Galectins in the tumor microenvironment: Focus on galectin-1

Neus Martínez-Bosch¹ and Pilar Navarro^{1,2}

¹Cancer Research Program, Hospital del Mar Medical Research Institute (IMIM), Unidad Asociada IIBB-CSIC, Doctor Aiguader 88; 08005-Barcelona, Spain

²Institute of Biomedical Research of Barcelona (IIBB-CSIC), Rosselló 161, 08036-Barcelona, Spain

Abstract

In the last decades, the focus of cancer research has moved from epithelial cells to the tumor milieu, in an effort to better understand tumor development and progression, and with the important end goal of translating this knowledge into effective therapies. The galectin family of glycan-binding proteins displays important functions in cancer development and progression. Numerous groups have made outstanding contributions to deepen our knowledge about the role of galectins in the tumor-stroma crosstalk, defining them as key players in modulating interactions between tumor cells and the extracellular matrix, fibroblasts, endothelium, and the immune system. While several members of the family have been of particular interest until now, others are still considered as future exploding stars. This chapter provides an overview for galectin-1, the first identified and still one of the most well-studied galectins, and highlights the very important implications in its regulation of the tumor microenvironment in many different tumor types. Besides, a glimpse of the role of other galectins in the tumor milieu is also provided. Gaining a deeper understanding about the numerous roles of galectin-1 will not only help us to better understand other galectins but also is likely to result in the development of more effective cancer therapies.

Keywords: Galectins, galectin-1, fibroblast activation, tumor microenvironment, angiogenesis, immune evasion, tumor-stroma crosstalk, cancer therapy

I. Galectins: a diverse family with important biological functions

Galectins were first referred as S-type lectins (thiol-dependent) by Drickamer *et al.* in 1988, to differentiate them from C-type lectins, which depended on Ca^{2+} . This classification proved to be inaccurate, as some members do not depend on reduced cysteine residues for carbohydrate binding (Whitney *et al.* 1986; Frigeri *et al.* 1990; Hirabayashi and Kasai 1991). These proteins were also called S-Lac lectins (soluble-lactose binding) (Leffler *et al.* 1989) and it was not until 1994 when the “galectin” name was given, in order to cluster all the identified β -galactoside-binding lectins and to homogenize the nomenclature of individual members (Barondes *et al.* 1994). The galectin family clusters a group of proteins that have a well-conserved carbohydrate recognition domain (CRD) and are classically described to bind lactosamine-containing structures found in glycoconjugates (Cummings *et al.* 2015). Today, galectins comprise 15 different proteins in mammals, 11 of which are found in humans (Figure 1). Members are classified in three different groups according to their structural hallmarks: 1) proto-type galectins (galectin-1 [Gal-1], Gal-2, Gal-5, Gal-7, Gal-10, Gal-11, Gal-13, Gal-14, and Gal-15), which have one CRD that can dimerize; 2) tandem-repeat galectins (Gal-4, Gal-6, Gal-8, Gal-9, and Gal-12), which contain two CRDs linked by a short peptide; and 3) a chimeric-type galectin (Gal-3), which has a large amino-terminus involved in oligomerization (Hirabayashi and Kasai 1993; Rabinovich and Conejo-García 2016) (Figure 1). Although they share general binding traits, galectins have a fine-tuned regulation at different levels, including at the redox environmental state, their subcellular localization, and their quaternary structure, all of which define ligand selectivity and interaction strengths.

Galectins are in both the cytoplasm and nuclei and can also be secreted outside the cell by a non-conventional mechanism (Hughes 1999), being deposited into the extracellular matrix (ECM) or bound to the cell membrane (Rabinovich et al. 2007; Haudek et al. 2010). They are expressed in numerous human tissues, including the placenta, intestine, lung, spleen, and heart (Johannes et al. 2018), and by many cell types, such as epithelial, endothelial, neuronal, and immune cells (Thiemann and Baum 2016).

The first hints that galectins play a role in cancer were observed in a tumor cell line (Teichberg et al. 1975), and data from the 1980s highlighted the importance of the family in mediating tumor-stroma crosstalk. Importantly, Raz and Lotan already speculated on the possible role of a galactoside-specific lectin on the surface of tumor cells in mediating metastasis through interaction of tumor cells with endothelial or immune system cells (Raz and Lotan 1981). These data were soon validated in several experimental models, and these lectins (which were still not called galectins at that moment) emerged as key players in mediating interactions between tumor cells and the host endothelial cells, immune cells, and ECM (Raz and Lotan 1987). Since then, extensive data have highlighted the importance of the galectin family in the tumor microenvironment, controlling key events in cancer development and progression such as fibroblast activation, angiogenesis, and the immune response (Sundblad et al. 2013).

Although other members of the galectin family have shown important responses in the regulation of the tumor microenvironment (and will be briefly addressed in section VII), this review will focus on Gal-1, as this protein stands out as one of the members

with best characterized functions in stroma activation, angiogenesis, and tumor immune evasion responses.

Gal-1 was the first member of the family to be identified and consists of a 14.5 KDa protein of 135 amino acids that is encoded by the *LSGALS1* gene located on chromosome 22q12 (Camby et al. 2006). Depending on the redox conditions, Gal-1 can be found as a monomer (oxidative conditions) or as a homodimer (reducing conditions), the subunits of which are held together by an hydrophobic core (Lopez-Lucendo et al. 2004). The CRD in this galectin recognizes N-acetyl-lactosamine (LacNAc) residues on glycans from cell surface receptors and extracellular proteins, such as integrins, CD43, CD45, fibronectin, mucin, and laminin, but Gal-1 is also able to interact with proteins independently of their sugar-binding moieties (Camby et al. 2006). Importantly, dimeric Gal-1 can establish cell surface microdomains or lattices with multiple glycoproteins found on cell membranes. These lattices are key to organizing membrane domains, mediating signaling at the cell surface, and determining receptor stabilization by controlling endocytosis (Garner and Baum 2008). For example, dimeric Gal-1 is able to control homotypic tumor cell adhesion by interacting with integrin receptors, or cell migration and invasion by recognizing glycans on ECM proteins, such as laminin or fibronectin (Hughes 2001).

II. Glycosylation and Gal-1 in the tumor microenvironment

Of the protein posttranslational modifications, glycosylation gives structural identity to the molecules by coating the cell membrane (which is known as the glycocalix). Importantly, this modification determines the cell's communication with its microenvironment, including with the ECM and other distinct cell types. Glycosylation

is a finely regulated process which is able to sense and quickly react to physiological and pathological contexts (Varki et al. 2015). Aberrant tumor glycosylation has been widely reported, and its implications for driving cancer development and progression have been partially deciphered, opening the gate to interesting applications in oncology (Pinho and Reis 2015). Some of the best known specific glycan traits in cancer include a general increase in sialic acid terminal residues, enhanced N-glycan branching, and overexpression or *de novo* synthesis of sialyl Lewis antigens and short O-GalNac glycans (Varki et al. 2015).

