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Glycans and Galectins: Sweet New Approaches in Pancreatic Cancer Diagnosis and Treatment

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1. Introduction

Pancreatic cancer is one of the tumors with worst prognosis. Its low survival rate is due to late diagnosis because of the lack of symptoms when the tumor initiates, being frequently diagnosed when it metastasizes to other organs. Thus, new early diagnostic biomarkers are an urgent need to improve pancreatic cancer survival rates. Aberrant protein glycosylation is common in tumoral cells, involving changes in glycosyltransferases and glycosidases that could be mediated by inflammatory cytokines and growth factors. These alterations are functionally important in cancer progression influencing cell migration and adhesion, metastatic capability and immune escape. These changes in protein glycosylation during tumor progression can lead to alterations in membrane proteins clustering and lectin binding, conferring functional advantages to tumoral cells. In this regard, differential reactivity towards endogenous lectins, especially galectins, has been reported in several cancers. Galectins are involved in a variety of biological processes including tumor growth and malignant transformation. This chapter focuses on the specific alterations in protein glycosylation and galectin expression and binding during pancreatic cancer progression, as well as their potential use as prognostic biomarkers and therapeutic targets. Interestingly, we have characterized the importance of the interaction between a glycoprotein (tissue plasminogen activator, tPA) and Galectin-1 (Gal-1) in pancreatic cancer, suggesting that strategies targeting this interplay might result in successful treatments.

2. Glycosylation in cancer

2.1 Glycans: General features

Glycosylation is one of the most common post-translational modifications and nearly half of all proteins in eukaryotes are glycosylated (Spiro, 2002). Glycans (oligosaccharides from glycoproteins) are classified considering their linkage to the protein backbone in N-Glycans (bound to the amide side chain of Asn) and O-Glycans (bound to the hydroxyl of Thr or Ser).

Studies focused on the carbohydrate moiety of proteins are methodologically complicated due to the extremely high diversity and flexibility of these structures. N-glycan content at

one particular site is frequently miscellaneous. Their structural diversity embraces the number and nature of monomeric units, their position, anomeric configuration and branching. Glycoproteins display site-occupancy heterogeneity (macroheterogeneity), which refers to the diversity on the presence or absence of glycan chains in specific aminoacids. Moreover, not all N-linked glycan sites are occupied. Apart from this source of variation, glycoproteins also present site-specific heterogeneity (microheterogeneity), which describes differences found regarding the carbohydrate content and structure present in a single glycosylation site.

Glycosylation of proteins can affect their folding, enhance solubility, intracellular trafficking, localization, secretion and rate of degradation (Hakomori, 2002). Apart from conferring specific properties to proteins themselves, glycans significantly affect protein/protein interactions, preventing the non-specific ones. In this direction, they mediate accurate cell/cell communication and signal transduction as well as the interaction between a cell and the extracellular milieu and soluble signaling molecules. Carbohydrate structures are key in many cell biological functions and indeed eighteen different types of congenital disorders of glycosylation (CDG) have been genetically defined (Freeze & Aebi, 2005).

2.2 Altered glycosylation in cancer

Typically, cancer has been associated with gain-of-functions in oncogenes or loss-of-function in tumor suppressor genes. However, there are many other mechanisms responsible for orchestrating all the events triggering cancer stepwise progression. In spite of the marked physiological glycan heterogeneity, cancer progression and metastasis have been characterized by significant alterations of the carbohydrate signature. Indeed, aberrant glycosylation is one of the cancer cell hallmarks (Varki et al., 2009), and certain structures are well-known markers of tumor development (Hakomori, 2002; Lau & Dennis, 2008). Besides, changes in glycosylation are presented not only by cancerous cells but also by cells surrounding the tumor (Rabinovich & Toscano, 2009). This specific pattern of glycosylation linked to neoplasia might affect protein functionality significantly, altering cell behavior in many different ways. Distinctive glycosylation profiles favor or impede interactions with different proteins.

Some of the best characterized glycan specific alterations in cancer are a general increase in sialic acid content, an increase in glycan branching and overexpression of specific carbohydrate antigens like sialyl Lewis antigens (SLe^a and SLe^x) (Fig.1). The tight regulation of enzymes during protein glycosylation is crucial and indeed, the population of sugars attached to each glycosylated site depends on the cell type in which the glycoprotein is expressed and in the physiological status of the cell. Inflammatory cytokines and growth factors such as IL-1β, TNF-α, IL-6 and EGF, mediate changes in concentration of glycosyltransferases and glycosidases, altering the proportion of the glycoforms present in a particular glycoprotein.

