The roles of Galectin-3 in autoimmunity and tumor progression

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Abstract Galectin-3, a unique chimera-type member of the β-galactoside-binding soluble lectin family, is widely expressed in numerous cells. Here, we discuss the role of Galectin-3 in T-cell-mediated inflammatory (auto) immunity and tumor rejection by using Galectin-3-deficient mice and four disease models of human pathology: experimental autoimmune encephalomyelitis (EAE), Con-A-induced hepatitis, multiple low-dose streptozotocin-induced diabetes (MLD-STZ diabetes) and metastatic melanoma. We present evidence which suggest that Galectin-3 plays an important pro-inflammatory role in Con-A-induced hepatitis by promoting the activation of T lymphocytes, NKT cells and DCs, cytokine secretion, prevention of M2 macrophage polarization and apoptosis of mononuclear cells, and it leads to severe liver injury. In addition, experiments in Galectin-3-"knock-out" mice indicate that Galectin-3 is also involved in immune-mediated β-cell damage and is required for diabetogenesis in MLD-STZ model by promoting the expression of IFN-gamma, TNF-alpha, IL-17 and iNOS in immune and accessory effector cells. Next, our data demonstrated that Galectin-3 plays an important disease-exacerbating role in EAE through its multifunctional roles in preventing cell apoptosis and increasing IL-17 and IFN-gamma synthesis, but decreasing IL-10 production. Finally, based on our findings, we postulated that expression of Galectin-3 in the host may also facilitate melanoma metastasis by affecting tumor cell adhesion and modulating anti-melanoma immune response, in particular innate antitumor immunity. Taken together, we discuss the evidence of pro-inflammatory and antitumor activities of Galectin-3 and suggest that Galectin-3 may be an important therapeutic target.

Keywords Galectin-3 · Con-A-induced hepatitis · Multiple low-dose streptozotocin-induced diabetes · Experimental autoimmune encephalomyelitis · Melanoma metastasis

Introduction

The galectins are evolutionarily conserved carbohydrate-binding proteins that have received attention in immunopathology due to their modulating activities on both pro- and anti-inflammatory immune responses [1, 2]. All galectins contain conserved carbohydrate-recognition domains (CRDs) of about 130 amino acids with affinity for β-galactosides [3]. To date, 15 mammalian galectins have been identified and classified into three groups: proto-type galectins (Galectins-1, -2, -5, -7, -10, -13, -14 and -15), which contain one CRD; tandem-repeat galectins (Galectins-4, -6, -8, -9 and -12), which have two different CRDs joined by a linker peptide of variable length; and the unique "chimera-type" Galectin-3, which contains a single CRD fused to non-lectin amino-terminal region [3–5].

Many galectins are either bivalent or multivalent with regard to their carbohydrate-binding activities. These molecules do not have specific individual receptors, but

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each of them is able to bind to a set of cell surface or extracellular matrix glycoproteins containing suitable oligosaccharides [5]. Sensitivity of cells to individual galectin family members may be affected depending on the repertoire of potentially glycosylated molecules expressed on the cell surface and the activities of specific glycosyltransferases that generate galectin ligands. These variables can change according to the differentiation and activation state of the cells [6].

Galectin-3, one of the members of the galectin family, is a ubiquitously expressed molecule with diverse physiological functions. Galectin-3 was initially identified as Mac-2, a cell surface antigen expressed on murine thyoglicollate-elicited peritoneal macrophages [7]. It consists of three structural domains: (1) an NH_2-terminal domain that contains a serine phosphorylation site, important for the regulation of intracellular signaling; (2) collagen-like sequence, sensitive to matrix metalloproteinase MMP-2- and MMP-9-induced proteolysis; and (3) a COOH-terminal domain containing a single CRD with Asp-Trp-Gly-Arg amino acid sequence (NWGR) responsible for the anti-apoptotic activity of Galectin-3 [8–12].

Depending on the type and proliferative status of cells, Galectin-3 can be found within the nucleus, in the cytoplasm, on the cell surface and in the extracellular compartment [13–15]. Galectin-3 binds and interacts with numerous ligands in the intra- and extracellular environment. In the presence of carbohydrate ligands, Galectin-3 is able to form pentamer through its NH_2-terminal domain, can cross-link cell surface glycoconjugates forming lattice-like structures and modulates a signaling cascade in the cells [16]. Thus, Galectin-3 regulates cell proliferation, differentiation and apoptosis in both normal and tumor cells [17, 18].

