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Overexpression of Galectin-1 and Galectin-3 in hepatocellular carcinoma

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Abstract

Galectins (Gals) are evolutionarily conserved proteins that bind to β-galactoside containing glycans. Abnormal expression of Gals is associated with the development, progression, and metastasis of different types of cancer. Among the 11 Gals identified in humans, the roles of Gal-1 and Gal-3 have been extensively investigated in various tumors. Here, we summarize the roles of overly expressed Gal-1 and Gal-3 in the pathogenesis of hepatocellular carcinoma (HCC). The overexpression of Gal-1 and Gal-3 correlates with tumor growth, HCC cell migration and invasion, tumor aggressiveness, metastasis, and poor prognosis. A potentially promising future treatment strategy for HCC may include the combination of immunotherapy with Gal-1 inhibition. Additional research is warranted to investigate targeting Gal-1 and Gal-3 for HCC treatment.

Keywords

Galectin-1 (Gal-1); Galectin-3 (Gal-3); Liver cancer; Hepatocellular carcinoma (HCC); Fibrosis; Cirrhosis; Non-alcoholic steatohepatitis (NASH); Metastasis; Epithelial-mesenchymal transition (EMT)

1. Introduction

Worldwide, more than 700,000 people are diagnosed with hepatocellular carcinoma (HCC) each year.[1] Since 1980 the incidence of HCC in the United States has more than tripled.[2] In most countries around the world, both the incidence and prevalence of HCC is 3 times higher among men.[3]

Regardless of the insult, most HCC cases (~80%) have an underlying association with cirrhosis due to chronic inflammation and advanced fibrosis. The most common associations

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Declaration of competing interest

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are infection with hepatitis B or C virus (HBV, HCV), alcohol consumption, obesity, or dietary exposure to aflatoxin.[4] Non-alcoholic steatohepatitis (NASH), a metabolic disorder resulting from insulin resistance that underlies fibrosis and cirrhosis, is another risk of HCC.[5] The long developmental process and asymptomatic nature of HCC results in late detection often at an advanced stage.[6] Therefore, this unfortunately often leads to a poor prognosis.

A complex interplay of different pro-inflammatory cytokines such as interleukin-6 (IL-6) or tumor necrosis factor-α (TNF-α) and anti-inflammatories (transforming growth factors (TGF)-α and β), transcription factors (NF-κB, STAT-3), and their associated pathways are involved in HCC development.[7] Several intracellular signaling pathways involved in abnormal proliferation, survival, differentiation, invasion, and metastasis are also involved in HCC development.[8]

Currently, molecular-targeted therapeutic strategies are focused on the use of inhibitors of vascular endothelial growth factor (VEGF), mesenchymal-epithelial transition factor (c-MET), a receptor tyrosine kinase for hepatocyte growth factor, signal-regulated kinase (ERK), Ras/Raf/mitogen-activated protein kinase/extracellular phosphoinositide 3-kinase (PI3K)/Akt, mammalian target of rapamycin (mTOR), Wnt/β-catenin, TGF-β, and epidermal growth factor receptor (EGFR) pathways, among others.[9,10]

In the last decade, there has been increasing evidence highlighting the involvement of Galectins (Gals), a family of glycan-binding proteins, in the pathogenesis of HCC. In this review, we summarize the most recent findings regarding Gal-1 and Gal-3 because both are overly expressed in HCC. In contrast to Gal-1 and Gal-3, reduced Gal-9 found in HCC is a risk factor for HCC patient survival. Additionally, the roles of Gal-9 in HCC have been summarized.[11] Therefore, Gal-9 is not included in the current review.