Glycan alterations are functionally important in cancer progression by affecting cell proliferation and survival, adhesion and migration, angiogenesis and metastatic capability, as well as the immune escape. For instance, a very common feature in cancer is the increased activity of β1-6-N-acetylglucosaminyltransferase V (GlcNAcT-V or MGAT5), which is in charge of β1-6 branching of both O and N-glycans. As a functional example of this fact,

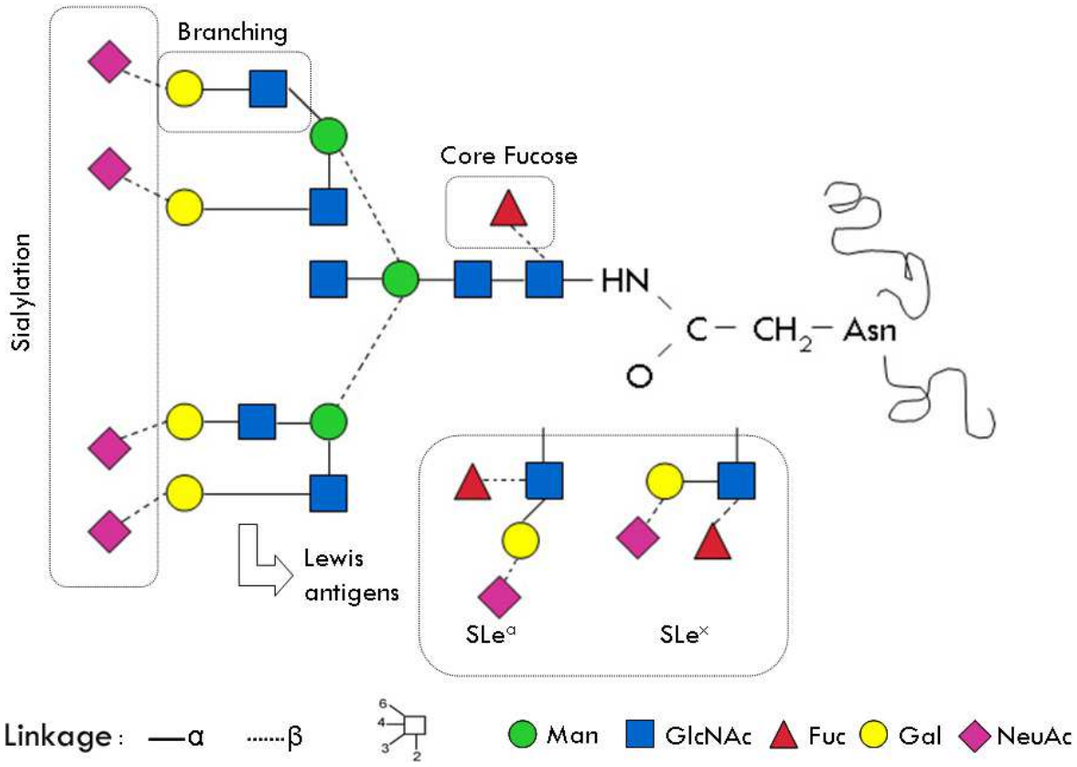

Fig. 1. Most frequent N-glycan altered patterns observed in tumorigenesis. Common features of cancer glycosylation include sialylation, increased β-1,6-branching, core fucosylation and sialyl-Lewis antigens.

increased branching in the β_1 subunit of $\alpha_5\beta_1$ integrin due to enhanced MGAT5 expression, inhibits integrin clustering, reducing the attachment of cancer cells to fibronectin and thus inducing migration (Guo et al., 2002). This enzyme is also involved in enrichment of the SLe^x group, which confers cells the ability to extravasate and metastasize. *In vivo*, progression of mammary tumors in MGAT5 knockout mice is significantly impaired (Granovsky et al., 2000). Various factors including oncogenes as Src, Her-2/neu, H-Ras, and V-sis and known cancer altered signaling pathways as Ras-Raf-Ets regulate MGAT5 transcription. What still remains to be determined is whether changes in glycosylation are a cause or a consequence of transformation. Cytokine regulation of glycosyltransferase activity suggests that signaling from the tumor microenvironment can be the responsible for cancer-associated glycosylation.

