, breast, prostate cancer cells to laminin, collagen IV, fibronectin	[25]
Integrin expression	
Increases $\alpha 6$ $\beta 1$ integrin expression in BT549	[24]
Increases $\alpha 4$ and $\beta 7$ integrin expression in Evsa-T	[23]
Reduces $\beta 1$ integrin in MDA-MB-231	[26]
Dissemination through blood flow	
Protects BT549 from anoikis	[27,28]
Tumor embolism	
Mediates homotypic cell aggregation	[29,30]
Extravasation	
Binds to T antigen and mediates tumor cell adhesion to endothelium	[14,31]

is the important goal of cancer research. In many cases, tumor cells are disseminated through the blood flow. This transvenous metastasis is a complex process, involving many cell-cell and cell-extracellular matrix interactions. The process of metastasis requires the following steps. (1) growth at the primary site, (2) angiogenesis at the primary site, (3) detachment from the primary site, (4) invasion through the extracellular matrix, (5) dissemination of cells through the blood flow, (6) tumor embolous formation in capillaries, (7) extravasation, (8) growth at the secondary sites (Figure 1) [19,20].

Angiogenesis and galectin-3

Angiogenesis is a complex multistep process and is associated with inflammation, wound healing, tumor growth, and metastasis [32]. The formation of new vessels is required for tumor growth at the primary and secondary sites and provides a gateway to the dissemination through the blood flow. Carbohydrate recognition may have a pivotal role in angiogenesis because angiogenic factors like fibroblast growth factor family and vascular endothelial growth factors bind initially to the extracellular matrix proteoglycans before binding to their cognate receptors, and some of the adhesion molecules bind to glycoconjugates present on the surface of the endothelial cells [33]. Galectin-1, with a carbohydrate affinity similar to that of galectin-3, has been shown to be mitogenic toward vascular endothelial and smooth muscle cells [34,35]. Recently we have demonstrated that galectin-3 is involved in tumor related angiogenesis [21]. Galectin-3 stimulates *in vitro* capillary tube formation by human umbilical endothelial cells (HUVEC) and *in vivo* neovascularization. This capillary tube formation was inhibited by 50 mM lactose, a competitive disaccharide, and 0.1% of pH-modified citrus pectin, a polysaccharide which competes with natural ligand recognition by galectin-3. These facts suggest the involvement of carbohydrate-binding in angiogenesis induced by galectin-3. Similarly, galectin-3 stimulated chemotaxis of the HUVEC and binding of galectin-3 to HUVEC was inhibited by lactose and modified citrus pectin. The binding of galectin-3 to HUVEC cell surface receptors is mediated by the carbohydrate-binding domain of galectin-3. Scatchard plot analysis revealed the presence of two galectin-3 receptors on the HUVEC cell surface: a high-affinity one with a K_D of 0.537×10^{-9} and a low-affinity one with a K_D of 7.161×10^{-9}. Their numbers per cell were 5.81×10^3 and 3.2×10^4. However, the endothelial cell surface receptors for galectin-3 are not yet identified.