2. Gals

Gals are an ancient family of evolutionarily conserved glycan-binding proteins or lectins and they are recognized by their β-galactoside structures.[12] To date, fifteen mammalian Gals have been detected and classified into three subgroups.[13,14] These proteins contain at least one carbohydrate recognition-binding domain (CRD) of ~130 amino acids with an affinity for β-galactosides and conserved sequence motifs.[15,16] The expression of Gal in different tissues varies. Specifically, Gal-1 and Gal-3 are highly expressed in immune cells, sensory neurons, as well as endothelial and epithelial cells.[17]

Gals do not possess a signal peptide for export through the classical secretory pathway; rather they are secreted to the extracellular milieu via a non-conventional secretory pathway, which is poorly understood.[18,19] For instance, Gal-1 is secreted from skeletal muscle during *in vivo* development as well as the differentiation of cultured myoblasts.[20] There is also evidence of Gal-3 secretion from macrophages, renal, and polarized intestinal epithelial cells.[21–23]

The main ligands for Gal-1 and Gal-3 are the Galβ1, 4GlcNAcβ-epitope which is widely present in complex, branched N-glycans of transmembrane glycoproteins.[24] These

glycoproteins are crucial to the biological effects of Gals. This area has been summarized in other review papers.[25,26] By binding to N-acetyllactosamine sequences, Gals form complexes with cell surface glycoconjugates and transmit signals inside the cell.[27,28] For example, Gal-1 can be internalized by Jurkat T cells in a carbohydrate-dependent mechanism, following dual pathways involving clathrin-coated vesicles and raft-dependent endocytosis.[29]

Within the intracellular milieu, Gals bind to ligands such as synexin, a cytosolic protein that mediates calcium-dependent membrane fusion, B-cell lymphoma-2 (Bcl-2), Ras, and Gem-associated protein 4 through protein-protein interactions and regulate intracellular processes including cell cycle progression, apoptosis, cell proliferation, and mRNA splicing (Table 1).[13,25,30–35] Together, Gals exert their effects through carbohydrate-dependent interactions with extracellular glycoconjugates as well as carbohydrate-independent interactions with cytosolic or nuclear targets.

Gals play an essential role in several physiological processes, *i.e.*, regulating cell adhesion, migration, cytokine synthesis, and survival. Elevated Gals are often found in cancers, *e.g.*, astrocytoma, melanoma, and prostate, thyroid, colon, head and neck, bladder, kidney, stomach, lung, bladder, uterine, breast, and ovary carcinomas.[17,36,37] Moreover, mounting evidence indicates that Gals play fundamental roles in cancer biology, including tumor transformation, tumor growth, angiogenesis, migration, metastasis, and tumor-immune escape.[38] Given these pleiotropic roles in the tumor microenvironment, Gals are being increasingly recognized as molecular targets for innovative cancer therapy.[8,39]

2.1. Gal-1

Like other Gals, Gal-1 has pleiotropic biological functions and is found both inside and outside cells with both intracellular and extracellular functions.[33] The extracellular functions require the carbohydrate-binding properties of dimeric Gal-1 (dGal-1). The single carbohydrate recognition domain of Gal-1 homodimerizes and binds to various glycoprotein ligands.[40] The intracellular functions require interactions with other proteins.[41] Additionally, Gal-1 binds to components of the extracellular matrix (ECM), such as laminin. Furthermore, Gal-1 binding is dependent on the amount of substrate present for cell adhesion.[41] These highlight the natural adhesive characteristic of Gal-1 (Fig. 1).

2.1.1. Expression and regulation of Gal-1 in HCC—Gal-1 mRNA is at higher levels in primary HCC compared to adjacent non-tumorous liver tissues and healthy human liver tissues.[42] Moreover, elevated expression of Gal-1 is found in metastatic lesions from patients with HCC.[43] Furthermore, activated human hepatic stellate cells (HSCs) express high levels of Gal-1.[44] In contrast, silencing the expression of Gal-1 down-regulates the expression of TGF-β and alpha-smooth muscle actin (α-SMA) in HSCs.[45] In cell cultures, the mRNA levels of Gal-1 are higher in more invasive and undifferentiated human HCC cell lines (JHH-6, HLF) compared with well-differentiated liver cancer cells (Huh7, HepG2) or embryonic primary liver cells.[42,46]

There are several mechanisms by which Gal-1 expression can be regulated. These include microRNAs (miRs), methylation, and cytokines. *miR-22* is a tumor suppressor that

effectively reduces the expression level of Gal-1.[47,48] There is an inverse association between Gal-1 and *miR-22* in both HCC and HSCs.[40,44,49,50] Silencing *miR-22* increases Gal-1, leading to enhanced cell growth, migration, and invasion of liver cancer cells. An effect that can be reversed by the Gal-1 inhibitor named OTX008.[40]