2.3 Glycosylation in pancreatic cancer

Specific alterations in pancreatic cancer glycoproteins have been described, such as increased N-glycan branching and increased fucosylation and sialylation (Zhao et al., 2007). Importantly, some of the aberrantly glycosylated proteins have been suggested as biomarkers (Lacunza et al., 2007; Okuyama et al., 2006; Peracaula et al., 2008). Lectin antibody microarrays have been used to detect unique glycosylation patterns in pancreatic cancer serum in high throughput strategies (Li et al., 2009; Wu et al., 2009). These assays proved efficient specificity and sensitivity and shed some light in distinguishing between

pancreatic cancer and chronic pancreatitis, a matter that has been for long unresolved. Major alterations in glycan-linked gene expression associated to pancreatic cancer epithelial to mesenchymal transition *in vitro* have been also reported (Maupin et al., 2010).

Data proposing some of the causes of altered glycosylation have emerged. Proinflammatory stimuli such as IFNγ, TNFα and IL-1α, in pancreatic cancer cells are responsible for altering Muc1, Muc5AC and Muc16 glycosylation in a cell type specific manner (Wu et al., 2009), and indeed, cytokine secretion has also been considered in pancreatic cancer diagnosis (Fearon et al., 1999; Wigmore et al., 2002).

One of the current pancreatic tumor markers is the monoclonal antibody CA19-9 (Ferrone et al., 2006), whose epitope is the SLe^a antigen in gangliosides and mucins. SLe^a physiologically functions in the extravasation of lymphocytes from the bloodstream by interacting with selectins on endothelial cells. In accordance with these data, its expression on the surface of pancreatic cancer cells has been linked to metastasis spread to other tissue sites (Aubert et al., 2000). Nevertheless, CA19-9 generally does not have the specificity and sensitivity required for general screening, being frequently restricted to monitor patient's progress after surgery. RNase-1 was long ago proposed as a tumor marker in pancreatic cancer but both its levels and its activity in serum failed in diagnosis. However, differences in glycosylation in this protein exist, finding neutral structures in healthy pancreas whereas charged structures (such as SLe^x and SLe^a antigens) and a significant increase in core fucosylation and sialylation are observed in pancreatic cancer (Peracaula et al., 2003). Increased core fucosylation is a general cancer feature and it is also common in pancreatic cancer. Serum haptoglobin and other acute phase proteins are also found to be more core fucosylated specifically in pancreatic cancer (Okuyama et al., 2006; Sarrats et al., 2010).

3. Galectins in cancer

3.1 The galectin family: Main features

Galectins belong to the lectin family of proteins, which are highly evolutionary conserved finding their members in all animal kingdoms and even in plants, fungi and viruses. All the proteins of the family share two main features: high affinity for β-galactosides and a well conserved carbohydrate recognition domain (CRD) of 130 aminoacids (Barondes et al., 1994). However, each galectin has a specific carbohydrate binding preference, as a result of their ability to accommodate different saccharides attached to galactose.

15 galectins have been described in mammals (11 of which are expressed in humans) and they can be structurally clustered in three groups (Fig.2): 1) Prototype galectins (1, 2, 5, 7, 10, 11) consist of a single CRD with a short N-terminal sequence; 2) Tandem-repeat galectins (4, 6, 8, 9) are composed of two differents CRDs joined by a short linker peptide sequence; and 3) Chimaeric galectins (Gal-3) have an extended N-terminal tail containing a consensus nine aminoacid residue-repeat rich in Pro, Tyr and Gly.

Galectins are differently distributed in animal tissue and its expression is modulated during differentiation and tissue development, changing in some physiological and pathological conditions (Yang et al., 2008), such as in cancer (Danguy et al., 2002). Galectins are secreted by a non-canonical pathway and display a wide variety of intra and extracellular functions.

Fig. 2. Galectin structural classification. Prototype galectins (Gal-1,2,5,7,10,11,13,14,15) have one CRD domain. Tandem repeat galectins (Gal-4,6,8,9,12) are composed of two different CRD. The only chimaeric galectin (Gal-3) has an extended N-terminal domain.

3.2 Gal-1: Structure and functions

The first protein discovered in the human galectin family was Gal-1 (Couraud et al., 1989; Gitt & Barondes, 1986), which is encoded by *LGALS1* gene located in chromosome 22q12-13.1. Splicing of its four exons results in a 0.6 Kb transcript that is translated into a protein of 135 aminoacids, without suffering any post-translational modification. Gal-1 expression might be modulated by histone acetylation and promoter methylation.