Importantly, galectins can be secreted from cells and thus stand out as important code-readers of these altered glycosylation patterns found in cancer cell membranes or even in the ECM. For instance, Gal-1 recognizes aberrant glycans on mucin-1 expressed on the surface of cancer cells (Jeschke et al. 2006), and it can also interact with poly-N-acetyllactosamines (poly-LacNAc) on ECM proteins, such as laminin or fibronectin, to regulate cell adhesion and migration (van den Brûle et al. 1995; van den Brûle et al. 2003). Furthermore, Gal-1 also communicates with different cells within the tumor microenvironment. For instance, Gal-1 recognizes glycosylated receptors on endothelial cells (ECs) such as neuropilin-1 (NRP-1) or vascular endothelial growth factor receptor 2 (VEGFR2), triggering cell signaling pathways that induce their proliferation, migration, and activation (Hsieh et al. 2008; Croci et al. 2014). Lately, a lot of the interest in Gal-1 as a potential target in cancer therapy has focused on the ability of Gal-1 to recognize receptors in the immune compartment (such as CD43, CD45, and CD7), which leads to specific T-helper type 1 (Th1) and T-cytotoxic cell apoptosis (Perillo et al. 1995; Pace et al. 1999; Hernandez et al. 2006) as well as other

immune evasion mechanisms (Martinez-Bosch et al. 2018b). These and other examples will be specifically addressed in the following sections.

III. Gal-1 expression in the tumor stroma

Gal-1 overexpression has been widely reported to be present in cancer, where in addition to being secreted by tumor cell types and deposited in the ECM, it has also been found to be expressed by diverse stromal components, including fibroblasts, ECs, neutrophils, macrophages, dendritic cells (DCs), and T-lymphocytes (Thijssen et al. 2015). In particular, Gal-1 has been reported in the stroma of prostate cancer, where it was identified as an independent predictor of recurrence (van den Brûle et al. 2001). Gal-1 has also been found overexpressed in the stroma in cervical cancer, where its levels positively correlate with pathological grade (Kohrenhagen et al. 2006). Gal-1 was also detected in the stroma of laryngeal carcinoma (Saussez et al. 2009) and epithelial ovarian cancer (van den Brûle et al. 2003), where peritumoral Gal-1 intensity positively correlates with poor progression-free survival (Kim et al. 2012) and low overall survival (Schulz et al. 2017). In breast cancer, Gal-1 levels in the stroma positively correlated with TNM stage and metastasis. Gal-1 was also expressed in the stroma of colon cancer tissue, at increasing levels as the tumor progressed (Sanjuan et al. 1997). In hepatocellular carcinoma (HCC) samples, Gal-1 also accumulated in the stroma and positively correlated with tumor size, TNM stage, vascular invasion, poor differentiation, metastasis, high rates of tumor recurrence, and low overall survival, identifying Gal-1 as an independent marker of poor prognosis in HCC (Spano et al. 2010; Wu et al. 2012; You et al. 2016). In pancreatic ductal adenocarcinoma (PDA), Gal-1 is highly overexpressed in tumors (localized in the abundant desmoplastic

reaction) (Berberat et al. 2001; Shen et al. 2004; Pan et al. 2009; Tang et al. 2012; Chen et al. 2012; Martinez-Bosch et al. 2018a), has an increased expression in advanced precursor lesions (Pan et al. 2009) and in poorly differentiated tumors (Berberat et al. 2001), and negatively correlates with longer survival times (Chen et al. 2012). Gal-1 is also expressed in the bone marrow microenvironment of multiple myeloma patients (Abroun et al. 2008). In Hodgkin and non-Hodgkin lymphomas, Gal-1 is present in the vascular walls of lymphomas but absent from normal tissue, and it correlates with vascular density (Renatus et al. 1997; D'Haene et al. 2005). In head and neck squamous cell carcinoma (HNSCC), Gal-1 is expressed in the stroma of tumor samples but absent from normal epithelium, and it correlates with the presence of alpha smooth muscle actin (α -SMA)–positive cancer-associated fibroblasts (CAFs) as well as with increased levels of several known HNSCC poor-prognosis factors (Valach et al. 2012). Multiple immunohistochemical studies with large patient cohorts have described Gal-1 expression in the stroma of gastric tumors. Interestingly, its expression positively correlates with tumor size (Chen et al. 2014), tumor location (Bektas et al. 2010), differentiation grade (Bektas et al. 2010; Chen et al. 2014), TNM stage (Bektas et al. 2010; Chen et al. 2014; Zheng et al. 2016; Chong et al. 2016b), histological grade (Zheng et al. 2016), invasion depth (Zheng et al. 2016; Chong et al. 2016b), lymph node metastasis (Bektas et al. 2010; Zheng et al. 2016; Chong et al. 2016b), lymphovascular invasion (Bektas et al. 2010), perineural and serosal invasion (Bektas et al. 2010), lower patient survival (Bektas et al. 2010; Chen et al. 2014; Zheng et al. 2016; Chong et al. 2016b), and with the expression of several markers, including vascular endothelial growth factor (VEGF) (Chen et al. 2014), Gli-1 (Chong et al. 2016b), vimentin, E-

cadherin (Chong et al. 2016a), and transforming growth factor beta (TGF- β) (Zheng et al. 2016) (Table 1).