It is well documented that extracellular Galectin-3 acts as an adhesion molecule by cross-linking adjacent cells, and cells and extracellular matrix [18]. Intracellularly, Galectin-3 is engaged in processes that are pivotal for basic cellular functions, such as pre-mRNA splicing [19, 20] regulation of cell growth, cell cycle progression and apoptosis [17]. The effect of Galectin-3 in the regulation of apoptosis depends on its subcellular localization: cytoplasmic Galectin-3 has anti-apoptotic activity maintaining mitochondrial integrity, whereas nuclear and extracellular Galectin-3 is pro-apoptotic molecule [12, 21, 22].

Galectin-3 is expressed in many immunocompetent and inflammatory cells, including macrophages, dendritic cells, eosinophils, mast cells, uterine NK cells and activated T and B cells [18]. Galectin-3 has been shown to induce apoptosis in T cells, including human T leukemia cell lines, human peripheral blood mononuclear cells (PBMC) and activated mouse T cells [23, 24]. Fukumori et al. [24] demonstrated that secreted Galectin-3 binds mainly to CD7

and CD29 molecules resulting in the activation of the mitochondrial pathway including cytochrome-c release and caspase-3 activation [24], but other authors provided evidence that this lectin also binds to CD45 [23]. Galectin-3 may play an inhibitory role in T-cell activation by forming complexes with TCR glycans, therefore limiting TCR clustering necessary for initiation of TCR-mediated signaling [25]. Additionally, it has been reported that Galectin-3 can trigger monocytes to produce superoxide anion [26] and serve as a chemoattractant for these cells [27]. Recombinant Galectin-3 was found to promote the adhesion of human neutrophils to laminin [28] and to endothelial cells [29]. Galectin-3 can also act as an opsonin and enhance the macrophage clearance of apoptotic neutrophils [30].

This review focuses on the role of Galectin-3 in different T-cell-mediated immunopathologies and infections, autoimmunity and tumor progression.

The role of Galectin-3 in T-cell-mediated immunopathology and infection

Over the past few years, Galectin-3 has been implicated in the regulation of many aspects of T-cell physiology such as T-cell activation, proliferation and apoptosis. We have studied the role of Galectin-3 in the model of T/NKT-cell-dependent inflammatory response in the liver. Concanavalin A (Con-A)-induced liver injury is a well-established murine model of T-cell-mediated hepatitis [31–34]. Apart from $CD4^+$ T cells and $CD8^+$ T cells, as well as natural killer (NK), natural killer T (NKT) cells, macrophages could also induce hepatocyte cell death by cell to cell contact, through secretion of pro-inflammatory cytokines or reactive oxygen species [35–39]. It has been suggested that Galectin-3 is involved in the pathogenesis of inflammatory and malignant liver diseases [40–43].

We provided the first evidence that Galectin-3 plays an important role in the pathogenesis of Con-A-induced hepatitis (Volarevic et al., Hepatology 2012, in press). Actually, we showed that Galectin-3 deficiency leads to a marked attenuation of Con-A-induced hepatitis associated with decreased number of effector cells in the liver: T lymphocytes (both $CD4^+$ and $CD8^+$), B lymphocytes, dendritic cells, NK and NKT cells. The level of TNF-alpha, IFN-gamma, IL-17 and IL-4 in the sera and number of TNF-alpha-, IFN-gamma-, IL-17- and IL-4-producing $CD4^+$ cells and IL-12-producing $CD11c^+$ dendritic cells were lower in Galectin-3-deficient mice. In contrast, number of IL-10-producing $F4/80^+$ macrophages (alternatively activated or M2-polarized macrophages) was significantly higher in the livers of Galectin-3-deficient mice. Several studies demonstrated that Galectin-3 activates

dendritic cells and macrophages, serves as a chemoattractant for these cells and plays an important role in the proliferation of activated T lymphocytes [44–46]. In our model, markedly reduced number of liver-infiltrating effector cells that we found in Galectin-3-deficient mice compared to wild-type C57BL/6 (WT) mice indicates important role of Galectin-3 in promoting liver inflammation. Thus, it seems that reduced inflammation noticed in the livers of Galectin-3-deficient mice could be due to both macrophage and T-cell attenuation. Accordingly, we found decreased number of IL-12-producing CD11c$^+$ DCs in livers of Galectin-3-deficient mice compared with WT mice, suggesting that Galectin-3 plays an important role in antigen presentation and activation of T lymphocytes in Con-A-induced hepatitis. Further, apoptosis of liver-infiltrating cells contributes to the lower number of mononuclear cells in the livers of Galectin-3-deficient mice since significantly higher percentage of late apoptotic Annexin V$^+$ PI$^+$ liver-infiltrating mononuclear cells and splenocytes were seen in Galectin-3-deficient mice compared with WT mice.