Gal-1 is a symmetrical dimer of 14.5 KDa subunits and it has a β-sandwich "jelly-roll" conformation involving two parallel β-sheets, which form a central hydrophobic core holding both amino and carboxy-terminus of each monomer. Gal-1 CRD has a binding grove that allows the presence of a tetrasaccharide (A, B, C and D). C site includes the eight

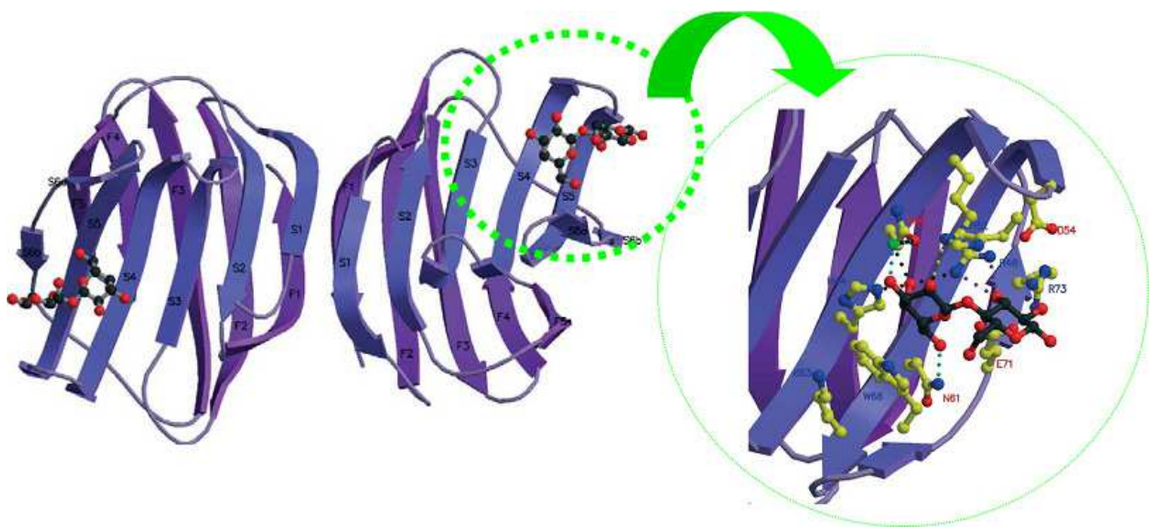

Fig. 3. Human dimeric Gal-1 jelly-roll structure complexed with lactose. Ribbon diagram prepared with MOLSCRIPT. Five-stranded (F) and six-stranded (S) sheets of each monomer are labelled in the image and the aminoacids involved in lactose binding are highlighted in the enlargement (His44, Asn46, Arg48, His52, Asn61, Trp68, Glu71 and Arg73). Adapted from (Lopez-Lucendo et al., 2004).

so well conserved aminoacids responsible for galactose binding (Fig.3), and this is common among all galectins. The rest of the sites are involved in galectin recognition specificity. Both Gal-1 and Gal-3 typically lodge a terminal LacNAc in site C-D but binding is inhibited by the presence of NeuAcα2-6 in the galactose located in B. Functional differences and binding avidities between Gal-1 and Gal-3 suggest the existence of additional determinants of binding specificity.

Gal-1 is found in the cytoplasm, membrane, extracellular matrix (ECM) and nucleus, being involved in a wide variety of cellular functions through its ability to recognize many different proteins (Elola et al., 2005). Extracellular functions depend on Gal-1 lectin activity whereas intracellular functions are usually independent and involve protein/protein interactions.

3.3 Role of galectins in cancer

Galectins have been reported to be clear modulators of tumor progression (Liu & Rabinovich, 2005) and their heightened expression usually correlates with tumor clinical aggressiveness and metastasis. Several members of the family have been involved in tumor progression, being Gal-1 and Gal-3 the best characterized ones (Danguy et al., 2002; Yang et al., 2008). These proteins display important functions in several aspects of cancer biology including cell adhesion, migration, tumor transformation, apoptosis, cell cycle progression, angiogenesis and immune response regulation. Indeed, galectin inhibitors have been well considered for cancer therapy (John et al., 2003; Sorme et al., 2003; Zou et al., 2005).