Tissue	Sample	Functions	Ref
Prostate	148 patients	Predictor of recurrence	van den Br�le et al. 2001
	100 patients	Increased expression in EC from capillaries infiltrating the tumor	Clausse et al. 1999
Cervical	20 benign cervical tissue, 40 intraepithelial lesions, and 20 invasive SCC	Increased expression according to pathologic grade	Kohrenhagen et al. 2006
Ovarian	66 tumors	Negative correlation with progression free survival	Kim et al. 2012
	156 tumors	Overall survival	Shulz et al. 2017
	30 tumors	Increased expression compared with normal stroma	van den Br�le et al. 2003
Gastric	108 tumors	Positive correlation with tumor size, differentiation grade, TNM stage, lymph node metastasis and VEGF expression	Chen et al. 2014
	111 tumors and adjacent normal tissue	Positive correlation with tumor invasion, lymph node metastasis, TNM stage and poor prognosis	Chong et al. 2016
	93 tumors	Positive correlation with tumor location, lymphovascular, perineural and serosal invasions, differentiation, stage and lymph node metastasis, and poor patient survival	Bektas et al. 2010
	134 tumors	Positive correlation with histological grade, invasion depth, lymph node metastasis, TNM stage and TGF� staining	Zheng et al. 2016
	162 paired gastric cancer tissues and noncancerous tissues	Negatively associated with E-cadherin expression but positively correlated with vimentin expression	Chong et al. 2016
Breast	105 tumors	Positive correlation with invasion, T stage, TNM stage, lymph node metastasis	Jung et al. 2007
	55 tumors	Positive correlations between Gal-1 positive cells and Scarff-Bloom-Richardson scale (histological grades 2 and 3)	Dalotto-Moreno et al. 2013
Colon	25 samples of mucosae, 15 adenomas, 25 carcinomas, and 11 metastases	Increased expression with tumor progression from normal mucosae to adenomas and carcinomas	Sanjuan et al. 1997
HCC	386 HCC	Positive association with tumor invasive characteristics, and poor tumor recurrence and overall survival	Wu et al. 2012
	197 HCC	Positive correlation with metastasis	Spano et al. 2010
	162 HCC and 12 normal liver samples	Positive correlation with tumor size, differentiation, TNM stage and distant metastasis. Negatively with CD3	You et al. 2016
Laryngeal carcinoma	53 LSCC of different stages (I,II,IV)	Positive correlation with EC, negative correlation with CD45. Increased expression in the tumor stroma compared to normal stroma	Saussez et al. 2009
PDA	33 normal pancreas, 21 adjacent benign CP, 45 adjacent CP, 17 dysplasias, and 43 PDA	Negative correlation with very long-term survival and longer survival	Chen et al. 2012
	66 PDA, 18 CP, 10NP	Expression: Normal<CP<PDA	Tang et al. 2012
	19 NP, 13 CP, 9 PanIN, 7 IPMN, and 30 PDA	Expression: Normal<CP ~ PDA	Martinez-Bosch et al. 2018a
	28 NP and 33 PDA	Overexpression in tumors compared to normal pancreas. Increased Gal-1 in poorly differentiated versus well-differentiated tumors	Berberat et al. 2001
	33 NP, 17 PanIN	Increased expression in advanced PanIN and PDA	Pan et al. 2009

	and 43 PDA		
	6 NP, 7 CP, 6 PDA	Overexpressed in tumors compared to normal and pancreatitis tissue	Shen Jet et al. 2004
Myeloma	30MM (multiple myeloma)	Gal-1 in the extracellular space	Abroun S et al. 2008
(non) Hodgkin lymphoma	25 normal lymphoid tissues, 42 non-Hodgkins and 14 Hodgkins lymphomas	Gal-1 in blood vessels	D'Haene N et al. 2005
HNSCC	31 patients with HNSCC and adjacent normal epithelium	Gal-1 overexpressed in the tumor stroma, correlating with α -SMA+ fibroblasts	Valach et al. 2012

IV. Gal-1 and fibroblast activation

Several reports have addressed how Gal-1 regulates physiological fibroblast function. Gal-1 induces rodent myofibroblast differentiation, activation (Goldring et al. 2002), proliferation, and migration (Maeda et al. 2003), and Gal-1 knockout (KO) mice show impaired wound healing responses (Lin et al. 2015). Mechanistically, Gal-1 promotes myofibroblast activation by triggering intracellular reactive oxygen species (ROS) production by regulating the NADPH oxidase 4 (NOX4) via the NRP-1/Smad3 signaling pathway in myofibroblasts (Lin et al. 2015). *In vitro*, TGF- β 1 activates fibroblasts by increasing Gal-1 expression through phosphoinositide 3-kinases (PI3K) and p38 mitogen-activated protein kinase (MAPK), and by retaining phosphorylated Smad2 in the nuclei, allowing cells to differentiate (Jin Lim et al. 2014). In liver, Gal-1 promotes hepatic stellate cell migration and invasion through its binding to NRP-1, and signaling through platelet-derived growth factor (PDGF) and TGF- β 1 (Wu et al. 2017a). In experimental mouse models with liver fibrosis, Gal-1 gene silencing in hepatic stellate cells results in downregulation of connective tissue growth factor (CTGF) and α -SMA, and thus inhibition of cell proliferation and migration and concurrent promotion of

apoptosis (Jiang et al. 2019). Indeed, reduced collagen and α -SMA expression were detected in liver fibrosis when studied in Gal-1 KO backgrounds (Wu et al. 2017a).

As Gal-1 is overrepresented in many tumor stroma microenvironments and its expression correlates with several clinicopathological tumor features (Table 1), it seems likely that it has an important role in this key tumor compartment. In oral squamous cell carcinoma (OSCC)–derived fibroblasts, Gal-1 downregulation decreases α -SMA and reduces ECM deposits *in vitro* (Wu et al. 2011) and impairs tumor progression and metastasis *in vivo* (Wu et al. 2011). In pancreatic cancer, the role of Gal-1 in activating CAFs (termed pancreatic stellate cells, PSCs) has been well established. In particular, our group has shown that genetic inhibition of Gal-1 in PSCs, or in different preclinical transgenic mouse models of pancreatic cancer, results in tumors with reduced α -SMA content and impairs tumor growth and metastasis, thereby increasing animal survival (Martínez-Bosch et al. 2014; Orozco et al. 2018). In addition, we have also shown that Gal-1 modulates Hedgehog signaling in PSCs (Martínez-Bosch et al. 2014). Further, Gal-1 modulates proliferation and enhances the release of monocyte chemotactic protein 1 (MCP-1) and cytokine-induced neutrophil chemoattractant-1 (CINC-1) in PSCs, thereby activating extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), activator protein 1 (AP-1), and the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) (Masamune et al. 2006). Recently, *in vitro* approaches have also shown that Gal-1 regulates proliferation and migration of PSCs and that the expression of Gal-1 positively correlates with increased levels of fibronectin, collagen type I, α -SMA, matrix metalloproteinase 2 (MMP-2), and tissue inhibitor of metalloproteinases 1 (TIMP-1), through the TGF- β 1/Smad pathway (Tang et al. 2018). Finally, in patients with

pancreatic cancer, Gal-1 staining in the tumor microenvironment has been proven by multivariate survival analysis to be an independent prognostic factor (Tang et al. 2014). Gal-1 role in inducing fibroblast activation has been addressed in several other tumor settings, such as in human breast cancer, in which Gal-1 knockdown reduces expression of α -SMA, fibroblast activation protein (FAP), and fibronectin, while overexpression of Gal-1 by transfection of breast normal fibroblasts induces differentiation towards a myofibroblast phenotype (Zhu et al. 2016). In HNSCC, Gal-1 overexpression correlates with the presence of α -SMA–positive activated CAFs in the tumor (Valach et al. 2012) (Figure 2).