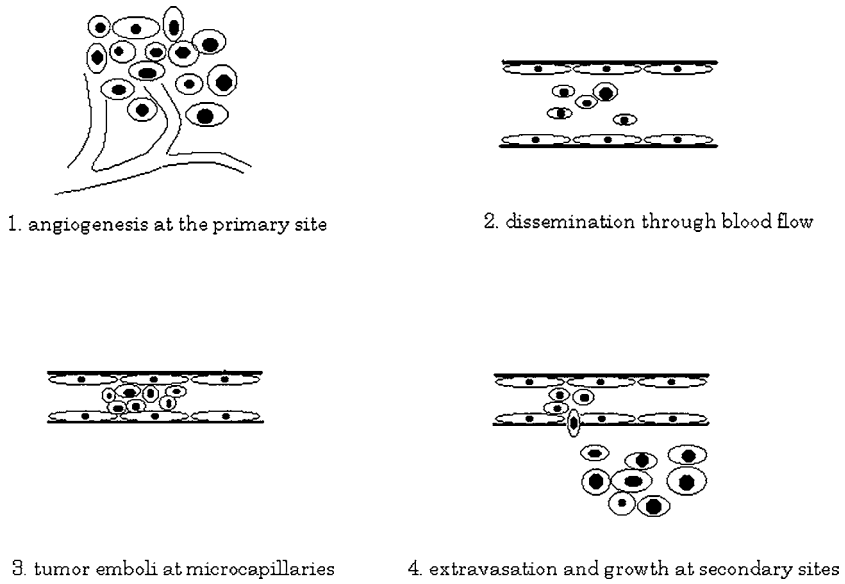

Figure 1. Metastatic cascade. The metastatic cascade involves several steps, including angiogenesis, dissemination through blood flow, tumor embolism formation and extravasation.

Cell-matrix interaction and galectin-3

Most of the mammalian cells are surrounded by extracellular matrix and express various cell adhesion molecules. Cell adhesion molecules mediate binding of cells to extra cellular matrix, homotypic cell adhesion, and heterotypic adhesion. Many cell adhesion molecules are transmembrane proteins, which have an extracellular domain, an intracellular domain and a transmembrane domain. Although galectin-3 lacks a transmembrane domain and a classical signal peptide for secretion, it is konwn to work on the cell surface and in the extracellular environment [36] and bind laminin, Lamp I and II, IgE and Mac-2 binding protein [14,36–38].

Laminin, the major basement membrane glycoprotein, regulates various cellular functions through its binding to integrins and non-integrin laminin binding proteins. Galectin-3 is one of the non-integrin laminin binding proteins [38]. Galectin-3 on the cell surface fails to modulate adhesion of melanoma cells to laminin [39]. Soluble galectin-3 doesn't alter melanoma cell adhesion to laminin but oligomerized galectin-3 induces melanoma cell spreading on laminin [40]. Exogenous galectin-3 promotes adhesion of neutrophils to laminin but this adhesion may be the result of the lectin induced activation of neutrophils [22]. Soluble galectin-3 inhibits melanoma, breast cancer, fibrosarcoma and prostate cancer cells adhesion to laminin [25]. Taken together, although galectin-3 binds to laminin, galectin-3 is not a key direct mediator of cell adhesion to laminin but rather may act indirectly by regulating other molecules such as integrins.

Integrins are expressed in all kinds of cells and associated with embryogenesis, wound healing, inflammation, apoptosis, cell proliferation, tumor growth and metastasis. Members of the integrin family work as receptors for fibronectin, laminin, vitronectin, collagen and other cell membrane protein. The role of integrins is not only binding of cells to matrix but also signal transduction which regulates cell proliferation, survival, differentiation, motility [41]. We and others have demonstrated that transfection of galectin-3 into breast cancer cell lines increased cell surface galectin-3 expression and enhanced cell adhesion to laminin and other ECM components [23,24]. At the same time, galectin-3 increased some integrins expression. In the galectin-3 transfected breast cancer cell line BT549, the expression of $\alpha 6\beta 1$ integrin, which is associated with tumor invasion [42], is up-regulated [24]. Recombinant galectin-3 was shown to bind $\alpha 1\beta 1$ integrin, a receptor for laminin, collagen [25]. Addition of recombinant galectin-3 induced endocytosis of integrin $\beta 1$ in MDA-MB-231 breast carcinoma cells [26]. Collectively, the main role of galectin-3 in cell-matrix interaction may be alteration of integrin expression and modulation of signals triggered by the adhesion to matrix.

Galectin-3 is a substrate for MMP-2 and MMP-9. Cleavage by MMP generates a 22 kDa fragment with carbohydrate recognition domain and 9 kDa fragment comprising of amino terminal end of galectin-3 [43]. This 22 kDa fragment binds 1.5–2 times more tightly to glycoconjugates but fails to self-associate [44]. Although the biological function of cleaved galectin-3 is unknown, there is a possibility that this cleavage may have some role in tumor metastasis because increased expression of MMP, especially MMP-2, is known to be associated with tumor aggressiveness [45].

