Review article

Molecular regulation of galectin-3 expression and therapeutic implication in cancer progression

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Abstract

Galectin-3, a multifunctional protein, distributes inside and outside cells and plays an important role in tumor cell adhesion, proliferation, differentiation, angiogenesis, and metastasis in multiple tumors. Changes in galectin-3 expression are commonly seen in cancer and pre-cancerous conditions. Therefore, to understand the molecular regulation of galectin-3 expression could aid the development of new approach for cancer treatment. This review summarizes different expression of galectin-3 in cancer cells and patients' serum, the regulation mechanism and the potential therapeutic targets of galectin-3 in cancer progression.

Keywords: galectin-3; regulation mechanism; transcription factors; inflammatory cytokines; Ras/MAPK pathway; cancer

1. Introduction

Galectins are a family of carbohydrate-binding proteins with a high affinity for β -galactoside. Members of the family have specific sequence homologies for specific carbohydrate-binding motifs. So far, 15 mammalian members of the galectin family have been isolated and identified [1]. 15 members have been classified into three subtypes: the prototype group, the chimera group and the tandem repeat group. Galectin-1, -2, -5, -7, -10, -11, -13, -14 and -15, belonging to prototypical group, contain one carbohydrate-recognition domain. The tandem repeat galectins (galectin-4, -6, -8, -9 and -12) have two CRDs in a single polypeptide chain. Galectin-3, the only member of vertebrate chimera type of galectin family, contains one CRD that is connected to an extended non-lectin N-terminal domain [2]. Among family members, galectin-3 was studied explicitly. Reports showed that galectin-3 had versatile multifunctions, such as cell-cell and cell-extracellular matrix adhesion, cell growth and differentiation, cell-cycle, signaling and apoptosis, as well as in angiogenesis [3].

Galectin-3, a 29- to 35-kDa protein, is synthesized in the cell cytoplasm and shuttles between the cytoplasm and nucleus. It can also be secreted to the cell surface and extracellular environment [4]. Structurally, galectin-3 is composed of two distinct domains: an N-terminal domain containing proline- and glycine-rich sequences and a globular C-terminal domain containing the carbohydrate recognition site. Human galectin-3 is coded by a single gene LGALS3 which is suited on chromosome 14, locus q21-q22. The promoter region of the human LGALS3 gene contains several regulatory elements: five putative Sp1 binding sites (GC boxes), five cAMP-dependent response element (CRE) motifs, four AP-1- and one AP-4-like sites, two NF- κ B-like sites, one sis-inducible element (SIE) and a consensus basic helix-loop-helix (bHLH) core sequence [3].

Galectin-3 is widely expressed in human tissues and is involved in diverse biological events, such as embryogenesis, cell adhesion, proliferation, apoptosis, mRNA splicing, and regulation of the immune system [5]. Changes in galectin-3 expression and subcellular and intercellular localizations are commonly seen in cancer and pre-cancerous conditions [6]. There is also a general shift of galectin-3

31 localization from nucleus to the cytoplasm in cancer development from adenoma to
32 carcinoma. Studies from various laboratories have suggested the possible involvement
33 of galectin-3 in tumorigenesis, angiogenesis, and tumor metastasis [7].
34 Overexpression of intracellular galectin-3 promotes neoplastic transformation, cell
35 cycle progression and inhibits apoptosis. Extracellular galectin-3 enhances tumour
36 cell adhesion to extracellular matrix (ECM) and promotes the escape of tumour cells
37 from the primary tumour sites [5]. Galectin-3 in the nucleus plays a pivotal role in the
38 regulation of cancer-related gene expression, including cyclin D1, TTF-1 and MUC2,
39 presumably associated with tumor progression [8]. However, the precise roles of
40 galectin-3 in physiological and pathological processes *in vivo* remain to be elucidated.