Gal-1 also has an immunosuppressive effect. Overexpression of Gal-1 promotes HSC-induced T cell apoptosis as well as interferon-gamma (IFN-γ) and IL-10 production, while *miR-22* expression inhibits Gal-1 in HSCs isolated from HCCs.[44] Furthermore, high Gal-1 expression, along with reduced CD3 counts, is associated with a poor prognosis among patients with HCC.[44] The immunosuppressive microenvironment in HCC promoted by HSC-generated Gal-1 has been shown to be inhibited by *miR-22*.[44] Thus, Gal-1 and *miR-22* potentially can be prognostic markers and therapeutic targets for HCC.[47,51]

Methylation is implicated in Gal-1 expression. CpG di-nucleotides surround the transcription start site of the *LGALS1* promoter. These are frequently found to be methylated in non-tumor liver specimens, whereas these same sequences are hypomethylated in HCC tissues.[52] The specific interactions of a methylation-sensitive factor with the regulatory elements is essential for the activation of the *LGALS1* gene in the liver cancer cell lines of HLF, Huh7, and HepG2, as well as human embryonic primary liver cells.[42]

In addition to methylation, Gal-1 expression and function can be regulated by TGFβ1. During carcinogenesis, tumor cells lose their cytostatic response to TGFβ1 and undergo epithelial-mesenchymal transition (EMT).[34] It has been shown that Gal-1 generated from liver cancer cells such as Huh7 and HepG2 can stimulate its own expression in SK-HEP-1 human liver sinusoidal endothelial cells.[34] The secreted Gal-1 induces sinusoidal endothelial cells proliferation and migration.[34] Additionally, TGFβ1 induces Gal-1 expression and secretion by HCC cells, thereby enhancing the adhesion of HepG2 cells to SK-HEP-1 and sinusoidal endothelial cells, which is a mechanism by which neoplastic hepatocytes escape from TGFβ-induced tumor growth inhibition.[34]

2.1.2. Functional roles of Gal-1 in liver carcinogenesis—Overexpression of Gal-1 plays a role in HCC cell migration, invasion, polarization, cell growth, proliferation, metastatic dissemination, and overall survival.[8,53,54] Retention in endoplasmic reticulum 1 (RER1) is a transmembrane protein localized at the Golgi apparatus. Knocking down the expression of Gal-1 reduces RER1. Indeed, in HCC specimens, there is a significant positive correlation between Gal-1 and RER1 expression.[40] Furthermore, the reduced cell motility of Gal-1-deficient cells can be reversed by RER1 expression. Thus, RER1 is a downstream effector of Gal-1.[40]

Athymic mice injected with Gal-1 overexpressing HepG2 cells have increased tumor volume and the number of draining-tumor lymph nodes compared to mice with no Gal-1.[33] In addition, overexpression of Gal-1 in Huh7 and Hep3B cells increases the number of invasive cells.[55] This has been observed in a nude mouse model in which Gal-1 overexpressing Hep3B and Huh7 cells were orthotopically transplanted, resulting in both liver tumors and pulmonary metastases.[55]

In a model of liver regeneration, Gal-1 deficiency (*LGALS1*$^{-/-}$) delays regeneration after 2/3 partial hepatectomy.[56] Moreover, the delayed regeneration is accompanied by reduced AKT phosphorylation and accumulation of the hepatocyte nuclear p21 protein in the Gal-1-knockout (KO) mice.[56] Taken together, these findings reveal that Gal-1 controls liver cell proliferation.

To assess the contribution of Gal-1 to the aggressiveness of HCC cells, cDNA of *LGALS1* from JHH-6 was cloned and transfected into human HCC Huh7 cells.[46] *LGALS1* overexpressed Huh7 cells have increased invasion and migration. Moreover, *LGALS1* overexpressed Huh7 cells have higher levels of phosphorylation of Syk protein compared with none-*LGALS1* cells.[46] These findings suggest that stimulation of Syk phosphorylation by Gal-1 mediates invasiveness in Huh7.[46,57] In addition, Gal-1 promotes cell adhesion and acts as a glycan-dependent matricellular modulator of human hepatoblastoma HepG2 cells. For instance, soluble Gal-1 significantly increased cell adhesion induced by laminin.[33] The pro-adhesive effects of Gal-1 are mediated explicitly by the integrins, PI3K and MAPK signaling pathways.[33] Blocking α1, α2, α3, αv, and β1 integrins can prohibit Gal-1-induced adhesion in HepG2 cells.[33]