Anoikis and galectin-3

Tumor cells inoculated i.v. are usually eliminated quickly. Only 0.1–0.01% of inoculated cells develop metastatic foci in the lungs [46]. Although the mechanisms of tumor cell destruction

still remain unclear, it was found that natural killer cells play a role in tumor cell elimination. In natural killer cell-depleted mice, tumor cell survival increased, but a vast majority of tumor cells died [47]. During dissemination through blood flow, tumor cells can't adhere to ECM, and under this condition, they may die as a result of anoikis. Anoikis is a kind of apoptosis, which is induced by disruption of cell-matrix interaction [48]. Selection of melanoma cells for anoikis resistance results in an increase in their metastatic potential [49]. We have recently shown that overexpression of galectin-3 protects cells from anoikis as well as other apoptotic stimuli [27,28,50]. When cultured in suspension, galectin-3 transfected BT549 respond to the loss of cell adhesion by inducing G1 arrest, though parental BT549, phosphorylation site mutant galectin-3 transfected BT549 and NWGR motif mutant galectin-3 transfected BT549 undergo anoikis. This implies that phosphorylation of galectin-3 and the NWGR motif, which is also present in the BH1 domain of Bcl-2 family protein, are required for the anti-apoptotic effect of galectin-3. However, how the phosphorylation and the NWGR motif act against the anoikis is not known yet. This galectin-3 mediated anoikis resistance may confer advantage during dissemination through blood flow on tumor cells.

Tumor Embolism and Galectin-3

The formation of secondary tumors by circulating cancer cells correlates with an increased tendency of the cells to form emboli by aggregation with other tumor cells or with host cells. Aggregation of tumor cells leads to the embolization in microcapillaries and the tumor embolous formation is followed by extravasation at secondary sites. Tumor cells injected as aggregates forms more lung colonies in mice than those injected as single cells [51]. A strong correlation has been demonstrated between the *in vitro* aggregation property and the *in vivo* metastatic potential [52]. We have shown that pH-modified citrus pectin inhibit aialofetuin induced homotypic aggregation of B16-F1 melanoma cells *in vitro* [53] and cell surface galectin-3 mediates homotypic cell adhesion by bridging through branched, soluble complementary glycoconjugates [29]. Mac-2-binding protein, originally identified as a ligand for galectin-3 [54], is a heavily N-glycosylated secreted protein and induces homotypic aggregation of melanoma cells. This aggregation is inhibited by lactose or anti-galectin-3 antibody. This suggests that Mac-2-binding protein interacts with galectin-3 on tumor cell surfaces, resulting in the formation of multicell aggregation [30]. Moreover, intravenous injection of B16-F1 melanoma cells with 0.5% MCP into synergenic mice resulted in a significant decrease of lung colonization [55]. In conclusion, galectin-3 induces homotypic aggregation, resulting in tumor embolism, and increases metastatic potential.

Nitric oxide-induced apoptosis and galectin-3

As a result of tumor embolism, microscopic infarcts develop, followed by re-establishment of blood flow. This ischemia-reperfusion injury causes the production of toxic reactive nitrogen and oxygen species such as nitric oxide. The highly metastatic cancer cells are reported to be resistant to ischemia-reperfusion injury [56]. Moon *et al.* found that galectin-3 transfected BT549 cells are more resistant to apoptosis induced by hepatic ischemia/reoxygeneration than galectin-3 null BT549 cells [57]. *In vivo* experiments showed that only galectin-3 transfected BT549 cells formed metastatic colonies in the liver after intrasplenic injection [58]. On the other hand, galectin-3 null BT549 cells didn't form colonies due to the cytotoxicity of nitric oxide. Thus, galectin-3 inhibits nitric oxide induced apoptosis and enhances liver metastasis.