41 In view of galectin-3 as a key factor in tumor progression and metastasis, many
42 researchers have tried to develop a new approach for cancer treatment by targeting
43 galectin-3. It is essential to understand the molecular regulation of galectin-3 in the
44 whole process of cancer progression. In this review, we summarize a brief overview of
45 different expression of galectin-3 in cancer cells and patients' serum, the regulation
46 mechanism and the potential therapeutic targets of galectin-3 in cancer progression.

47 2. Different expression of galectin-3 in cancer cells and patients serum

48 2.1. Complicated expression of galectin-3 in cancer cells

49 In normal cells, the expression of galectin-3 has been detected in activated
50 macrophages, basophils, mast-cells, certain epithelial cells and sensory neurons [3]. In
51 cancer cells, complicated expressions of galectin-3 were observed (Table 1). In the
52 cancers of pancreas, liver, tongue and thyroid, gastric, the expression of galectin-3 is
53 increased [9-14]. This increase of galectin-3 expression is correlated with neoplastic
54 progression or metastatic potential in some cancers. For example, the expression of
55 galectin-3 was significantly higher in the primary lesions of papillary carcinomas with
56 metastases than that in those without metastases [15]. However, contradictory
57 observations have been reported. In breast cancer, prostate cancer, endometrial cancer,
58 expression of galectin-3 was significantly decreased [16-19]. In addition, in

intrahepatic cholangiocarcinoma, galectin-3 expression is up-regulated in the early stage of carcinoma and down-regulated in later stage [19]. More complicated expressions of galectin-3 were reported in colon cancer. Irimura *et al.* reported that galectin-3 expression in colon cancer was increased in advanced stage, however, Lotz *et al.* observed a 5- to 10-fold decrease in galectin-3 mRNA as well as a reduction in the amount of protein expressed in colon cancer compared with normal colonic mucosa [20-21]. These discrepancies could be explained by a more rigorous analysis of localization of galectin-3. During progression of colon cancer, endometrial cancer and prostate carcinoma, a translocation of galectin-3 from the nucleus to the cytoplasm was observed [16,21,22]. This implies that the decrease of galectin-3 expression may be related to alteration of the cytoplasmic/nuclear expression pattern.

2.2. Level of galectin-3 in patients serum

Low serum level of galectin-3 was detected in healthy individuals. The concentrations of galectin-3 in serum from patients with breast, gastrointestinal, lung, head and neck were at least 1.5 times higher than that of healthy individuals. Compared with localized tumors, galectin-3 serum levels in patients with metastatic tumor were significantly elevated [23-25]. Barrow *et al.* found that the level of galectin-3 in colorectal cancer patients serum without (3.6 ng/ml) or with (median 14.4 ng/ml) liver metastases were significantly higher than healthy individuals (median 1.3 ng/ml) [26]. These evidences indicated that circulating galectin-3 may play a crucial role in tumor progression and metastasis.

Recent studies have shown that circulating galectin-3 levels may contribute significantly to the metastatic spread of disseminating cancer cells by enhancing their ability to adhere to blood vessel endothelium and by helping their avoidance of immune surveillance [27]. Circulating galectin-3 enhances tumour cell homotypic aggregation and prevents anoikis by interacting with muc-1 [28]. However, the source of increased serum galectin-3 in cancer patients remains unknown [27]. Thus, targeting the galectin-3 in the circulation may hold significant promise for future

development of novel therapeutic agents to prevent metastasis and reduce cancer-associated fatality.