Further, our results show that deletion of Galectin-3 gene most probably, due to lack of intracellular Galectin-3, enhanced apoptosis of mononuclear cells. Moreover, pretreatment of WT mice with selective inhibitor of Galectin-3 (provided by Nilsson and Leffler Ref. [47]) led to attenuation of the liver injury and milder infiltration of IFN-gamma-, IL-17- and IL-4-producing CD4$^+$ T cells and increase in total number of IL-10-producing CD4$^+$ T cells, and F4/80$^+$ CD206$^+$ alternatively activated (M2 polarized) macrophages and prevented apoptosis of liver-infiltrating mononuclear cells. We propose that Galectin-3 plays an important pro-inflammatory role in Con-A-induced hepatitis by promoting the activation of T lymphocytes, NKT cells and DCs, cytokine secretion, prevention of M2 macrophage polarization and apoptosis of mononuclear cells that leads to severe liver injury.

In addition, Galectin-3 plays a major role in the removal of circulating advanced lipoxidation endproducts (ALE) in the liver, and deletion of Galectin-3 accelerates non-alcoholic steatohepatitis or prevents the development of ALE-induced liver injury [43].

Recent studies suggest that galectin family members may serve as pathogen recognition receptors [48, 49]. For example, Galectin-3 can bind to glycans expressed by *Neisseria gonorrhoeae*, *Leishmania major*, *Shistosoma mansoni* and *Trypanosoma cruzi* [50–53]. The studies on mouse infectious disease models have also revealed the pro-inflammatory role of Galectin-3. For instance, in a study of *Toxoplasma gondii* infection, Galectin-3-deficient mice developed lower inflammatory response in the intestines, liver and brain but not in the lungs compared with similarly infected WT mice [54]. Further, Galectin-3-deficient mice mounted a higher Th1-polarized response [54].

Thus, in these infections, Galectin-3 suppressed Th1 response. On the other hand, when infected by *Schistosoma mansoni*, Galectin-3-deficient mice developed lower numbers of T and B lymphocytes in the spleen as well as a lower extent of liver granulomas in comparison with WT mice [46]. Additionally, Galectin-3-deficient mice were more susceptible to infection by *Paracoccidioides brasiliensis* and developed a Th2-polarized immune response compared to WT mice [55]. After stimulation with *P. brasiliensis* antigens, macrophages derived from Galectin-3 mice exhibited higher levels of TLR2 mRNA and IL-10 production compared to WT mice [55]. It seems that the effect of Galectin-3 in Th1/Th2 polarization depends on type of the infectious agent.

Breuilh et al. [46] suggest that although Galectin-3 deficiency in dendritic cells does not affect their differentiation and maturation, it greatly influences the strength, but not the nature of the acquired immune response. This view has been challenged by the analysis of dendritic cell phenotype during induction of experimental autoimmune encephalomyelitis (EAE) [56]. It appears that Galectin-3 is a modulator of the immune/inflammatory response during helminthic infection and that Galectin-3 expression in dendritic cells is pivotal in the control of the magnitude of T-cell priming [46].

The role of Galectin-3 in T-cell-mediated autoimmunity

In order to dissect out the role of Galectin-3 in T-cell-mediated autoimmunity, we used Galectin-3-deficient mice in experimental model of diabetes mellitus: multiple low-dose streptozotocin-induced diabetes (MLD-STZ diabetes) and multiple sclerosis: experimental autoimmune encephalomyelitis (EAE).

MLD-STZ model of type 1 diabetes is characterized by delayed and sustained hyperglycemia [57]. The initial destruction of some β cells due to MLD-STZ, similarly to viral infection, induces the activation of autoreactive T cells to multiple diabetogenic epitopes. This "epitope spreading" leads to β-cell loss until biochemical and histological evidence of disease is present.