Gal-1 is an inducer of EMT, a key process for tumor invasion and metastasis in HCC.[58–60] Through a PI3/Akt-dependent mechanism, Gal-1 overexpression results in an increased level of transcription factor Snail, which acts as an E-cadherin repressor, a major inducer of EMT.[60] Overexpression of Gal-1 in HepG2 cells increases their resistance to anikos and a loss of apico-basal polarity, which are hallmarks of EMT. Moreover, up-regulation of Gal-1 can reduce the protein levels of Zona-occludens thereby decreasing tight junctions.[60]

Gal-1 appears to control both hepatic pro-carcinogenic as well as anti-inflammatory activities.[61] To understand the mechanisms by which Gal-1 contributes to inflammation-induced HCC, multidrug-resistant (Mdr2)-KO mice were studied.[61] This mouse model mimics human HCC in harboring chronic hepatitis preceding tumor development and aberrant tumor gene expression.[62] Moreover, Mdr2-KO mice express high levels of Gal-1 in the liver.[61] Loss of Gal-1 leads to higher liver injury, fibrosis and inflammation found in Gal-1 and Mdr2 double knockout (DKO) model with C57BL/6 and FVB/N genetic backgrounds from an early age.[63] Moreover, aged DKO mice demonstrate hepatocarcinogenesis and enhanced tumor size earlier than Mdr2-KO mice.[63] The HCC development modulator osteopotin as well as the oncogenic proteins Ntrk2 and S100A4 are increased in the DKO mice compared to Mdr2-KO mice.[63] These findings show that Gal-1 has a protective effect against HCC initiation but pro-carcinogenic effects at the later stage.[63] Therefore, using this particular mouse model, it was concluded that anti-Gal-1 treatments may not be applicable at all stages of chronic liver inflammation-mediated HCC.[63]

Gal-1 is overly expressed in regulatory T cells, and the expression is increased even further after activation.[64] In a Gal-1 homozygous null mutant mouse model, a reduction in regulatory activity of $CD4^+CD25^+$ T cells was observed.[64] Analysis of tissue microarray data from 386 HCC patients demonstrated a significant positive association between Gal-1 expression and the number of $FoxP3^+$ lymphocytes. HCC patients with high Gal-1 and

FoxP3$^+$ Tregs had a more pronounced recurrence and overall poorer prognosis.[65] The interaction of FoxP3$^+$ Tregs and Gal-1 may play a role in the suppression of anti-tumor immune responses against HCC in tumor microenvironments.

Increased Gal-1 leads to resistance in cisplatin treatment, thereby prohibiting HCC cell death.[52] Gal-1 also induces autophagic flux by suppressing the mammalian target of rapamycin complex (mTORC) signaling and activating BCL-2 interacting protein 3 (BNIP3) in HCC cells and thus facilitating chemoresistance.[52] Furthermore, inhibition of autophagy or knockdown of the *Atg5* prevents Gal-1-induced cisplatin resistance in HepG2 and Huh7 cells.[52]

Gal-1 expression and secretion has been found to be significantly upregulated in sorafenib-resistant Huh7 cells (Huh7^R).[43] The knockdown of Gal-1 in Huh7^R cells leads to reduced proliferation, metastasis, and also restores sorafenib sensitivity.[43] Higher Gal-1 expression in Huh7^R causes higher tumorigenic and metastatic effects than in non-resistant Huh7. Inhibition of AKT/PI3K and mTOR reduce the expression of Gal-1 in Huh7^R, which suggests involvement of these pathways in the overexpression of Gal-1 in Huh7^R cells.[43] Reduced sensitivity to sorafenib is also observed in Hep3B cells through PI3K/Akt signaling.[55] Knockdown of Gal-1 in HCCLM3 and MHCC97H cells increases the sensitivity of the cells to sorafenib-induced apoptosis.[55] Additionally, elevated serum Gal-1 levels in HCC patients are associated with poor sorafenib treatment outcomes.[43] Moreover, the overall survival of HCC patients with elevated Gal-1 in normal livers and HCC tissues is 5 months shorter than those patients with reduced Gal-1 (14 *vs.* 9 months).[55]