Gal-1 expression has been identified as a prognostic factor for tumor progression in many different neoplasms (Demydenko & Berest, 2009). Gal-1 involvement in tumor progression is focused on different aspects: neoplastic transformation, tumor cell proliferation and survival, angiogenesis, metastasis and evasion from the immune response (Fig.4).

Inhibition of Gal-1 expression impairs transformation in glioma cells (Yamaoka et al., 2000). Among all Gal-1 partners, H-Ras could be the one closer linked to tumor transformation (Paz et al., 2001) although this interaction is lectin independent. Gal-1 is also very important in fibroblast activation in different tumor settings (Fitzner et al., 2005; Masamune et al., 2006), and indeed, Gal-1 knockdown in cancer associated fibroblasts inhibits *in vivo* tumor progression (Wu et al., 2011).

Gal-1 effects in cell proliferation are controversial. It is mitogenic in several cell types, such as in mammalian vascular cells and hepatic stellate cells, but it is also able to hamper cell growth in other cell types, such as in stromal bone marrow cells. Intracellular Gal-1 can induce not only cell cycle arrest but also apoptosis in cancer cells. Gal-1 concentration seems to be key when deciding the final outcome: high doses (μM) of Gal-1 inhibit cell proliferation independently of its lectin activity whereas low doses (nM) are mitogenic through its ability to recognize carbohydrates (Adams et al., 1996). Apart from this dose response effect, the cell type and cell activation status, the distribution of monomeric versus dimeric forms and Gal-1 compartimentalization, might be also affecting the overall result on cell cycle progression.

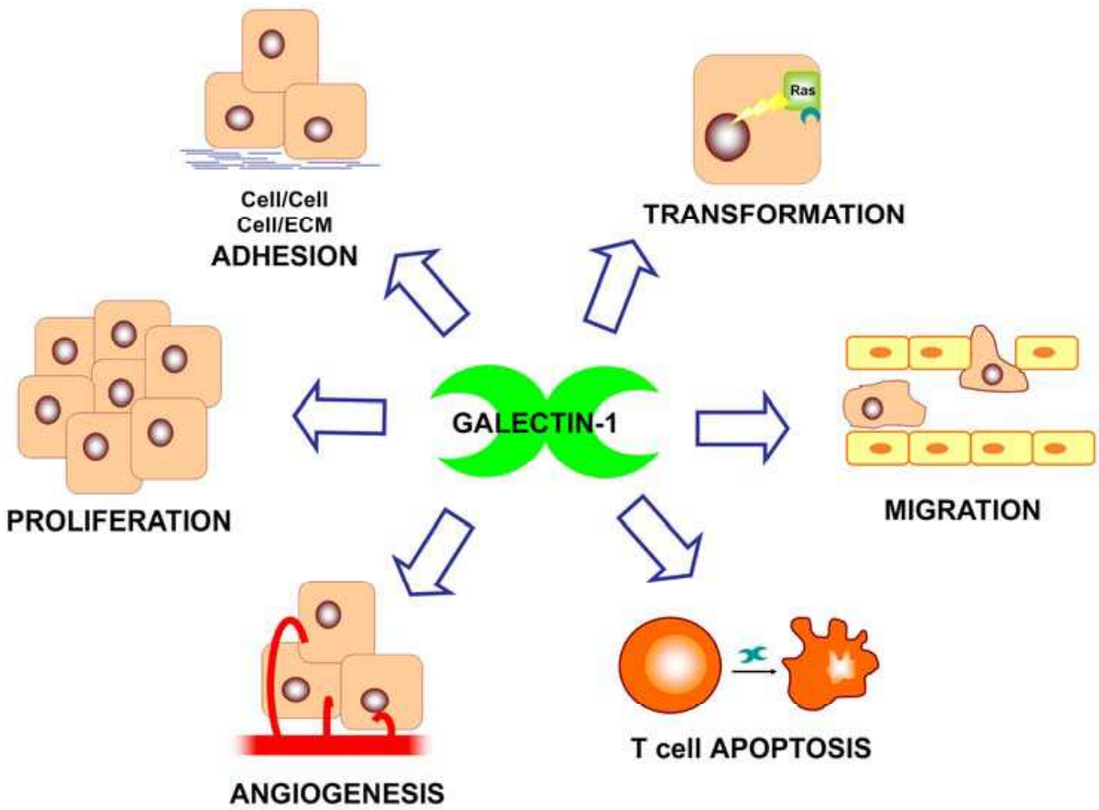

Fig. 4. Gal-1 is involved in many different tumor progression events. Gal-1 participates in cell transformation, proliferation, adhesion, migration, angiogenesis and T cell apoptosis.