In addition to its autochthonous functions in activated fibroblasts, Gal-1 has an important paracrine effect over the cancer cell compartment. For instance, in breast cancer, fibroblast-derived Gal-1 promotes cancer cell migration and invasion by induction of MMP-9 (Zhu et al. 2016), a pattern that has also been observed in human breast cancer patients (Jung et al. 2007). Fibroblast-derived Gal-1 has similar pro-tumoral functions in gastric cancer, via integrin β 1 binding (He et al. 2014). Further, this axis is also responsible for Gli-1 upregulation in gastric cancer cells, resulting in epithelial-mesenchymal transition (EMT) (Chong et al. 2016). In ovarian cancer, Gal-1 secreted from fibroblasts modulated cancer cell invasion through MMP-2 (Kim et al. 2012). In pancreatic cancer, fibroblast-derived Gal-1 increases tumor proliferation and invasion by upregulating MMP-2 and MMP-9 in cancer cells (Xue et al. 2011; Tang et al. 2014). Besides, PSC increases the *in vitro* and *in vivo* tumorigenic capacity of pancreatic tumoral cells via paracrine secretion of Gal-1 and gene expression analysis in a pancreatic cancer cell line (RWP-1) upon addition of recombinant Gal-1 brought additional hints on the molecular mechanisms driving these effects by identifying

genes involved in cell proliferation, migration, metastasis and cell metabolism (Orozco et al. 2018). Finally, in OSCC, Gal-1 regulates cancer cell migration through MCP-1 fibroblast secretion and binding to C-C chemokine receptor type 2 (CCR2) in tumor cells (Wu et al. 2011).

Paracrine effects of fibroblast-secreted Gal-1 are not restricted to cancer cells but also rely on signals from other cells of the tumor microenvironment. In pancreatic cancer, Gal-1 from PSCs drives tumor immune privilege by promoting T-cell apoptosis and Th2 cytokine secretion (Tang et al. 2012; Martínez-Bosch et al. 2014; Martinez-Bosch et al. 2018b; Orozco et al. 2018). In gastric cancer, Gal-1 derived from CAFs interacts with ECs to induce proliferation, migration, and tube formation, resulting in increased angiogenesis and tumor progression (Tang et al. 2016).

V. Gal-1 in the tumor endothelium

Gal-1 staining in the endothelium was already described in 1986 (Gabius et al. 1986), and it has been long known to be upregulated upon EC activation (Baum et al. 1995b; La et al. 2003; Thijssen et al. 2006, 2008), and in particular in cancer-associated capillaries in several tumors, such as prostate (Clausse et al. 1999), breast, Ewing sarcoma, colon (Thijssen et al. 2006, 2008), HNSCC, OSCC (Hsieh et al. 2008), and lung carcinomas (Lotan et al. 1994). Overexpression of Gal-1 in tumors can be explained by their typical hypoxic microenvironment, as Gal-1 has been reported to be upregulated upon hypoxia (Le et al. 2005; Croci et al. 2012). For example, Gal-1 is upregulated in colorectal cancer cell lines by hypoxia inducible factor 1 (HIF-1)–dependent mechanisms (Zhao et al. 2010). Alternatively, in Kaposi's sarcoma, hypoxia-mediated increases of Gal-1 expression is driven by ROS-dependent activation of NF- κ B (Croci et al. 2012).

Gal-1 modulates EC activity, inducing their proliferation, migration, and tubulogenesis *in vitro* (Thijssen et al. 2006, 2010; Le Mercier et al. 2008; Hsieh et al. 2008; Ito et al. 2011; Laderach et al. 2013; Tang et al. 2016; Manzi et al. 2016). Indeed, ECs derived from Gal-1 KO mice show reduced tubular networks compared with wild type mice (Mathieu et al. 2012). One of the mechanisms that has been proposed for Gal-1-mediated angiogenesis is signaling through endoplasmic reticulum transmembrane kinase/ribonuclease inositol-requiring enzyme 1 α (IRE1 α), resulting in regulation of oxygen-regulated protein 150 (ORP150) (Le Mercier et al. 2009), a chaperone which modulates VEGF maturation (Ozawa et al. 2001) (Figure 2).

In addition to the relevance of endogenous Gal-1 in EC biology, several reports have also highlighted the importance of exogenous Gal-1 in endothelium functions, in particular in cancer angiogenesis. Indeed, Thijssen et al. (Thijssen et al. 2010) reported that Gal-1 secreted by tumor cells can be uptaken by ECs and signal through H-Ras and the downstream Raf/MAPK/ERK pathway to induce EC migration and proliferation (Thijssen et al. 2010) (Figure 2). Along this line, different cancer cells knocked down for Gal-1 behave similarly, irrespective of being implanted into wild-type or Gal-1 KO mice, highlighting the importance of tumor-secreted Gal-1 for angiogenesis (Mathieu et al. 2012; Croci et al. 2012, 2014). Indeed, tumor-derived Gal-1 enhances EC viability and protects them from apoptosis in the oxidative stress situations that frequently occur in the tumor microenvironment (Ito et al. 2011).

Importantly, most of the mechanisms concerning Gal-1 exerting pro-angiogenic effects in tumors have orbited around the VEGFR family of proteins. Gal-1 interaction with NRP-1, a known co-receptor for VEGF (Soker et al. 1998); results in VEGFR2 activation

and downstream signaling stress-activated protein kinase-1/c-Jun NH2-terminal kinase (SAPK/JNK) (Figure 2), thereby inducing migration and adhesion of ECs in a model of OSCC (Hsieh et al. 2008). Importantly, Croci et al. described that Gal-1 can directly bind to VEGFR2 when displays cancer-specific glycosylation patterns, triggering its activation in the absence of VEGF, resulting in anti-VEGF-resistant tumors (Croci et al. 2014). Extracellular Gal-1 increases vascular permeability through interactions with NRP-1 and VEGFR1, triggering Akt activation and impairing vascular endothelial-cadherin at cell/cell junctions (Wu et al. 2014). Further, Gal-1 and Gal-3 in combination exert an enhanced pro-angiogenic effect through VEGFR1 activation, probably linked to impaired receptor endocytosis (D’Haene et al. 2013). Gal-1 can also promote neovascularization by modulating platelet VEGF release through protein kinase C (PKC) and ERK signaling (Etulain et al. 2014). Besides the VEGF-VEGFR pathway, other molecular mechanisms have been related to activation of ECs by tumor-secreted Gal-1. For example, microarray analyses comparing glioblastoma cells knocked down for Gal-1 as compared to the parental cell line reveal reduced levels of microvascular differentiation gene 1 (MDG1), a marker of EC activation (Berger et al. 2003) and other hypoxia-regulated pro-angiogenic molecules (Le Mercier et al. 2009). In the same direction, Gal-1 inhibition in multiple myeloma cell lines decreases MMP9 and chemokine (C-C motif) ligand 2 (CCL2) and increases semaphorin 3A (SEMA3A) and (C-X-C motif) chemokine 10 (CXCL10) (Storti et al. 2016). Gal-1 also impairs vasculogenesis *in vitro* and *in vivo* through expression of brain-expressed X-linked gene (BEX2) in an oligodendroglioma model (Le Mercier et al. 2009).