3. Regulation of galectin-3 expression in cancer progression

The expression of galectin-3 on both transcriptional and translational level can be regulated by various stimuli (Figure 1). Numerous factors are involved in the complex regulative mechanism of galectin-3. For example, very recent data indicated that in pituitary as well as in other tumors, expression of galectin-3 in adenomas producing prolactin and adrenocorticotrophic hormone (ACTH) is related to the promoter methylation status of the galectin-3 gene (LGALS3). LGALS3 was unmethylated in cells expressing galectin-3, whereas its promoter was methylated in galectin-3 null cells. However, the follicle-stimulating hormone/luteinizing hormone (FSH/LH)-producing and null cell adenomas with unmethylated LGALS3 gene did not express galectin-3 protein, suggesting that the promoter methylation is not the only factor regulating galectin-3 protein expression [29]. Regulation of galectin-3 expression is not directly induced by a specific factor, but it would be dependent on cells' differentiation status or tissue type. Various factors, like RUNX (runt-related protein) family, homeodomain-interacting protein kinase 2 (HIPK2), nuclear factor κ B (NF- κ B), inflammation cytokines, and some intracellular signal pathways are involved in the regulation of expression of galectin-3.

3.1. Transcription factors

3.1.1. RUNX protein family

RUNX protein family includes RUNX1, RUNX2, and RUNX3 which are all transcription factors containing a highly conserved region of 128 amino acids designated as the "runt homology domain RHD" which is responsible for DNA binding, protein-protein interaction, ATP binding, and contributes to nuclear localization [30]. RUNXs also play an important role in tumor proliferation and progression, but whether they are oncogenes or tumor suppressor genes remains

controversial [31].

Zhang *et al.* reported that RUNX1 and RUNX2 upregulated the expression of galectin-3 in human pituitary tumor cells by direct binding to the promoter region of LGALS3 and different RUNX family member binds different DNA sequences in the LGALS3 promoter region. RUNX1 binds to an upstream region on LGALS3 promoter which contains the predicted RUNX1 binding consensus sequence “TCTGGT”; RUNX2 binds to a downstream region on LGALS3 promoter which contains the predicated RUNX2 binding consensus sequence “CACCGCC” [31]. Recent study showed that RUNX1 also leads to the up-regulation of galectin-3 expression by binding to the galectin-3 promoter in human pituitary cell line HP75 cells. When HP75 cells were treated with transforming growth factor- β (TGF β 1), which acts as a potent driver of cancer progression through the induction of epithelial-mesenchymal transition EMT, intracellular galectin-3 protein was decreased and RUNX1 up-regulated. Therefore, RUNX1 may be functioning in a negative feedback manner as a result of the effect of TGF β 1 on decreasing galectin-3 protein levels [32].

3.1.2. Homeodomain-interacting protein kinase 2 (HIPK2)

HIPK2, the best characterized member of HIPK family, acts as co-regulator of an increasing number of transcription factors and modulates many different basic cellular processes such as apoptosis, proliferation, DNA damage response, differentiation, and development. Most of these effects are mediated by phosphorylation and activation of the oncosuppressor protein p53, which mediated apoptosis [33].

Cecchinelli *et al.* showed that DNA damage induced by UV irradiation was responsible for the activation of HIPK2 and that this event, in turn, stimulated the phosphorylation of specific serine/threonine residues in p53 protein. Finally, phosphorylated p53 protein induced downregulation of the potent anti-apoptotic molecule galectin-3, by suppressing its expression directly at the promoter level. The decrease in galectin-3 mRNA and protein levels facilitated the occurrence of apoptosis

[34]. Lavra *et al.* showed that RNA interference (RNAi) or overexpression of the p53 activator HIPK2 in papillary thyroid carcinomas (PTC) derived K1 cells causes upregulation or downregulation respectively of galectin-3 expression. In reverse, expression of a non-repressible galectin-3 prevented HIPK2- and p53-induced apoptosis. These results revealed a new apoptotic pathway induced by HIPK2-activated p53 and requiring repression of the anti-apoptotic factor galectin-3 [35].

3.1.3. NF- κ B

NF- κ B, a heterodimeric transcription factor, plays an important role in inflammatory and stress responses as well as in tumor cell resistance to apoptosis. Recently, NF- κ B is increasingly recognized as a hallmark of inflammatory responses and inflammation- associated cancer which involved in many steps of initiation and progression of cancer [36].