Gal-1 has been actively involved in the long range dissemination of tumoral cells or metastasis (Camby et al., 2006), as it participates in adhesion, migration, motility and invasion. Gal-1 can decrease tumor cell adhesion to the ECM, resulting in cell detachment from primary sites and invasion. Alternatively, the dimeric nature of Gal-1 allows crosslinking integrins on the cell surface of tumoral cells to proteins on the ECM, mediates tumoral cell/cell interactions favoring aggregation and their interaction with endothelial cells, facilitating tumor cell dispersion on the blood stream and establishment at distal sites during metastasis. In addition, Gal-1 has been also involved in invasion through adhesion independent mechanisms by upregulating well known ECM degradators like MMP-2, MMP-9, or by reorganizing the actin cytoskeleton through Cdc42 or RhoA upregulation.

Gal-1 also plays a key role in angiogenesis as it is able to stimulate the growth of vascular endothelial cells. The lectin is overexpressed in activated tumor endothelium and it is involved in endothelial cell function (by NRP-1 interaction and VEGFR-2 activation). Gal-1 deficiency impairs tumor growth and angiogenesis *in vivo* (Le Mercier et al., 2009; Thijssen et al., 2006). Moreover, Gal-1 modulates the expression of BEX2 and several hypoxia related genes involved in angiogenesis. Paracrine mechanisms involving the uptake by endothelial cells of Gal-1 secreted from tumoral cells have been linked to endothelial cell activation and tumor angiogenesis stimulation, through Ras and Erk1/2 activation (Thijssen et al., 2010).

Finally, Gal-1 is involved in the tumor immune response promoting an immunosuppressive environment at tumor sites by inhibiting full T cell activation, triggering T cell growth arrest and apoptosis and protecting the tumor by negatively regulating Th1 and proinflammatory cytokines. These effects are mediated by Gal-1 recognition of cell surface glycoproteins present on T cell membranes such as CD2, CD3, CD7, CD43 and CD45 (Galvan et al., 2000; Pace et al., 1999).

3.4 Galectins in pancreatic cancer

In pancreatic cancer, Gal-1 and Gal-3 are found to be overexpressed (Berberat et al., 2001; Chung et al., 2008; Grutzmann et al., 2004; Schaffert et al., 1998).

Gal-3 expression is faint in ductal cells of normal pancreas but it is high in intrapapillary mucin neoplasms, chronic pancretatitis, cancerous pancreatic tissue and metastatic cells, suggesting its role in cancer cell proliferation and metastasis formation. However, decreased Gal-3 expression has been linked to advanced stage, tumor de-differentiation and metastasis in ductal adenocarcinomas, implying a fine tuned regulation of its levels in different steps of tumor progression. Gal-3 secreted by pancreatic cells plays a role in pancreatic stellate cell proliferation and in pancreatic cancer cell proliferation and invasion *in vitro*. A negative correlation between anoikis and Gal-3 presence has been established, too. Besides, the interaction between Gal-3 and Muc4 has been proven to be functional to dock tumor cells to the endothelial surface, what might present a possible mechanism to explain Gal-3 involvement in metastasis.

Gal-1 has found to be overexpressed in pancreatic tumors compared to normal tissue (Berberat et al., 2001; Grutzmann et al., 2004; Iacobuzio-Donahue et al., 2003; Shen et al., 2004) (Fig.5). Interestingly, its expression levels correlate not only with histology but also with T stage, N stage and global AJCC stage of pancreatic cancer disease (Chung et al., 2008), suggesting that Gal-1 might also participate in tumor progression and that its presence does not seem to be a random event. Gal-1 expression by immunohistochemical analysis has been reported to be mainly restricted to the ECM and fibroblasts in and around the cancer mass, but not to pancreatic cancer cells, suggesting its importance in the so characteristic desmoplastic reaction. Gal-1 is also found in the stroma of PanIN-2 and PanIN-3 (Pan et al., 2009) and in chronic pancreatitis (Wang et al., 2000).