Given the importance of neovascularization in tumor development and progression, much preclinical effort has been centered on deciphering the effects of Gal-1

downregulation or blockade in tumors. Further, Gal-1 has been linked to metastasis through its involvement in establishing heterotypic interactions between ECs and tumor cells (Lotan et al. 1994; Lehr and Pienta 1998; Thijssen et al. 2007). Gal-1 triggers tumor angiogenesis *in vivo* in experimental models (Thijssen et al. 2006), and tumors grown or developed in Gal-1 KO mice show impaired angiogenesis (Thijssen et al. 2006; Martínez-Bosch et al. 2014; Büchel et al. 2016; Orozco et al. 2018). Further, Gal-1 knockdown in tumor cells inoculated into nude mice result in impaired tumor angiogenesis in Kaposi's sarcoma (Crocì et al. 2012), prostate cancer (Laderach et al. 2013), kidney cancer (Huang et al. 2014), myeloma (Storti et al. 2016), melanoma, and breast cancer xenografts (Ito et al. 2011). Gal-1 downregulation in Lewis lung carcinoma or T-cell lymphoma cancer cell lines render them sensitive to anti-VEGF therapy by impairing Gal-1–driven angiogenesis (Crocì et al. 2014). Likewise, Gal-1 expression positively correlates with VEGF and Gal-1 knockdown in gastric CAF-impaired tumor growth and angiogenesis *in vivo* (Tang et al. 2016).

Altogether, information generated by these preclinical studies has laid the foundation for the development of Gal-1 pharmacological inhibitors as a novel antiangiogenic therapeutic arsenal. Accordingly, strategies closer to clinical translation have also been assessed in preclinical setups, such as the use of Gal-1 antibodies, which in Kaposi's sarcoma result in tumor regression due to impaired angiogenesis (Crocì et al. 2012), and in melanoma and Lewis lung carcinoma syngenic models restore a functional vascular network rendering tumors sensitive to anti-VEGF therapy (Crocì et al. 2014). Similarly, intratumor injections of a Gal-1 antibody impairs vascular permeability and tumor growth in OSCC xenografts (Wu et al. 2014). In glioblastoma, introducing anti-Gal-1 siRNA into mouse brains significantly decreases angiogenesis and enhances the

effects of temozolamide (Le Mercier et al. 2008). Anginex, an angiostatic peptide that targets Gal-1, shows impaired angiogenesis and tumor growth in a teratocarcinoma syngenic model, which of course could not be observed in a Gal-1 KO background (Thijssen et al. 2006). Very recently, synthetic glycomimetic compounds (Phostine 3.1a) targeting the VEGFR2 and Gal-1 interaction have shown effectiveness in impairing angiogenesis *in vitro* and *in vivo* in a glioblastoma model (Bousseau et al. 2019). Intratumor injections of thiodigalactoside (TDG) (a non-metabolized small drug that targets the amphipathic β -sheet of Gal-1) reduces EC content and tumor growth in melanoma and breast cancer models (Ito et al. 2011). OTX008 (a calixarene compound targeting the CRD of Gal-1) restores tumor vessel normalization and impaired tumor growth in HNSCC (Koonce et al. 2017) and ovarian xenografts (Zucchetti et al. 2013; Astorgues-Xerri et al. 2014b), where the drug potentiates cytotoxic and targeted therapies (Zucchetti et al. 2013; Astorgues-Xerri et al. 2014b). Finally, several reports using different drugs that do not directly target Gal-1 have unveiled Gal-1 to be nonetheless responsible for the pharmacological effects. For instance, in a mouse model of prostate cancer, hemin treatment decreases Gal-1 tumor levels and thus impairs angiogenesis (Jaworski et al. 2017), and anti-VEGF and anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA4) combined therapy in human melanoma elicits humoral Gal-1 immune responses resulting in improved outcomes and overall survival (Wu et al. 2017b).

VI. Gal-1 in tumor immune evasion

Many reports have highlighted the effects of Gal-1 over a wide variety of immune cell types (Rabinovich and Conejo-García 2016). In fact, the role of this protein in

regulating the immune system homeostasis was one of the first identified functions for Gal-1 (Perillo et al. 1995). Gal-1 recognizes different glycosylated receptors in immune cells and can trigger a wide variety of cellular processes, including polarization, maturation, activation, differentiation, and apoptosis (Rabinovich and Toscano 2009; Rabinovich and Conejo-García 2016). In T-lymphocytes, Gal-1 binds to the CD43, CD45, CD7, and CD4 receptors on the cell membrane and regulates their function (Pace et al. 1999; Stillman et al. 2006).

Gal-1 plays a pleiotropic immunosuppressive role by directly targeting different immune cell types. For this reason, it has crucial physiological functions in immune-privileged sites, such as in testis (Pérez et al. 2015), eyes (Ridano et al. 2017) and placenta, where it is key in maintaining fetomaternal tolerance in pregnancy (Blois et al. 2007). Anti-inflammatory functions of Gal-1 are found both in innate and adaptive immune responses. In fact, Gal-1 has been associated to the regulation of most of the innate immune cells. For instance, Gal-1 has been linked to inhibition of neutrophil activation and trafficking upon inflammation (Cooper et al. 2008; Iqbal et al. 2011), as well as to phagocytic neutrophil removal through exposure of cell surface phosphatidylserine (Stowell et al. 2009). In macrophage polarization, Gal-1 favors an M2 profile by regulating metabolism of L-arginine, by decreased nitrogen oxide (NO) production, and/or by promoting the arginase pathway (Correa et al. 2003). Additionally, Gal-1 impairs the expression of major histocompatibility complex II (MHC-II) as well as of Fcγ receptor (FcγR) on macrophages recruited to inflammation sites through ERK1/2 signaling pathway, thereby regulating the phagocytic potential (Barrionuevo et al. 2007). The pro-resolving conversion of macrophages by Gal-1 has also been linked to 12/15-lipoxygenase expression (Rostoker et al. 2013). In

monocytes, Gal-1 induces chemotaxis and can also modulate FcγRI expression, dependent phagocytosis, and MHC-II expression and can interfere with antigen presentation (Barrionuevo et al. 2007). Gal-1 was reported to induced migration and maturation of DCs (Fulcher et al. 2006, 2009; Thiemann et al. 2015), conferring tolerogenic potential by regulating IL-2 production and inducing IL-10–mediated T-cell tolerance (Blois et al. 2007; Iltis et al. 2009). In the adaptive immunity, Gal-1 can modulate B-lymphocyte PI3K signaling, activation, proliferation, and differentiation (Tsai et al. 2014). However, the most important role of Gal-1 in controlling the immune system concerns T-cell function. Gal-1 can regulate T-cell effector function by inducing growth arrest and apoptosis (Baum et al. 1995a; Rabinovich et al. 1998, 2002; Blaser et al. 1998) to specifically induce Th1, Th17 and CD8+ T-cell apoptosis, highlighting an anti-inflammatory Th2 response (Rabinovich et al. 1999b, 2002; He and Baum 2004; Toscano et al. 2006, 2007; Blois et al. 2007). Further, Gal-1 is able to impair proper T-cell activation (Chung et al. 2000) and proinflammatory cytokine production (Rabinovich et al. 1999a, b; Santucci et al. 2003; Cedeno-Laurent et al. 2012c). Gal-1 also regulates T-cell differentiation (de la Fuente et al. 2014), trafficking, and transendothelial migration (Rabinovich et al. 1999a; He and Baum 2006b; Norling et al. 2008). The immunosuppressive activity of forkhead box P3 (FoxP3)–positive T-regulatory (Treg) cells is also controlled by Gal-1 (Garín et al. 2007; Baatar et al. 2009), allowing expansion of T-regulatory type-1 cells secreting interleukin 10 (IL-10) (Toscano et al. 2006; Iltis et al. 2009; Cedeno-Laurent et al. 2012a). Indeed, following intradermal parasite infection, Gal-1 KO mice fail to activate Gal-1–driven tolerogenic circuits and FoxP3+ regulatory T-cells, resulting in a Th1 Th17–favored profile (Poncini et al. 2015).