2.1.3. Gal-1 inhibitor and HCC treatment—To improve diagnosis and treatment of HCC, gene silencing and targeted inhibition of Gal-1 have been studied. A combined treatment using OTX008 (5 mg, 3 days, administrated intraperitoneally) and sorafenib (30 mg/kg/day, administrated orally) in nude mice has been shown to have improved therapeutic outcomes when compared to sorafenib alone.[40] In 2012, a phase 1 clinical trial aimed at evaluating the effects of OTX008 for the treatment of advanced solid tumors was carried out (ClinicalTrials.gov: NCT01724320). The results from this trial were promising in that they confirmed the efficacy of OTX008 in reducing serum Gal-1 in patients with colorectal carcinoma.[40] Similar studies in patients with HCC are pending.[66]

As stated earlier, hepatic fibrosis is a significant precursor to the development of HCC. It is therefore of interest to note that silencing the expression of Gal-1 improves hepatic fibrosis in a mouse model using CCl_4-induced liver injury. Moreover, inhibiting Gal-1 expression in mouse HSCs reduces cell cycle progression, proliferation, and migration but induces the apoptosis of HSCs in the fibrotic livers.[45]

2.2. Gal-3

2.2.1. Structure and general functions of Gal-3—Human Gal-3 is coded by a single gene the *LGALS3*, which is located on chromosome 14, locus q21–q22.[67] 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-κB-like sites, one sis-inducible element (SIE), and a consensus basic helix-loop-helix (bHLH) core sequence.[68,69]

Gal-3 has three distinct domains consisting of a short NH2-terminal domain including a repeated collagen-like sequence, a serine phosphorylation site, and a COOH-terminal domain containing one carbohydrate recognition-binding domain (CRD) which contains an Asp-Trp-Gly-Arg motif.[15] The Asp-Trp-Gly-Arg motif is also present in the Bcl-2 family of apoptosis regulators and is responsible for the anti-apoptotic activity of Gal-3.[31] In solution, Gal-3 largely occurs as a monomer.[70] Although in the absence of its binding partners, it can form homodimers by self-association through its CRDs.[71] In the presence of carbohydrate ligands, Gal-3 can polymerize up to pentamers through its N-terminal domain.[71,72]

Gal-3 plays many roles in different biological functions, including the adhesion, proliferation, and differentiation of tumor cells, angiogenesis, cancer progression, and metastasis (Fig. 1).[35] Several ligands are associated with Gal-3 modulating cell/ECM adhesion such as laminin, collagen IV fibronectin vitronectin, and integrin α1β1 and Mac-1 (αMβ2, CD11b/CD18). Ligand association with Gal-3 modulating cell/ECM adhesion has been summarized in another review paper.[26,73]

Gal-3 is synthesized in the cell cytoplasm.[69] Cytosolic Gal-3 is targeted to the plasma membrane and released into the extracellular space, where it plays a role in the regulation of the migration and adhesion of cells.[73] The translocation of Gal-3 from the nucleus to the cytoplasm involves a nuclear export sequence located within its CRD and occurs through nucleoporin NP98.[74,75] However, the N-terminal domain is also required for the secretion of Gal-3 to the extracellular milieu.[76] Nuclear Gal-3 plays a role in pre-mRNA splicing and the activation of transcription factors.[77]

2.2.2. Expression of Gal-3 in liver diseases—The serum concentration of Gal-3 in alcoholic cirrhosis, non-alcoholic cirrhosis, and toxic hepatitis patients is significantly higher than that of healthy people.[78] Moreover, the concentration of serum Gal-3 is higher in alcoholic cirrhosis (57 subjects) in comparison with toxic hepatitis patients (22 subjects).[79] There is a significant correlation between serum Gal-3 level and the severity of cirrhosis.[79] Gal-3 protein expression level increases in mouse models of NASH with the highest expression in macrophages surrounding lipid laden hepatocytes.[80]

The overexpression of Gal-3 at the mRNA and protein levels was observed in a study of 165 paraffin-embedded HCC specimens.[81] Gal-3 expression correlated with histological differentiation and vascular invasion in patients with HCC.[82] High Gal-3 HCC patients tended to relapse early and experience a shorter overall survival.[81,82] Other clinicopathological parameters, *e.g.*, CD34, tumor size, serum level of alpha-fetoprotein (AFP), and histological differentiation, significantly correlated with a high expression of Gal-3.[81] However, there was no association between the overall survival and sex, age, liver cirrhosis, serum level of AFP and hepatitis B virus surface antigen (HBsAg), metastasis, or recurrence.[81] These data suggest that Gal-3 is a useful marker for predicting the overall survival of patients with HCC.