Galectin-3 on endothelial cell surface

In the processes of inflammation, immune cells circulating through blood extravasate and migrate to the site of inflammation. In the first step of extravasation, cells bind to endothelial cells followed by penetrating through the layers of endothelial cells and basement membrane. This binding between circulating cells and endothelial cells is mediated by specific interactions between cell surface lectins and carbohydrates present on glycoproteins, glycolipids and glycosaminoglycans. Galectin-3 as well as galectin-1 is also expressed on the surface of endthelial cells of various organs [59] Both galectins are involved in extravasation in inflammation. Anti galectin-1 antibody inhibits adhesion of lymphoma cells to endothelial cells [59].

Metastasis of cancer also requires extravasation of cells, like inflammation. Thomsen-Friedenreich antigen (T antigen), which has β-galactose as the terminal residue, is involved in adhesion of tumor cells to endothelium. An inhibitory peptide and antibody against T antigen inhibited adhesion of tumor cells to endothelial cells [31]. Recombinant galectin-3 binds to T antigen in a dose-dependent manner. Moreover, when tumor cells and endothelial cells bind together, galectin-3 on endothelial cells clusters at the sites of the contact with tumor cells and galectin-1 on tumor cells accumulates at the sites of cell-cell contacts predominantly [31]. Thomsen-Friedenreich antigen on the surface of tumor cells and galectin-3 on the surface of endothelial cells play a leading role in docking cancer cells onto endothelial cells [60]. Therefore, not only galectin-3 on tumor cell surface but also galectin-3 on endothelial cell surface contribute to metastasis.

Clinical significance of galectin-3

A series of clinical evidence have been reported to support correlation between galectin-3 expression and malignant transformation. Consequently, diagnostic and prognostic significance of galectin-3 have been shown for some types of tumors, but there is conflicting data for other types (Table 2). Metastasis is one of the most important factors of death in patients with malignant tumors, and tumor biomarkers which can predict metastasis and prognosis are important for clinical management of malignancies. Iurisci *et al*. reported that circulating levels of galectin-3 in the sera of patients with breast, gastrointestinal,

lung, or ovarian cancer, melanoma, and Hodgkin's lymphoma were elevated and circulating levels of galectin-3 reflect biological aspects of tumor behavior associated with a metastasizing phenotype [61]. Gillenwater *et al*. have reported that galectin-3 localized to superficial mucosal layers in head and neck squamous cell carcinoma (HNSCC), and adjacent to keratin pearls in invasive carcinoma [15]. Xu *et al*. have revealed that malignant thyroid tumors of epithelial cell organ and metastatic lymphnodes of papillary carcinomas exhibit high levels of galectin-3 as well as galectin-1 while neither benign thyroid tumors nor normal adjacent tissues express them [12]. Irimura *et al*. have revealed a direct correlation in human colon cancers between galectin-3 expression and tumor progression [6]. In our study on colon cancers, galectin-3 expression elevates according to Dukes' stage and its overexpression correlates with decreased survival of patients [16]. In contrast, Castronovo *et al*. reported that normal breast tissue expressed high levels of galectin-3 by immunoperoxidase staining, whereas galectin-3 was downregulated in breast cancer cells [8]. In ovarian cancer, Van den Brule *et al*. reported that galectin-3 expression was downregulated in cancer cells compared with the normal tissues [13]. We have revealed that galectin-3 is also downregulated in prostate cancer. Approximately 60–70% of the normal tissue examined demonstrated heterogenous expression of galectin-3, while in stage II tumors, there was a dramatic decrease in galectin-3 expression in both PIN and tumor sections, with only 10.5% of these samples expressing this protein [9]. These results suggest that galectin-3 has tumor progressive or inhibitory effects, depending on tumor cell types. The variations in galectin-3 expression *in vivo* may depend on tumor-specific factors.