Modulation of immune system response by Gal-1 together with its frequent overexpression in many tumors prompted numerous studies to explore Gal-1's role in tumor immune evasion. Importantly, in 2004, almost ten years before immunotherapy was named breakthrough of the year, Rubinstein et al. published that Gal-1 was driving tumor immune escape in melanoma, as blockade of Gal-1 *in vivo* induced the generation of T-cell-mediated responses, including enhanced secretion of Th1 cytokines and tumor regression (Rubinstein et al. 2004). Since then, many articles have described that Gal-1 can induce tumor immune suppression in different tumor types, trying to decipher the mechanism by interrogating both innate and adaptive tumor immune responses (Figure 2). In ovarian and lung cancer models, for instance, a major role of the lectin was reported to be to regulate DC tumor-promoting pro-inflammatory activities (Kuo et al. 2012; Tesone et al. 2016). In Kaposi's sarcoma, specific antibodies targeting Gal-1 *in vivo* showed enhanced tumor-infiltrating NK1.1⁺ natural killer (NK) cells (Crocì et al. 2012). Along the same direction, in glioblastoma, Gal-1 downregulation in tumor cells impairs myeloid accumulation and tumor progression due to decreased CCL2 and VEGF expression (which are known macrophage and MDSC chemoattractants) as well as enhanced interferon gamma (IFN- γ) production from CD8⁺ T cells (Figure 2). These data were confirmed by Baker and colleagues, who have shown that Gal-1 knockdown in glioma cells leads to recruitment of Gr-1⁺CD11b⁺ myeloid cells as well as NK1.1⁺ NK cells in tumors, thereby impairing tumor growth (Baker et al. 2016) (Figure 2). Recent *in vitro* and *in vivo* data also have shown that Gal-1 knockdown in glioblastoma results in diminished M2 macrophages and MDSCs as well as decreased immunosuppressive cytokine production (You et al. 2016; Chen et al. 2019). In mice, intranasal delivery of siRNAs against Gal-1 also

reduces MDSCs and Tregs, increases CD4⁺ and CD8⁺ T cells, and impairs M2 polarization in macrophages (Van Woensel et al. 2017) (Figure 2).

Most articles have described the impact of Gal-1 on the T-cell population in tumors. In pancreatic cancer, for instance, co-culturing experiments of PSCs with T-cells show that Gal-1 induces T-cell apoptosis and stimulates secretion of IL-6 and IL-10 while decreasing lymphotoxin (TNF- β) and IFN- γ , thus favoring a Th2 immunosuppressive profile (Tang et al. 2015) (Figure 2). These data were validated in preclinical transgenic models by our group; we reported that, in c-myc-driven pancreatic tumors, Gal-1 ablation results in tumors with increased intratumoral T lymphocytes and increased neutrophil populations (Martínez-Bosch et al. 2014). Similarly, in a K-Ras driven system, Gal-1 KO tumors show enhanced infiltrating T-cells and decreased MDSCs (Orozco et al. 2018). Other descriptive tissue analyses report increased T-cells due to decreased apoptosis in low Gal-1 expressing tumors of lung syngenic models (Banh et al. 2011), a link that has been also corroborated in melanoma preclinical models and in laryngeal squamous cell carcinoma patients (Saussez et al. 2009). In neuroblastoma, Gal-1 downregulation in tumor cells impairs tumor growth and metastasis due to increased accumulation of infiltrating CD4⁺ and CD8⁺ T-cells with enhanced IFN- γ secretion, cytotoxic T cell function, and DC maturation, and reduced T-cell apoptosis (Soldati et al. 2012). In a model of sarcoma, myeloid-derived suppressor cells (MDSCs) were shown to promote Gal-1 secretion in $\gamma\delta$ lymphocytes in toll-like receptor 5 (TLR5) responsive tumors, resulting in immune evasion (Rutkowski et al. 2015). However, Gal-1 regulation of T-cell infiltrates is not only a result of its pro-apoptotic effect. In prostate cancer for instance, Gal-1 inhibits transendothelial migration of T-cells through CD43 clustering (He and Baum 2006b), and Gal-1 inhibition with hemin

enhances CD8⁺ T-cell proliferation and antigen-specific cytotoxicity *in vivo* (Jaworski et al. 2017). As mentioned above, Gal-1 also affects the intratumoral Treg population (Figure 2). More specifically, silencing Gal-1 in breast cancer syngenic animals reduces the presence of intratumoral and peripheral FoxP3⁺ Tregs, impairing its function and suppressing its activity, thus resulting in impaired tumor growth and metastasis (Dalotto-Moreno et al. 2013). Interestingly, in hematological tumors, Gal-1 has also been reported to induce immunosuppression (Cedeno-Laurent et al. 2012b, a, c) but is also relevant for the tumor cells themselves. For instance, in leukemia, Gal-1 derived from myeloid cells modulates B-cell receptor (BCR) signaling in tumor B-cells through Gal-1, thereby regulating tumor cell activity and favoring cancer progression (Crocì et al. 2013). Gal-1 in neoplastic Reed-Sternberg cells in Hodgkin lymphoma impairs T-cell viability and maintains expansion of FoxP3⁺ Treg cells, to support an immunosuppressive Th2 milieu (Juszczynski et al. 2007; Cedeno-Laurent et al. 2012c) (Figure 2).

The relevance of tumor versus host Gal-1 in immunosuppression was addressed by regulating Gal-1 expression in cell lines and injecting them into wild-type or Gal-1 KO mouse models. Interestingly, in neuroblastoma, T-cell-derived Gal-1 regulated intratumoral infiltrates without affecting tumor growth, which was however affected by tumor-derived Gal-1 (Büchel et al. 2016); this indicates important issues that need to be considered before developing therapeutic Gal-1 targeting strategies. In lung cancer and glioblastoma, for instance, tumor Gal-1 also proved to be more determinant than host-derived Gal-1 in tumor growth and metastasis (Banh et al. 2011; Verschuere et al. 2014). Although the reasons of this difference are unknown, the authors discussed that this may be due to enhanced Gal-1 expression levels from

tumor cells as compared to T-cells (Banh et al. 2011). Importantly, driving tumor immune evasion by Gal-1 seems to be one of the major functions of this protein in cancer, as the effects of reducing tumor growth and increasing survival observed after Gal-1 downregulation are not observed in immunodeficient mouse models (Banh et al. 2011; Croci et al. 2014; Martínez-Bosch et al. 2014).