2.2.3. Functional roles of Gal-3 in liver carcinogenesis—Gal-3 is overly expressed in liver cancer cells including HepG2, Hep3B, Huh7, and Sk-Hep1 compared with that in a normal liver cell line named Lo2.[81] The overexpression of Gal-3 prevents apoptosis in Hep3B cells, which is accompanied by reduced expression of poly (ADP-ribose) polymerase (PARP), caspase-3/9, BAX, and Bcl-2.[81] In contrast, knockdown of Gal-3 induced PARP and caspase-3/9 as well as BAX, and reduced Bcl-2, revealing an anti-apoptotic effect.[81]

Gal-3 knockdown by siRNA significantly inhibits cell growth, migration, and invasion, and also induces apoptosis of HepG2, Hep3B, Huh7, and Sk-Hep1 liver cancer cells.[81] Moreover, knockdown of Gal-3 in HepG2, Bel-7402, Hep3B, Huh7 cells prolong the wound closure time and reduces invasion capability suggesting its role in metastasis.[81] Furthermore, there is a positive correlation between Gal-3 expression and micro-vessel density (MVD), suggesting its role in angiogenesis.[81] However, inhibiting Gal-3 expression did not significantly change the number of G1, S, or G2 phase cells when HepG2, Bel7402, Hep3B, and Huh7 cell lines were used.[81]

The role of Gal-3 in NASH is controversial in Gal-3 KO mouse models. One study showed that Gal-3 KO mice fed an atherogenic diet were resistant to NASH and steatosis after 24 months.[78] In contrast, another study showed that Gal-3 KO mice at six months of age spontaneously developed NASH and there was evidence of neoplastic nodule formation at 15 months.[83]

There are few clinical trials regarding the use of Gal-3 inhibitors in the treatment of colon, breast, testes, ovary cancer, and multiple myeloma carcinoma, which are detailed in other review papers.[84,85] However, there was only one Gal-3 inhibitor (belapectin) studied in liver diseases.[86] The second phase of a clinical trial (NCT02462967) found no significant differences between NASH patients ($N = 108$) who received belapectin (2 and 8 mg/kg) biweekly for 52 weeks and the placebo group ($N = 54$).[86] Belapectin is safe, but there is no significant impact on fibrosis or non-alcoholic fatty liver disease activity score, and liver-related outcomes did not differ significantly between the studied cohorts.[86]

3. Conclusions and future perspective

Current literature shows that Gal-1 and Gal-3 levels are increased in HCC cells compared to their normal counterparts. The up-regulation of Gal-1 clearly correlates with HCC adhesion, tumor growth, migration and invasion, tumor aggressiveness, metastasis, post-operative recurrence, as well as poor prognosis. The overexpression of Gal-3 is mainly associated with HCC anti-apoptosis, angiogenesis, and metastasis. Overexpression of Gal-1 and Gal-3 significantly associate with poor overall survival and may be considered as predictive prognostic factors.[87] Moreover, those Gals not only play a key role in HCC, but also in other liver pathologies including chronic inflammation and fibrosis.

There are many studies concerning the importance of Gal-1 and Gal-3 in HCC. Data obtained from *in vitro* cell culture or murine xenograft models are significant and informative; however, the results need to be translated into orthotopic animal models as

well as clinical settings. Moreover, exploitation of therapeutic options in Gals requires a clear understanding of the mechanism of action and binding targets that may be involved in cancer progression. Gal-1 combined with immune intervention shows promising results. The results suggest that the main function of the increased Gals is to interfere with the anti-tumor effects of T cells in the well-established HCC. Therefore, additional combination treatments should be considered to advance the field.