It has been reported that NF- κ B interacted with galectin-3 promoter to increase the expression level of galectin-3 in Human T lymphoma cells infected by human T lymphotropic virus-1 [37]. In glioblastoma cells, the expression of galectin-3 was downregulated by NF- κ B inhibitor, suggesting that NF- κ B may be a key mediator in the regulation of galectin-3 [38]. Another study showed that Nucling, which is a stress-inducible protein associated with apoptosomes, inhibited the expression of galectin-3 by interfering with NF- κ B activation *via* the nuclear translocation process of NF- κ B/p65, thus induce apoptosis [39]. Furthermore, the increased galectin-3 expression is tightly correlated with a longer duration of inflammatory lesions. For example, galectin-3-deficient mice had fewer inflammatory cells after intraperitoneal challenge with reduced levels of NF- κ B activation [40].

3.1.4. Hypoxia-inducible factor-1 (HIF-1 α)

Hypoxia-inducible factors (HIFs) mediated the major transcriptional response to hypoxia, some of the HIF target genes regulate processes involved in tumorigenesis,

such as increased glucose uptake and glycolysis, angiogenesis, invasion, metastasis and radiation resistance [41].

Wesley *et al.* found that the hypoxia-mimetic, cobalt chloride (CoCl₂)-treated neuroblastoma Neuro-2a cells showed enhanced levels of HIF-1, which commonly expressed in many cancer cell types. In correlation with the increased HIF-1 levels, galectin-3 expression was increased at both mRNA and protein levels. Silencing of HIF-1 expression by small hairpin interfering RNA in Neuro-2a cells blocked the increase of galectin-3 expression indicating that HIF-1 regulated galectin-3 expression in Neuroblastoma cells [42]. Moreover, Zeng *et al.* found that intracellular galectin-3 expression is upregulated under low oxygen/hypoxic conditions in HeLa cells and mouse embryonic fibroblasts (MEFs) from HIF-1 α wildtype but not HIF-1 α -null mice. They further confirmed that HIF-1 α combined with hypoxia response elements there was enhanced galectin-3 promoter activity, suggesting that HIF-1 α serves as a major regulator of galectin-3 expression [43].

3.2. Inflammatory cytokines

Tumors are usually characterized by an inflammatory microenvironment, which enables cancer growth and progression by supplying growth factors, pro-angiogenic mediators, extracellular matrix modifying enzymes, and signaling molecules, all favoring cancer cell invasion and metastatization [44]. Galectin-3, a powerful pro-inflammatory signal, was produced and secreted by certain cells in large quantities as a response to various inflammatory stimuli [3].

The expression and activity of galectin-3 was induced in T-cells when T lymphocytes were activated by common gamma-chain signaling cytokines, like interleukins 2, 4 and 7, whereas the inflammatory cytokines including TNF- α and IFN- γ did not [45]. However, there was a contradictory report showed that TNF- α or IFN- γ could induce the galectin-3 expression in a human monocyte-like cell line U937, whereas interleukin-4 (IL-4) did not [46]. Moreover, granulocyte-macrophage colony-stimulating factor (GM-CSF), a hematopoietic cytokine, has also

197 been shown to induce the expression of galectin-3 [47, 48]. Galectin-3 mRNA and
198 protein levels are in a moderate, transient increase during *in vitro* differentiation of
199 human monocytes into immature dendritic cells (iDCs), by culturing monocytes in the
200 presence of IL-4 and GM-CSF. Culturing monocytes with IL-4 alone strongly
201 increased galectin-3 mRNA levels, whereas GM-CSF induces a low increase in
202 galectin-3 mRNA. The combined data indicate that GM-CSF reduces IL-4 induced
203 galectin-3 mRNA levels during the generation of iDC. Remarkably, stimulation of
204 monocytes with GM-CSF results in secretion of significant amounts of galectin-3 in
205 the medium, whereas iDCs do not release detectable amounts of galectin-3, indicating
206 a suppressive role of IL-4 on GM-CSF induced galectin-3 secretion [49].