Tumor-derived Gal-1 is not only able to promote an immunosuppressive tumor microenvironment but also to generate a systemic immunosuppression in the animal (Dalotto-Moreno et al. 2013). Indeed, in lung metastases models, inhibiting Gal-1 with TDG or reducing endogenous Gal-1 levels in tumor cell lines enhances peripheral T-cell immune responses and impairs metastases. As expected based on its important role in evading the tumor immune response, blocking Gal-1 has shown preliminary synergistic effects with immunotherapy in preclinical models. Screening The Cancer Genome Atlas (TCGA) reveals that Gal-1 levels in glioblastoma patients negatively correlates with the Th1/Treg and cytotoxic T lymphocytes (CTL)/Treg ratios and overall survival (Van Woensel et al. 2017). Accordingly, silencing Gal-1 improves DC vaccine and programmed cell death protein 1 (PD-1) blocking therapies in mice with glioma tumors (Verschuere et al. 2014; Van Woensel et al. 2017). Inhibitory disaccharides targeting Gal-1 also improve vaccine immunotherapy in breast cancer preclinical models (Stannard et al. 2010). In non-Hodgkin lymphoma, a forward exome screen of primary tumors identified Gal-1 as a marker of resistance to CD20 immunotherapy; this finding was validated in an *in vivo* preclinical model (Lykken et al. 2016).

VII. Other galectins in the tumor microenvironment

Besides Gal-1, other galectins have garnered the attention of researchers who are trying to understand the tumor microenvironment, mainly due to their role in angiogenesis and regulation of the tumor immune response (Cerliani et al. 2017; Méndez-Huergo et al. 2017).

The role of galectins in cancer fibroblast activation appears to be monopolized by Gal-1. However, Gal-3 can also be secreted (Bänfer et al. 2018; Popa et al. 2018) and is found in the stromal compartment of tumors (Logullo et al. 2007; Schulz et al. 2017). Thus, in addition to its important roles in fibroblast biology in fibrotic disease (Henderson et al. 2006; Li et al. 2014) and arthritis (Filer et al. 2009), Gal-3 also has predominant functions in CAF activation. Indeed, in 2018, Zhao and colleagues described that recombinant Gal-3 can induce proliferation, invasion, and inflammatory cytokine secretion by PSCs, which contributes to tumor growth and metastasis in preclinical mouse models (Zhao et al. 2018).

Gal-3 is also one of the best characterized members of the galectin family with respect to tumor blood vessel formation. Different reports have shown that Gal-3 induces EC morphogenesis, chemotaxis, and differentiation, leading to angiogenesis (Nangia-Makker et al. 2000, 2002a, b; Markowska et al. 2010; Funasaka et al. 2014). Several mechanistic studies have tried to elucidate how Gal-3 triggers angiogenesis in tumors; these studies have shown that Gal-3 retains VEGFR2 on the membrane of ECs (Markowska et al. 2011), directly interacts with Jagged-1 (JAG1) in the EC cell membrane and activates Notch-1 (Santos et al. 2017), and induces VEGF expression from macrophages (Machado et al. 2014). Furthermore, Gal-3 regulates tumor cell

adhesion to ECs, promoting metastasis (Nangia-Makker et al. 2002b; Shekhar et al. 2004; Funasaka et al. 2014).

Gal-8 has also been reported to play a key role in tumor angiogenesis and was classified as a pro-angiogenic molecule in 2011 by Delgado *et al.*, who reported that this galectin controls EC migration and angiogenesis *in vitro* and *in vivo*. CD166 was identified as the receptor for Gal-8 in EC membranes (Delgado et al. 2011; Troncoso et al. 2014). Regarding Gal-9 role in tumor angiogenesis, increased levels of this lectin have been found in blood vessels from lung, liver, breast, and kidney carcinomas (Heusschen et al. 2014). Interestingly, ECs can express different splicing forms of Gal-9, and the expression of this protein is regulated during EC activation, although the precise role of Gal-9 in angiogenesis is not yet well understood (Thijssen et al. 2008). Angiogenesis induced by these different galectin members can also be triggered in an indirect way by cytokines or soluble factors. For example, both Gal-3 and Gal-8 were shown to promote VEGF release by platelets, and Gal-8 also induces endostatin secretion, leading to angiogenesis (Etulain et al. 2014). Similarly, through its function as eosinophil chemoattractant or DC expansion, Gal-9 might induce the release of pro-angiogenic factors by these cells (Heusschen et al. 2014).

Moving to tumor immunity regulation, other galectin family members besides Gal-1 have raised a lot of interest for cancer, being even considered as emerging immune checkpoints. Gal-3, for example, has modulatory effects on T-cell survival and activation (Fukumori et al. 2003; Stillman et al. 2006; Peng et al. 2008), NK function (Tsuboi et al. 2011; Wang et al. 2014), and DC expansion (Kouo et al. 2015). In particular, the axis of Gal-3/lymphocyte-activation gene 3 (LAG-3) has been postulated

to be a novel cancer immune checkpoint (Melero et al. 2015). Gal-9 gained the attention of oncologists when it was identified as a partner of the T-cell exhaustion marker T-cell immunoglobulin and mucin-domain containing molecule-3 (TIM-3)(Zhu et al. 2005). Thus, the Gal-9 and TIM-3 interaction induces effector T-cell exhaustion or apoptosis, leading to tumor immune evasion; therefore, Gal-9 has been also recently added to the list of cancer immune checkpoints (Melero et al. 2015). Recent preclinical data in mouse models of liver cancer have demonstrated that therapy with antibodies against LAG-3 (i.e. targeting the Gal-3 axis) or TIM-3 (i.e. targeting the Gal-9 axis) are able to restore cancer immune surveillance and increase tumor-infiltrating T-cell number and functionality (Zhou et al. 2017). These data open new gates to assessing novel immunotherapy combinations that may result in increased percentage of responders or even allow resistance to be overcome; they further provide a rationale for studying these new immune checkpoints *per se*. Whether other family members have a role in promoting tumor immune suppression has not yet been elucidated, although *in vitro* data have already proven that Gal-2, Gal-4, and Gal-8 can also exert pro-apoptotic functions over T-cells (Tribulatti et al. 2007; Paclik et al. 2008; Norambuena et al. 2009).

Still, as we have experienced for Gal-1, special precaution and profound understanding of the context is vital, as other galectins also present apparently controversial outcomes depending on the model. For instance, breast cancer immunocompetent preclinical models show that absence of Gal-3 in the host mouse in fact boosts tumor growth and bone marrow metastasis (Pereira et al. 2016).