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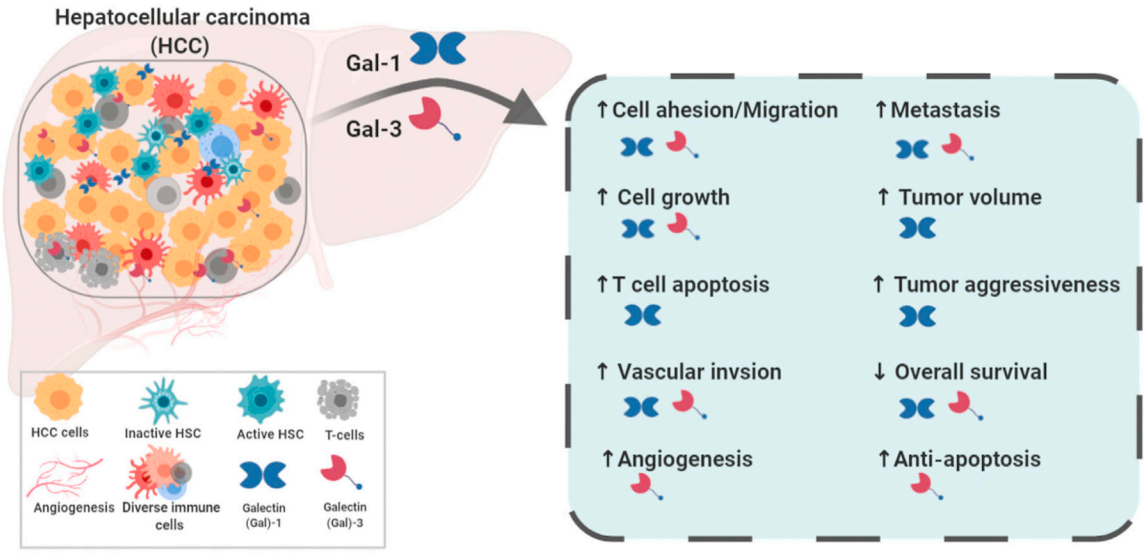

Fig. 1. Gal-1 and Gal-3 in HCC development.

Gal-1 and Gal-3 are overly expressed in HCC. Gal-1 is involved in tumor-associated hepatic stellate cells (HSCs), favoring tumor cell proliferation, adhesion to the extracellular matrix (ECM) as well as tumor progression, T cell apoptosis, metastasis, and overall survival. Gal-3 plays a role in the progression of HCC by regulating angiogenesis, apoptosis, adhesion to ECM, invasion, migration, metastasis, and overall survival. Fig. 1 was generated using tools available at BioRender.com. Abbreviations: Gal, Galectin; HCC, hepatocellular carcinoma; HSC, hepatic stellate cell.

Table 1

The characteristics of Gal-1 and Gal-3.

Gal	Molecular weight (kD)	Intercellular localization (%)	Ligands	Functions	References
Gal-1	14.5	Cytoplasmic (62) Nuclear (22) Mitochondria (4) Endoplasmic reticulum (4) Secretory vesicle (4)	H-Ras, Gem-associated protein, protocadherin-24, Monomeric actin, laminin	• Correlates with tumor aggressiveness and metastases. Enhances the risk of post-operative recurrence • Increases HCC cell adhesion to extracellular matrix, migration, and invasion • Increases tumor growth and metastasis in draining-tumor lymph nodes • Suppresses anti-tumor immune responses	13,25,33,34
Gal-3	29 to 35	Cytoplasmic (26) Nuclear (48) Mitochondria (9)	ATP synthase, protocadherin-24, CD95 (APO-1/Fas), K-Ras, Bcl-2, Gemin, TTF-1, Alix/AIP-1, laminin, collagen IV fibronectin vitronectin, α1β1 integrin and α1Mβ1, CD11b/18, Mac-1 antigen in human macrophages	• Correlates with histological differentiation and vascular invasion • Likely promotes angiogenesis • Inhibits apoptosis	13,25,30,35

Abbreviations: ATP, adenosine triphosphate; Bcl-2, B-cell lymphoma-2; CD, cluster of differentiation; Gal, Galectin; HCC, hepatocellular carcinoma; TTF-1, thyroid transcription factor-1.