207 3.3. Ras/MAPK pathway

208 The Ras-Raf-MEK-ERK-MAPK (Ras/MAPK) pathway comprises several key
209 signaling components and phosphorylation events that play crucial role in
210 tumorigenesis. These activated kinases transmit extracellular signals that regulate cell
211 growth, differentiation, proliferation, apoptosis and migration functions. Alteration of
212 Ras/MAPK in the pathway has been reported in various human cancers as a result of
213 abnormal activation of receptor tyrosine kinases or gain-of-function mutations mainly
214 in the Ras or Raf genes. This pathway is considered potential therapeutic targets for
215 cancer treatment [50].

216 Kim *et al.* reported that the Ras-Raf-MEK1 pathway involved in the
217 Phorbol-12-myristate-13-acetate (PMA) stimulated galectin-3 gene expression in
218 macrophages. And the finding that expression of MEK3, a specific activator of p38
219 MAPK, enhanced galectin-3 promoter activity suggests the involvement of the
220 Ras-Raf-MEK1-MEK3-p38 pathway in galectin-3 gene expression in macrophages
221 [51].

222 The expression of galectin-3 was increased in basic fibroblast growth factor (bFGF)
223 and nerve growth factor (NGF) treated PC12 rat pheochromocytoma cell, the ras-
224 and MAPK-dependent and -independent signaling mechanisms may involve in the

upregulation of galectin-3 mRNA level. However, an activation of the Ras/MAPK pathway is essential, but not sufficient for galectin-3 expression in the presence of factors other than NGF [52]. Similarly, in Ras-transfected non-tumorigenic cell lines, the elevated galectin-3 expression is only associated with transformed cell transfectants, suggesting that the induction of galectin-3 expression occurs as a consequence of cell transformation rather than the introduction of Ras [53].

Moreover, some regulatory factors are crosstalked, for example, both GM-CSF and Ras/MARK pathway are involved in the regulation of galectin-3 expression. The Ras/MAPK pathway would be activated in response to GM-CSF treatment in microglial cells [52, 54].

3.4. Other cytokines regulate the expression of galectin-3

3.4.1. β 1 integrins

Integrins are a family of heterodimeric transmembrane receptors for components of the ECM, such as fibrinogen (FN), laminin (LN), collagen (Col), and vitronectin (VN) [55]. Integrins cluster in specific cell-matrix adhesions to provide dynamic links between extracellular and intracellular environments by bi-directional signaling and by organizing the ECM and intracellular cytoskeletal and signaling molecules. Through bidirectional “outside-in” and “inside-out” signaling, integrins regulate multiple biological processes, such as adhesion, apoptosis, proliferation, differentiation, migration, and metastasis [56]. Galectin-3 is known to bind to LN, Lamp I and II, IgE and Mac-2 binding proteins. In addition, galectin-3 can also mediate the cell adhesion to ECM by regulating integrins [57]. Galectin-3 may regulate tumor cell migration and invasion by promoting integrin clustering on the cell surface, mediating the endocytosis of integrins or activating integrin directly. Overexpression of galectin-3 enhances β 1 integrin-mediated cell adhesion to fibronectin (FN) and laminin (LN), as well as cell migration. In turn, β 1 integrin induces galectin-3 expression by demethylation of the Lgals3 promoter, which promotes cell migration. The functional feedback-loop between β 1 integrins and

galectin-3 involves in the epigenetic induction of galectin-3 expression during integrin-induced EMT and cell scattering [58].

3.4.2. Epidermal growth factor (EGF)

EGF is a growth factor that stimulates cell growth, proliferation, and differentiation by binding to its receptor EGFR [59]. It has been reported that high concentrations of EGF induced apoptosis of the cells with high level EGFR though the precise mechanism remains unknown [60].

High concentrations of EGF stimulation reduced the expression of galectin-3 at both mRNA and protein levels to promote apoptosis in HepG2 cells with high levels of EGFR *via* inhibiting Ras/ERK and PI3K/Akt pathway [61].