We have demonstrated that the lack of Galectin-3 induced resistance to the induction of MLD-STZ diabetes in susceptible C57BL/6 mice [58], which was associated with the lack of significant mononuclear infiltration in the pancreatic islets and with retention of higher insulin content when compared with WT mice. Galectin-3 deficiency on immune and accessory effector cells is responsible for the attenuation of diabetogenesis. We also found that accessory cells in Galectin-3-deficient mice produce lower levels of inflammatory cytokines. More importantly, immune cells in the draining lymph node of Galectin-3-deficient mice

exhibit lower expression of IFN-gamma and iNOS and do not express TNF-alpha and IL-17 after MLD-STZ treatment. Further, we observed that macrophages produce less TNF-alpha and NO in Galectin-3-deficient mice compared with WT mice [58]. Thus, Galectin-3-deficient mice do not produce relevant cytokines, and their macrophages are ineffective in intracellular [59] and extracellular killing.

The roles of TNF-alpha and IFN-gamma in β-cell damage are well established [60], and it could be assumed that the attenuation of their production affects diabetogenesis in vivo. Apart from IFN-gamma, there is evidence that IL-17 can contribute to the pathogenesis of autoimmune inflammation [44] and that IL-23/IL-17 axis also plays a role in diabetogenesis [57].

Interestingly, our ongoing work suggests that the deletion of Galectin-3 facilitates type 2 diabetes progression (unpublished data). Obesity-induced insulin resistance and dysfunction of islet β cells are characteristic of the disease. Additionally, accumulation of amyloid peptide in the islets stimulates NLRP-3-dependent cleavage of caspase-1 and production of IL-1β in resident dendritic cells and invading macrophages [61]. This leads to β-cell loss and immune-mediated accumulation of pro-inflammatory T cells. It appears that visceral fat-associated inflammation is facilitated by Galectin-3 deletion (unpublished data). Thus, in contrast to the β-cell loss in type 1 diabetes, in obesity-induced type 2 diabetes, Galectin-3 may exert protective effect.

In the central nervous system (CNS), expression of Galectin-3 is upregulated in prion-infected brain tissue [62, 63] and experimental pneumococcal meningitis [64]. However, the role of Galectin-3 in autoimmune neurological disease remains unclear. By using Galectin-3-deficient mice, we investigated the role of Galectin-3 in EAE [56], T-cell-mediated autoimmune disease in which both Th1 and Th17 cells are responsible for inflammatory-mediated demyelination [65–69]. We showed that Galectin-3-deficient mice immunized with myelin oligodendrocyte glycoprotein (MOG$_{35-55}$) peptide developed markedly attenuated EAE compared with similarly immunized WT mice [56]. The disease attenuation was accompanied by reduced cellular infiltration of monocytes and macrophages in the CNS but increased apoptosis in the CNS infiltrates. Following Ag stimulation in vitro, lymph node cells from the immunized Galectin-3-deficient mice produced less IL-17 and IFN-gamma than did those of the WT mice. In contrast, there was an increased serum level of IL-10, IL-5 and IL-13 in Galectin-3-deficient mice. Furthermore, the CNS of the Galectin-3-deficient mice contained higher frequency of Foxp3$^+$Treg cells. Additionally, bone marrow-derived dendritic cells from Galectin-3-deficient mice produced more IL-10 in response to LPS or bacterial lipoprotein compared with WT marrow-derived dendritic cells.

Moreover, Galectin-3-deficient dendritic cells induced Ag-specific T cells to produce more IL-4, IL-5 and IL-10, but less IL-17, than did WT dendritic cells. These findings, therefore, demonstrate that Galectin-3 plays an important pathogenic role in EAE by preventing cell apoptosis in the CNS and enhancing IL-17 and IFN-gamma synthesis but decreasing IL-10 production [56]. Recent findings suggest that Galectin-3 exerts cytokine-like regulatory action, amplifying the inflammatory cascade in the brain [45]. Jeon and colleagues also showed that extracellular Galectin-3 is able to activate immune and inflammatory signaling events through phosphorylation of STAT1, STAT3 and STAT5 as well as JAK2 (reviewed in [45]).

In apparent contrast to these data, Demetriou and colleagues observed that inflammatory demyelization and neurodegeneration are enhanced in mice, which display natural deficiencies in multiple N-glycosylation pathway enzymes (reviewed in [70]). These differences may be related to the fact that other related galectins (Galectin-1 and Galectin-9) suppress EAE [56, 71].

Earlier report [25] has shown that reduction in Galectin-3 functions through the deletion of Mgat5 (β1, 6N-acetylglucosaminyltransferase V) in mice and leads to heightened susceptibility to EAE. The glycosylation deficiency in Mgat5$^{-/-}$ mice affects other pathways and cell types that may also contribute to the observed autoimmunity. Mgat5-modified glycans also reduce clusters of fibronectin receptors, therefore causing accelerated focal adhesion turnover in fibroblasts and tumor cells, a functionality that may affect leukocyte motility [25].