VIII. Future trends and directions

In this chapter, we discuss the current knowledge about Gal-1 functions in the tumor microenvironment. These effects of Gal-1 driving fibroblast activation, angiogenesis, and tumor immune suppression are deeply entangled with one another and cannot be understood separately (Figure 2). In lung cancer, for instance, Gal-1 secreted from tumor cells is important for fibroblast activation and induction of tryptophan catabolism by AKT signaling pathway and TDO2 (tryptophan 2,3-dioxygenase) and kynurenine (Kyn) upregulation, which contribute to immune evasion by impairing DC differentiation and T-cell function (Hsu et al. 2016). In fact, fibroblast activation promotes the secretion of chemokines and cytokines that are responsible for aberrant blood vessel formation in tumors and for establishing an immunosuppressive milieu (Masamune et al. 2006; Tang et al. 2012, 2016). Furthermore, tumor immune evasion driven by Gal-1 is not only a direct effect of Gal-1 over immune cell types but also a consequence of a deficient blood vessel network. Indeed, in 2014, Croci and colleagues reported that anti-Gal-1 antibody administration in immunocompetent melanoma and lung preclinical mouse models results in increased intratumoral infiltrates and tumor-draining lymph node cells, which is at least partially due to vessel normalization (Croci et al. 2014). Tumor cells induce expression of Gal-1 by EC, which then inhibits T-cell transendothelial migration (as discussed above) (He and Baum 2006a). Interestingly, tumor growth as well as angiogenesis are more severely impaired upon TDG treatment in immunocompetent mice as compared to immunodeficient animals (Ito et al. 2011), highlighting the strong link between Gal-1-mediated immune regulation and cancer cell proliferation and vessel formation.

The tumor microenvironment has over the years emerged as a key governor of tissue malignancy that drives tumor development and progression (Hanahan and Weinberg

2011) and is often the cause of ineffective therapies. Thus, strategies targeting the tumor soil have gained the interest of both basic and clinical researchers in virtually all tumor types, with thousands of clinical trials being designed and performed (EBioMedicine 2018; Degroote et al. 2018; Cuoco et al. 2018; Zandberg and Ferris 2018; Roma-Rodrigues et al. 2019; van Mackelenbergh et al. 2019). Although clinical trials are still in their infancy, robust preclinical data suggest that galectins may represent interesting targets in this tumor compartment (Ingrassia et al. 2006; Thijssen et al. 2015; Méndez-Huergo et al. 2017; Wdowiak et al. 2018; Dings et al. 2018). Among them, Gal-1 emerges as a top candidate with high potential, as this protein is a pleiotropic molecule that remodels the tumor microenvironment as a whole –including fibroblast activation, angiogenesis, and immune evasion– to allow uncontrolled tumor progression. Accordingly, development of Gal-1 inhibitors has currently a strong interest in cancer therapy and has shown promising results in several preclinical models (Ito et al. 2012; Astorgues-Xerri et al. 2014a). Moreover, taking into account the critical role of Gal-1 in immune evasion through its direct interaction with cell membrane receptors of effector T-cells triggering cell apoptosis, the Gal-1–CD7/CD43/CD45 axis could be considered as a new immune checkpoint (like Gal-3/LAG-3 and Gal-9/TIM-3; (Melero et al. 2015)); therefore, Gal-1 inhibitors could enlarge the list of novel cancer immunotherapies.

However, several issues are crucial for successful and proper drug development, such as having a profound understanding of the particular tumor type and galectin repertoire, deciphering redundant versus specific roles of each member, the endogenous versus exogenous roles of the protein, drug specificity, and so on. Classical *in vivo* models trying to study targets at the tumor microenvironment have

shown limited translational success (Sharpless and Depinho 2006; Richmond and Su 2008). More recently, patient-derived cancer models, including organoids and patient-derived xenografts (PDXs), have emerged in cancer preclinical studies, but they still lack the autochthonous tumor microenvironment (Weeber et al. 2017; Williams 2018). With the advent of immunotherapy, many groups have joined efforts to develop more suitable and more sophisticated models to allow the tumor as a whole to be investigated, such as using humanized mice (De La Rochere et al. 2018) and *ex vivo* systems that retain the whole native tumor microenvironment, such as 3D microfluidic cultures, tumor tissue explants, “tumor-on-a-chip”, and multicellular tumor spheroids (Jenkins et al. 2018; Roma-Rodrigues et al. 2019). Further experiments in systems with better recapitulation of the tumor’s surrounding will for sure be necessary for completing preclinical steps before moving Gal-1 therapy into the clinics.

In addition to the emerging attention on Gal-1 inhibitors as a novel cancer therapy, Gal-1 also offers other translational applications. For example, increasing data have unveiled the potential use of detecting Gal-1 levels for cancer diagnosis (Thijssen et al. 2015), as Gal-1 can be detected in biological fluids and, for many of the tumors expressing Gal-1, in the stroma (Table 1). Further, increased levels of the protein have been detected in plasma or serum from patients with cancer of thyroid (Saussez et al. 2008), colorectal (Watanabe et al. 2011), pancreatic cancer (Martinez-Bosch et al. 2018a), Hodgkin lymphoma (Ouyang et al. 2013), glioma (Verschuere et al. 2013), or OSCC (Aggarwal et al. 2015). Moreover, in the era of personalized medicine, Gal-1 may also work for selecting patients who respond better to anti-angiogenic or immunotherapies, and it could represent a useful biomarker for therapy response.

Further preclinical and clinical data will be necessary to answer whether reality meets expectations to all of us working in the galectin world.

Table Legends

Table 1. Data on Gal-1 expression in the tumor microenvironment. This table summarizes the articles in which Gal-1 has been described in the tumor stroma by immunohistochemistry, describing the amount of samples analyzed and the functions and clinical correlations observed.

Figure Legends

Figure 1. Galectin family in mammals. Fifteen galectins have been described in mammals, 11 of which have also been found in humans (located in the central core of the diagram). Galectins are organized in three groups: proto-type galectins that display one CRD and function as monomers or dimers; tandem-repeat galectins that have two CRDs linked; and chimeric-type galectins formed by Gal-3 with a long amino-terminal domain that allows oligomerization.

Figure 2. Gal-1 functions in the tumor microenvironment. Information regarding the roles of Gal-1 in fibroblast activation, induction of angiogenesis, and tumor immune suppression is shown in this overview picture. Precise data and references are found in the main text.

Acknowledgments

This work was supported by grants from the Spanish Ministry of Economy and Competitiveness/ISCIII-FEDER (PI17/00199), the Carmen Delgado/ Miguel Pérez-Mateo

AESPANC-ACANPAN 2016 grant, and the Generalitat de Catalunya (2017-SGR-225) to P.N. We are also grateful to A. Flotats for help in graphic design and V. Raker for English proofreading and editing.

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FIGURES

Figure 1

Figure 2