3.4.3. Synexin

Synexin (annexin VII), a 51-kDa member of the annexin family of proteins, has been proposed to act as a Ca^{2+} channel and as a Ca^{2+} -activated GTPase, thus regulating intracellular vesicle fusion and membrane trafficking [62, 63]. Yu *et al.* found that galectin-3 interacts with synexin following apoptotic stimuli. This galectin-3-synexin interaction helps galectin-3 to translocate to the perinuclear membrane. When synexin expression was down-regulated, intracellular galectin-3 levels also decreased, whereas extracellular galectin-3 levels increased (5-fold) [64].

4. Potential therapeutic targets of galectin-3 in cancer progression

Many researchers have tried to develop a new approach for cancer treatment by targeting galectin-3. Yoshii *et al.* proved that thyroid papillary carcinoma cells transfected galectin-3 antisense cDNA leads to a marked reduction of the malignant phenotype [65]. Numerous studies have focused on the development of galectin-3 inhibitors including peptide antagonists [66], lactulose amines [67], and galactose-based inhibitors [68,69]. In addition to these synthetic molecules, a natural product, pectin, has emerged as a good source for generating high affinity galectin-3

inhibitors with low toxicity. Iurisci *et.al* found that modified citrus pectin, a competitive inhibitor for natural ligands of galectin-3, inhibited the spontaneous metastasis of rat prostate adenocarcinoma cells and human breast carcinoma cells [23,70]. Moreover, Domic *et.al* showed that curcumin can reduce basal level of galectin-3, while the cell pretreated with curcumin reduced the increased expression of galectin-3 induced by ultraviolet radiation or alkylating agents in glioblastoma cells [71]. Dabelić *et.al* reported that anti-inflammatory drugs (hydrocortisone and dexamethasone) reduced expression of galectin-3 both on the mRNA and the protein level in human monocytic cell [72].

5. Conclusion

Galectin-3 is expressed in many human tumors and cell lines, and involved in many biological processes including cell growth, adhesion, apoptosis, tumor progression, and metastasis. In this review, we have summarized different expression of galectin-3 in cancer cells and patients' serum, the regulation mechanism and the potential therapeutic targets of galectin-3 in cancer progression. However, the molecular regulation mechanisms responsible for the expression level of galectin-3 in tumor cells are not yet clear.

It has been speculated that the regulation of galectin-3 expression is not directly induced by the action of a specific oncogene, but it would be dependent on cells' differentiation status or tissue type. As far as we know, bFGF, inflammatory cytokines IL-2, IL-4, and IL-7, TNF- α , IFN- γ and GM-CSF can induce expression of galectin-3 in various cancer cells. The increased galectin-3 expression is tightly correlated with RUNX1, RUNX2, NF- κ B, HIF-1 α and β 1 integrins. On the other hand, TGF β 1, EGF, HIPK2 is related to the decreased expression of galectin-3. However, there might be a crosstalk between these factors regulating galectin-3.

Although targeting regulation of galectin-3 is only under the fundamental research and has not yet turned into substantial therapeutic strategy, an understanding of the regulation of galectin-3 can lead insight into the development of novel therapeutic

agents for cancer treatment.

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535

Figures

Figure 1

Table 1. Different expression of galectin-3 in different types of cancers

	Up-regulation	Down-regulation	Conflicted reports
Cancer Tissue	Pancreatic cancer; Hepatic carcinoma Tongue cancer; Thyroid carcinoma; Gastric carcinoma; Intrahepatic cholangio- carcinoma (early stage)	Breast carcinoma; Prostate carcinoma; Endometrial carcinoma; Intrahepatic cholangioc- arcinoma (later stage);	Colon carcinoma Up-regulation in later stage Down-regulation in during neoplastic progression
Patients' Serum	Breast carcinoma; Gastrointestinal carcinoma; Lung carcinoma; Head and neck carcinoma;		