In other experimental models of autoimmune and chronic inflammatory diseases including rheumatoid arthritis [72, 73] and atherosclerosis [74], Galectin-3 principally acts as a pro-inflammatory molecule. However, Iacobini et al. [75] indicates a protective role for Galectin-3 in the uptake and effective removal of modified lipoproteins, with concurrent downregulation of RAGE-dependent pro-inflammatory pathways responsible for the initiation and progression of lipid-induced atherosclerosis.

In agreement with our findings in EAE and MLD-STZ diabetes, in the model of antigen-induced arthritis, Forsman and colleagues [73] demonstrated that Galectin-3-deficient mice displayed reduced disease severity compared with disease in WT mice. Reduced arthritis in Galectin-3-deficient mice was associated with decreased systemic production of pro-inflammatory cytokine IL-6 and TNF-alpha, and the frequency of IL-17-producing T cells, indicating that Galectin-3 may be new molecular target for therapeutic treatment of rheumatoid arthritis [73].

Taken together, our data in EAE and MLD-STZ diabetes and recent data in experimental arthritis suggest that intervention using specific Galectin-3 inhibition may be considered the therapy of human autoimmune diseases.

This assumption should be tested using newly developed synthetic inhibitor of Galectin-3 [47].

The role of Galectin-3 in tumor progression

Accumulating experimental and clinical evidence have revealed that Galectin-3 expressed in tumor cells plays an important role in the processes relevant to tumorigenesis such as malignant cell transformation, invasion and metastasis [5, 17] (Fig. 1). It has been demonstrated that human breast carcinoma cells lose their malignant phenotypes in cell culture after inhibition of Galectin-3

expression [76, 77]. In addition, inhibition of Galectin-3 expression results in slower tumor growth in vivo [76].

There are some indications that Galectin-3 has an important role in the initiation of tumor cell transformation possible through its interactions with oncogenic Ras proteins [78]. Galectin-3 preferentially binds to K-Ras and causes the activation of phosphatidylinositol 3-kinase (PI3K) and Raf1, modulating gene expression at the transcriptional level [78, 79]. Experimental evidence from studies of human breast cancer cells in vitro indicates that Galectin-3 may influence tumorigenesis through the regulation of cell cycle [17, 80]. Galectin-3 downregulates the expression of cyclin E and cyclin A and upregulates the
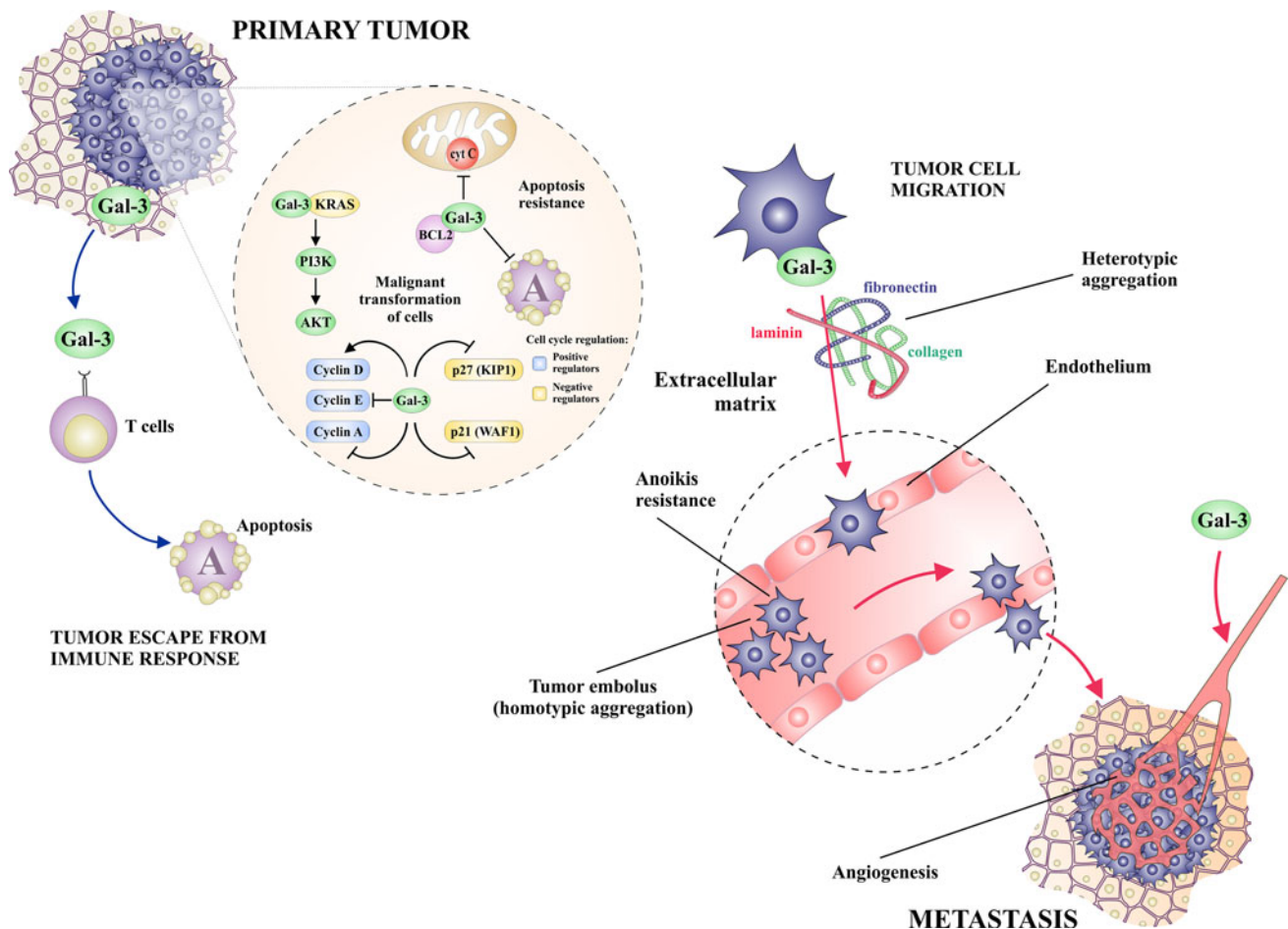