Galectin-3 is one of the promising target proteins for cancer treatment. We have reported a novel therapy using modified citrus pectin (MCP). Citrus pectin (CP) is a complex polysaccharide obtained from citrus fruits. MCP is produced from CP via pH and temperature modification that breaks it into shorter, non-branched, galactose-rich, carbohydrate chains. Although CP is unable to interact with galectin-3, the modification makes MCP a ligand for galectin-3 [55]. Therefore, MCP can work as a competitive inhibitor for natural ligands of galectin-3. MCP has been shown to inhibit homotypic aggregation of tumor cells, angiogenesis and tumor cell adhesion to endothelial cells *in vitro* [21,55,62]. Intravenous injection of 0.5% MCP inhibited experimental lung metastasis of B16-F1 melanoma cells although CP promoted the metastasis [55]. Oral intake of MCP inhibited the spontaneous metastasis of rat prostate adenocarcinoma cells and human breast carcinoma cells [61,62]. Recent studies have revealed that galectin-3 null mutant mice are relatively healthy [64], suggesting galectin-3 inhibitors may be therapeutically valuable without causing severe side effects.

Future prospects of galectin-3

As we described above, galectin-3, found in the intracellular and extracellular milieu, is a multifunctional protein involved in regulation of cell growth, adhesion, differentiation, angiogenesis, and apoptosis [21–24,27,65]. These data suggest several rewarding aspects of inquiry. To clarify a definitive function of galectin-3, assessment of the natural functional galectin-3 ligand on tumor cells will be required. Furthermore, it is important to investigate the *in vivo* extracellular and intracellular roles of galectin-3 in cell signaling. Answering these questions should greatly further our limited understandings not only of galectins but also of a mechanism of cancer progression and metastasis.

References

1 Barondes SH, Cooper DN, Gitt MA, Leffler H, Galectins. Structure and function of a large family of animal lectins, *J Biol Chem* **19**(269), 20807–10 (1994).

2 Gong HC, Honjo Y, Nangia Makker P, Hogan V, Mazurak N, Bresalier RS, Raz A, The NH2 terminus of galectin-3 governs cellular compartmentalization and functions in cancer cells, *Cancer Res* **15**(59), 6239–45 (1999).

3 Herrmann J, Turck CW, Atchison RE, Huflejt ME, Poulter L, Gitt MA, Burlingame AL, Barondes SH, Leffler H, Primary structure of the soluble lactose binding lectin L-29 from rat and dog and interaction of its non-collagenous proline-, glycine-, tyrosine-rich sequence with bacterial and tissue collagenase, *J Biol Chem* **268**, 26704–11 (1993).

4 Liu FT, Hsu DK, Zuberi RI, Kuwabara I, Chi EY, Henderson WR, Jr, Expression and function of galectin-3, a beta-galactoside-binding lectin, in human monocytes and macrophages, *Am J Pathol* **147**, 1016–28 (1995).

5 Lotan R, Ito H, Yasui W, Yokozaki H, Lotan D, Tahara E, Expression of a 31-kDa lactoside-binding lectin in normal human gastric mucosa and in primary and metastatic gastric carcinomas, *Int J Cancer* **56**, 474–80 (1994).

6 Pacis RA, Pilat MJ, Pienta KJ, Wojno K, Raz A, Hogan V, Cooper CR, Increased content of an endogenous lactose-binding lectin in human colorectal carcinoma progressed to metastatic stages, *Cancer Res* **51**, 387–93 (1991).

7 Schoeppner HL, Raz A, Ho SB, Bresalier RS, Expression of an endogenous galactose-binding lectin correlates with neoplastic progression in the colon, *Cancer* **75**, 2818–26 (1995).

8 Castronovo V, Van Den Brule FA, Jackers P, Clausse N, Liu FT, Gillet C, Sobel ME, Decreased expression of galectin-3 is associated with progression of human breast cancer, *J Pathol* **179**, 43–8 (1996).

9 Pacis RA, Pilat MJ, Pienta KJ, Wojno K, Raz A, Hogan V, Cooper CR, Decreased galectin-3 expression in prostate cancer, *Prostate* **44**, 118–23 (2000).

10 Konstantinov KN, Robbins BA, Liu FT, Galectin-3, a beta-galactoside-binding animal lectin, is a marker of anaplastic large-cell lymphoma, *Am J Pathol* **148**, 25–30 (1996).