Fig. 1 The role of tumor cell-associated Galectin-3 in malignant transformation and tumor progression. Galectin-3 can contribute to tumorigenesis and tumor progression through several different mechanisms. It has an important role in the initiation tumor cell transformation through its interactions with oncogenic Ras proteins (K-Ras) and causes the activation of phosphatidylinositol 3-kinase (PI3K) and Raf1, modulating gene expression at the transcriptional level. Galectin-3 may also influence tumorigenesis through the regulation of cell cycle. This molecule downregulates the expression of cyclin E and cyclin A and upregulates the expression of cell cycle inhibitors p21 and p27 (*top left*). The cell surface Galectin-3 acts as an adhesion molecule in homotypic cell–cell and heterotypic cell–matrix interactions and is involved in the formation of tumor emboli and attachment of tumor cells to endothelium during metastasis (*bottom right*). The intracellular Galectin-3 has anti-apoptotic activity and is able to protect metastatic tumor cells against apoptosis induced by the loss of cell anchorage (anoikis) (*bottom right*). On the other hand, the tumor cell surface Galectin-3 may contribute to tumor immune escape by inducing apoptosis of TILs (*top left*). Galectin-3 can also regulate tumor cell migration and invasion by involving activation or expression of integrins (*top right panel*). Galectin-3 also has angiogenic activity, and it promotes new capillaries formation in vivo (*bottom right*)

expression of cell cycle inhibitors p21 (WAF1) and p27 (KIP1) [81] (Fig. 1). Subsequent studies demonstrated that interactions of Galectin-3 with β-catenin enhance the expressions of cyclin D and c-myc and promote cell cycle progression [80, 82].

The establishment of metastasis is a final qualitative step in the progression of malignant tumors. Changes in cell adhesion, increased migration, invasion, survival of metastatic cells in blood/lymphatic circulation and angiogenesis are necessary for the successful establishment of metastasis. The cell surface Galectin-3 acts as an adhesion molecule in homotypic cell–cell and heterotypic cell–matrix interactions [83–85], and it is believed that tumor cell-associated Galectin-3 is involved in the formation of tumor emboli and attachment of tumor cells to endothelium during metastasis [17, 86, 87] (Fig. 1). The interaction between free circulating Galectin-3 and transmembrane mucin protein MUC1 also promotes the formation of embolus and survival of disseminating tumor cells in the circulation [88]. An increase in tumor cell aggregation, as a result of the increased interaction between circulating Galectin-3 and tumor-associated MUC1 in cancer patients, provides a survival advantage to the disseminating tumor cells in the circulation [88].

Resistance to anoikis and anticancer drug resistance are considered to be a hallmark of metastatic tumor cells [89, 90]. It is believed that Galectin-3 is able to protect metastatic tumor cells against apoptosis induced by the loss of cell anchorage (anoikis) [81, 91], and it also participates in the regulation of apoptotic pathways that are important for the anticancer drug resistance [92, 93] (Fig. 1).

Galectin-3 can also affect tumor metastasis by exerting its effect on tumor cell motility and tumor invasion. For example, it is shown that Galectin-3 overexpression in lung cancer cell line results in enhanced cell motility and invasiveness in vitro [94]. It seems that Galectin-3 regulates tumor cell migration and invasion by activation or expression of integrins [17, 91] (Fig. 1). It is well known that integrins have a critical role in controlling tumor cell migration and therefore tumor cell invasion [95]. Recent study has shown that Galectin-3 upregulates the expression of protease-activated receptor-1 (PAR-1) and MMP-1, thereby promoting gastric cancer metastasis [96].

Angiogenesis is essential for tumor growth and metastatic dissemination and constitutes an important point in the control of cancer progression. It was reported that Galectin-3 has angiogenic activity in vitro, as it induces migration of endothelial cells [97]. In addition, overexpression of Galectin-3 in transfected clones of human prostate cancer cells (LNCaP) as well as overexpression of Galectin-3 in human breast cancer promotes the formation of new capillaries in vivo, resulting in enhanced tumor growth in mice [97, 98] (Fig. 1).

There is evidence that human melanoma elicits a spontaneous T-cell response against tumor and that anti-tumor T cells can accumulate at metastatic sites [99–101]. However, in patients with advanced cancer, tumors progress despite the presence of tumor-infiltrating lymphocytes (TILs), indicating that the tumor-specific T cells become ineffective either because tumor cells have become resistant to the immune attack or because TILs have become functionally impaired [102, 103]. In this regard, a recent study indicated an important role for Galectin-3–N-glycan interactions in mediating anergy of tumor-specific cytotoxic T lymphocytes (CTLs) by favoring the segregation of CD8 from TCR molecule [104]. Based on these findings, Demotte et al. [104] suggested that intratumoral Galectin-3 could impair T-cell function and that Galectin-3 ligands could improve antitumor immunity in vivo. On the other hand, Galectin-3 expression in human melanoma biopsies also correlated with apoptosis of TILs, therefore contributing to tumor immune escape [105] (Fig. 1).

It was therefore of interest to analyze the role of Galectin-3 expression on the host cells in regulating metastatic process in vivo. We recently provided the evidence that deletion of Galectin-3 in the host leads to a marked attenuation of metastasis in B16-F1 malignant melanoma model [106]. Galectin-3-deficient mice were more resistant to metastatic malignant melanoma as evaluated by decreased number and size of metastatic colonies in the lung. Related to this finding is the observation [107] that the incidence of lung tumors was significantly lower in Galectin-3-deficient mice after intraperitoneal injection of chemical carcinogen. Thus, Galectin-3 in the host could be important in lung tumorigenesis as well as in metastasis. Clinical evidences have also shown increased serum levels of Galectin-3 in patients with malignant melanoma [108, 109]. We postulated that Galectin-3 may be involved in tumor cell adhesion as the adhesive interaction of metastatic tumor cells appears to be obligatory for the successful creation of metastatic foci in the distant organs [110]. In vitro assays showed lower number of attached malignant cells in tissue sections derived from Galectin-3-deficient mice. It appears that endothelial Galectin-3 might be important for the adhesion of tumor cells due to its interaction with numerous carbohydrate ligands expressed on tumor cells [85, 86, 111].