11 Hsu DK, Hammes SR, Kuwabara I, Greene WC, Liu FT, Human T lymphotropic virus-I infection of human T lymphocytes induces expression of the beta-galactoside-binding lectin, galectin-3, *Am J Pathol* **148**, 1661–70 (1996).

12 Xu XC, El-Naggar AK, Lotan R, Differential expression of galectin-1 and galectin-3 in thyroid tumors. Potential diagnostic implications, *Am J Pathol* **147**, 815–22 (1995).

13 van den Brule FA, Buicu C, Berchuck A, Bast RC, Deprez M, Liu FT, Cooper DN, Pieters C, Sobel ME, Castronovo V, Expression

of the 67-kD laminin receptor, galectin-1, and galectin-3 in advanced human uterine adenocarcinoma, *Hum Pathol* **27**, 1185–91 (1996).
14 Inohara H, Raz A, Identification of human melanoma cellular and secreted ligands for galectin-3, *Biochem Biophys Res Commun* **201**, 1366–75 (1994).
15 Gillenwater A, Xu XC, El-Naggar AK, Clayman GL, Lotan R, Expression of galectins in head and neck squamous cell carcinoma, *Head Neck* **18**, 422–32 (1996).
16 Lee EC, Woo HJ, Korzelius CA, Steele GD, Jr, Mercurio AM, Carbohydrate-binding protein 35 is the major cell-surface laminin-binding protein in colon carcinoma, *Arch Surg* **126**, 1498–502 (1991).
17 Lotz MM, Andrews CW, Jr, Korzelius CA, Lee EC, Steele GD, Jr, Clarke A, Mercurio AM, Decreased expression of Mac-2 (carbohydrate binding protein 35) and loss of its nuclear localization are associated with the neoplastic progression of colon carcinoma, *Proc Natl Acad Sci USA* **90**, 3466–70 (1993).
18 Raz A, Meromsky L, Zvibel I, Lotan R, Transformation-related changes in the expression of endogenous cell lectins, *Int J Cancer* **39**, 353–60 (1987).
19 Kohn EC, Development and prevention of metastasis, *Anticancer Res* **13**, 2553–9 (1993).
20 Raz A, Lotan R, Endogenous galactoside-binding lectins: A new class of functional tumor cell surface molecules related to metastasis, *Cancer Metastasis Rev* **6**, 433–52 (1987).
21 Nangia Makker P, Honjo Y, Sarvis R, Akahani S, Hogan V, Pienta KJ, Raz A, Galectin-3 induces endothelial cell morphogenesis and angiogenesis, *Am J Pathol* **156**, 899–909 (2000).
22 Kuwabara I, Liu FT, Galectin-3 promotes adhesion of human neutrophils to laminin, *J Immunol* **156**, 3939–44 (1996).
23 Matarrese P, Fusco O, Tinari N, Natoli C, Liu FT, Semeraro ML, Malorni W, Iacobelli S, Galectin-3 overexpression protects from apoptosis by improving cell adhesion properties, *Int J Cancer* **85**, 545–54 (2000).
24 Warfield PR, Makker PN, Raz A, Ochieng J, Adhesion of human breast carcinoma to extracellular matrix proteins is modulated by galectin-3, *Invasion Metastasis* **17**, 101–12 (1997).
25 Warfield P, Regulation of cellular adhesion to extracellular matrix proteins by galectin-3, *Biochem Biophys Res Commun* **246**, 788–91 (1998).
26 Ochieng J, Galectin-3 mediates the endocytosis of beta-1 integrins by breast carcinoma cells, *Biochem Biophys Res Commun* **289**, 845–50 (2001).
27 Kim HR, Lin HM, Biliran H, Raz A, Cell cycle arrest and inhibition of anoikis by galectin-3 in human breast epithelial cells, *Cancer Res* **59**, 4148–54 (1999).
28 Yoshii T, Fukumori T, Honjo Y, Inohara H, Kim HR, Raz A, Galectin-3 phosphorylation is required for its anti-apoptotic function and cell cycle arrest, *J Biol Chem* **277**, 6852–7 (2002).
29 Inohara H, Raz A, Functional evidence that cell surface galectin-3 mediates homotypic cell adhesion, *Cancer Res* **55**, 3267–71 (1995).
30 Inohara H, Akahani S, Koths K, Raz A, Interactions between galectin-3 and Mac-2-binding protein mediate cell-cell adhesion, *Cancer Res* **56**, 4530–4 (1996).