Further, we also noticed that lack of Galectin-3 correlates with higher serum levels of IFN-gamma and IL-17 in tumor-bearing hosts [106]. In addition, in WT mice but not in Galectin-3-deficient mice, injection of melanoma cells resulted in significant increases in the percentage and total number of CD4$^+$Foxp3$^+$ T cells. We found clear difference in the number of CD4$^+$Foxp3$^+$ T cells, which was not accompanied with differences in the number of CD4$^+$ and CD8$^+$ T cells. Obviously, this does not exclude the
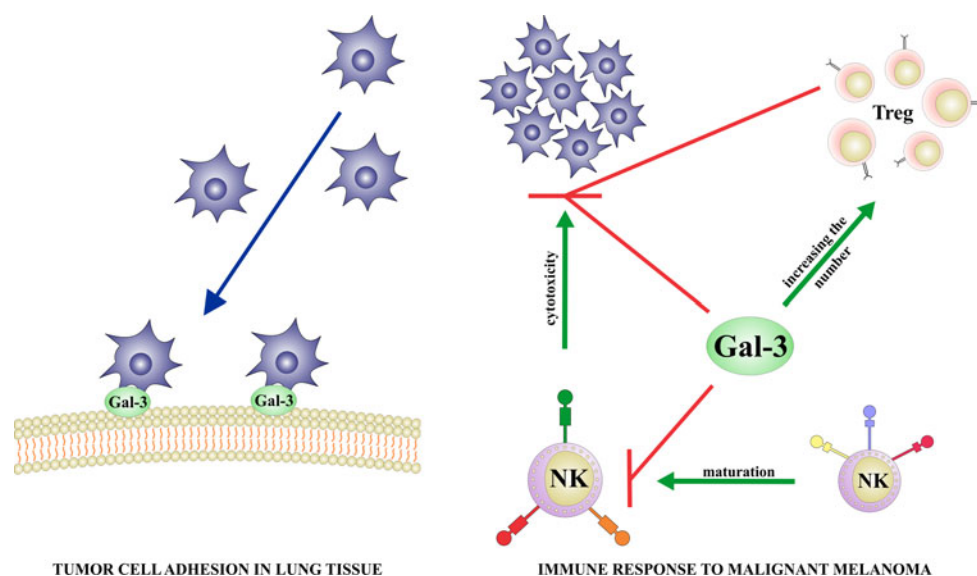
Fig. 2 Hypothetical role of Galectin-3 in malignant melanoma metastasis. In our tumor system, expression of Galectin-3 may facilitate melanoma metastasis by at least two independent mechanisms. Galectin-3 may act as an adhesion molecule resulting in binding of melanoma cells onto lung tissue and appear obligatory for successful creation of metastatic foci (*left panel*). Additionally, Galectin-3 may have an important role in the modulation of anti-melanoma immune response by affecting maturation of cytotoxic NK cells (see text) and their cytotoxic activity and enhancing the number of Treg cells that probably contribute to the suppression of NK function (*right panel*)

possibility that the number of tumor-specific Th1 cells and $CD8^+$ cytotoxic cells is different. This assumption is strengthened by the clear difference in the serum IL-17 and IFN-gamma in the tumor-bearing host. While $CD8^+$ T cell and adherent cell cytotoxicity were similar, there was greater cytotoxic activity of splenic NK cells of Galectin-3-deficient mice compared with WT mice. Despite the reduction in total number of $NK1.1^+$ cells, Galectin-3-deficient mice constitutively have a significantly higher percentage of effective cytotoxic $CD27^{high}CD11b^{high}$ NK cells as well as the percentage of immature $CD27^{high}CD11b^{low}$ NK cells. In contrast, $CD27^{low}CD11b^{high}$ less functionally exhausted NK cells, and NK cells bearing inhibitory KLRG1 receptor were more numerous in WT mice [106]. However, it is possible that most relevant role of Treg cells in our tumor system is suppression of NK function [112, 113]. In fact, we observed significant increase in the NK cell cytotoxicity and maturation of cytotoxic NK cells in Galectin-3-deficient mice. Our data therefore demonstrated that lack of Galectin-3 affects tumor metastasis by at least two independent mechanisms: by a decrease in binding of melanoma cells onto target tissue and by enhanced NK-mediated antitumor response [106].

As illustrated in Fig. 1, Galectin-3 expressed in the tumor cells can contribute to tumor progression through many different mechanisms. In addition, based on our findings [106], we postulated that the expression of Galectin-3 may also facilitate melanoma metastasis by

affecting tumor cell adhesion and modulating anti-melanoma immune response, in particular innate antitumor immunity (Fig. 2). These findings suggest that blockade of Galectin-3 might have therapeutic benefits.

Thus, the knowledge summarized in this review indicates that Galectin-3 has a broad spectrum of immuno-regulatory effects in T-cell-mediated inflammatory processes, autoimmune diseases and tumor progression. Thus, selective inhibition of Galectin-3 may be a useful therapeutic approach in the treatment of autoimmune and malignant diseases.

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