31 Glinsky VV, Huflejt ME, Glinsky GV, Deutscher SL, Quinn TP, Effects of Thomsen-Friedenreich antigen-specific peptide P-30 on beta-galactoside-mediated homotypic aggregation and adhesion to the endothelium of MDA-MB-435 human breast carcinoma cells, *Cancer Res* **15**(60), 2584–8 (2000).
32 Folkman J, Angiogenesis in cancer, vascular, rheumatoid and other disease, *Nat Med* **1**, 27–31 (1995).
33 Nangia-Makker P, Baccarini S, Raz A, Carbohydrate-recognition and angiogenesis, *Cancer Metastasis Rev* **19**, 51–7 (2000).
34 Moiseeva EP, Javed Q, Spring EL, de Bono DP, Galectin 1 is involved in vascular smooth muscle cell proliferation, *Cardiovasc Res* **45**, 493–502 (2000).
35 Sanford GL, Harris-Hooker S, Stimulation of vascular cell proliferation by beta-galactoside specific lectins, *FASEB J* **4**, 2912–8 (1990).
36 Cherayil BJ, Weiner SJ, Pillai S, The Mac-2 antigen is a galactose-specific lectin that binds IgE, *J Exp Med* **170**, 1959–72 (1989).
37 Rosenberg I, Cherayil BJ, Isselbacher KJ, Pillai S, Mac-2-binding glycoproteins. Putative ligands for a cytosolic beta-galactoside lectin, *J Biol Chem* **266**, 18731–6 (1991).
38 Woo HJ, Shaw LM, Messier JM, Mercurio AM., The major non-integrin laminin binding protein of macrophages is identical to carbohydrate binding protein 35 (Mac-2), *J Biol Chem* **265**, 7097–9 (1990).
39 van den Brule FA, Buicu C, Sobel ME, Liu FT, Castronovo V, Galectin-3, a laminin binding protein, fails to modulate adhesion of human melanoma cells to laminin, *Neoplasma* **42**, 215–9 (1995).
40 van den Brule FA, Liu FT, Castronovo V, Transglutaminase-mediated oligomerization of galectin-3 modulates human melanoma cell interactions with laminin, *Cell Adhes Commun* **5**, 425–35 (1998).
41 Parise LV, Lee J, Juliano RL, New aspects of integrin signaling in cancer, *Semin Cancer Biol* **10**, 407–14 (2000).
42 Dedhar S, Integrin mediated signal transduction in oncogenesis: An overview, *Cancer Metastasis Rev* **14**, 165–72 (1995).
43 Ochieng J, Fridman R, Nangia Makker P, Kleiner DE, Liotta LA, Stetler Stevenson WG, Raz A, Galectin-3 is a novel substrate for human matrix metalloproteinases-2 and -9, *Biochemistry* **33**, 14109–14 (1994).
44 Ochieng J, Green B, Evans S, James O, Warfield P, Modulation of the biological functions of galectin-3 by matrix metalloproteinases, *Biochim Biophys Acta* **1379**, 97–106 (1998).
45 Chen WT, Critical appraisal of the use of matrix metalloproteinase inhibitors in cancer treatment, *Oncogene* **19**, 6642–50 (2000).
46 Fidler IJ, Gersten DM, Hart IR, The biology of cancer invasion and metastasis, *Adv Cancer Res* **28**, 149–250 (1978).
47 Gorelik E, Wiltrout RH, Okumura K, Habu S, Herberman RB, Role of NK cells in the control of metastatic spread and growth of tumor cells in mice, *Int J Cancer* **30**, 107–12 (1982).
48 Francis H, Disruption of epithelial cell-matrix interactions induces apoptosis, *J Cell Biol* **124**(4), 619–26 (1994).
49 Gorelik E, Anoikis and metastatic potential of cloudman S91 melanoma cells, *Cancer Res* **61**, 1707–16 (2001).
50 Akahani S, Nangia Makker P, Inohara H, Kim HR, Raz A, Galectin-3: A novel antiapoptotic molecule with a functional BH1 (NWGR) domain of Bcl-2 family, *Cancer Res* **57**, 5272–6 (1997).
51 Thompson SC, The colony forming efficiency of single cells and cell aggregates from a spontaneous mouse mammary tumour using the lung colony assay, *Br J Cancer* **30**, 332–6 (1974).
52 Raz A, Bucana C, McLellan W, Fidler IJ, Distribution of membrane anionic sites on B16 melanoma variants with differing lung colonising potential, *Nature* **284**, 363–4 (1980).

53 Inohara H, Raz A, Effects of natural complex carbohydrate (citrus pectin) on murine melanoma cell properties related to galectin-3 functions, *Glycoconj J* **11**, 527–32 (1994).
54 Rosenberg I, Cherayil BJ, Isselbacher KJ, Pillai S, Mac-2-binding glycoproteins. Putative ligands for a cytosolic beta-galactoside lectin, *J Biol Chem* **266**, 18731–6 (1991).
55 Platt D, Raz A, Modulation of the lung colonization of B16-F1 melanoma cells by citrus pectin, *J Natl Cancer Inst* **84**, 438–42 (1992).
56 Edmiston KH, Shoji Y, Mizoi T, Ford R, Nachman A, Jessup JM, Role of nitric oxide and superoxide anion in elimination of low metastatic human colorectal carcinomas by unstimulated hepatic sinusoidal endothelial cells, *Cancer Res* **58**, 1524–31 (1998).
57 Moon BK, Lee YJ, Battle P, Jessup JM, Raz A, Kim HR, Galectin-3 protects human breast carcinoma cells against nitric oxide-induced apoptosis: Implication of galectin-3 function during metastasis, *Am J Pathol* **159**, 1055–60 (2001).
58 Song YK, Billiar TR, Lee YJ, Role of galectin-3 in breast cancer metastasis: Involvement of nitric oxide, *Am J Pathol* **160**, 1069–75 (2002).
59 Lotan R, Belloni PN, Tressler RJ, Lotan D, Xu XC, Nicolson GL, Expression of galectins on microvessel endothelial cells and their involvement in tumour cell adhesion, *Glycoconj J* **11**, 462–8 (1994).
60 Glinsky VV, Glinsky GV, Rittenhouse-Olson K, Huflejt ME, Glinskii OV, Deutscher SL, Quinn TP, The role of Thomsen-Friedenreich antigen in adhesion of human breast and prostate cancer cells to the endothelium, *Cancer Res* **61**, 4851–7 (2001).
61 Iurisci I, Tinari N, Natoli C, Angelucci D, Cianchetti E, Iacobelli S, Concentrations of galectin-3 in the sera of normal controls and cancer patients, *Clin Cancer Res* **6**, 1389–93 (2000).
62 Pienta KJ, Naik H, Akhtar A, Yamazaki K, Replogle TS, Lehr J, Donat TL, Tait L, Hogan V, Raz A, Inhibition of spontaneous metastasis in a rat prostate cancer model by oral administration of modified citrus pectin, *J Natl Cancer Inst* **87**, 348–53 (1995).
63 Nangia-Makker P, Hogan V, Honjo Y, Baccarini S, Tait L, Bresalier R, Raz A, Inhibition of human cancer cell growth and metastasis in nude mice by oral intake of modified citrus pectin, *J Natl Cancer Inst* **94**, 1854–62 (2002).
64 Colnot C, Fowlis D, Ripoche MA, Bouchaert I, Poirier F, Embryonic implantation in galectin 1/galectin 3 double mutant mice, *Dev Dyn* **211**, 306–13 (1998).
65 Inohara H, Akahani S, Raz A, Galectin-3 stimulates cell proliferation, *Exp Cell Res* **245**, 294–302 (1998).