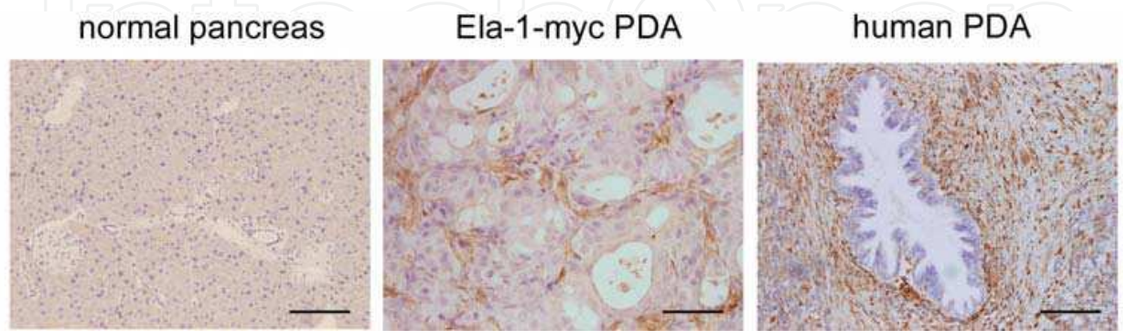

Fig. 5. Gal-1 is overexpressed in precursor lesions and pancreatic cancer. Gal-1 immunohistochemistry in mouse pancreatic normal tissue, in Ela-1-myc pancreatic ductal adenocarcinoma (PDA) lesions and in human pancreatic cancer tissue. Scale bars correspond to 200 μm.

Interestingly, although Gal-1 did not appear in the list of genes consistently misregulated in pancreatic cancer that were gathered in 12 core signaling pathways (Jones et al., 2008), 54 of the genes found overexpressed encoded secreted or cell surface proteins, putative and already known Gal-1 binding targets, like laminin. Thus, Gal-1 overexpression might be involved in the functional outcome of these overrepresented molecules, playing a role in some of the key identified signaling pathways such as homophilic cell adhesion, integrin signaling and regulation of invasion. Gal-1 could have been excluded from the reported list because this important global genomic analysis was based on tumoral epithelial cells, leaving out the stroma, whose population seems to be the one predominantly affected by Gal-1 increased levels.

Gal-1 could be involved in tumor progression in pancreatic cancer by remodeling the ECM in the formation of the desmoplastic reaction. Indeed, Gal-1 is able to induce activation (increased collagen synthesis), proliferation and chemokine production (MCP-1 and CINC-1) of pancreatic stellate cells, through Erk1/2, Jnk, NF-κB and AP-1 activation. At the same time, activated pancreatic stellate cells secrete Gal-1, which can be acting autocrinely and might be also regulating the tumor immune response (Fitzner et al., 2005; Masamune et al., 2006).

As it has been described above, Gal-1 displays a wide variety of biological functions which bring up a high degree of complexity when trying to understand its involvement in cancer. Thus, Gal-1 might not always tilt the balance in the same direction. In pancreatic cancer cells, for example, stable transfection of the tumor suppressor p16/Ink4a can induce Gal-1 expression and its affinity for the fibronectin receptor, resulting in increased susceptibility towards anoikis (Andre et al., 2007). Another Gal-1 antitumoral role is presented by the fact that it is downregulated in gemcitabine resistant pancreatic cancer cells (Kuramitsu et al., 2010). The ability of Gal-1 to induce opposite effects regarding proliferation and adhesion, as well as its reduced expression found in some tumors (Choufani et al., 1999), hint at Gal-1 as a double side coin and question its nature as a protumoral molecule. Many variables might be influencing the final outcome, such as cell type and activation status, Gal-1 levels and localization, as well as its quaternary structure.

3.5 Gal-1 establishing protein/glycan interactions

Gal-1 interactions involving its CRD domain and lectin activity are involved in many of Gal-1 important functions (Table 1). N-glycans from cell surface glycoproteins are the major ligands for Gal-1 and Gal-3, although they also bind to mucins, proteoglycans and the ECM. Although both proteins have high affinity for β-galactosides and indeed they share many interacting partners such as CD45, laminin, fibronectin and integrins, a fine specificity level results in binding differences. The general rule is that Gal-3 prefers repeating lactosamine units whereas Gal-1 recognizes independent lactosamine disaccharides with low affinity (Kd=50 μM) but deeply increases avidity when presented in multiantennary repeating units (Kd=5 μM) and when the lectin is surface bound to cell membranes or to the ECM. Indeed, Gal-1 is involved in microdomain (lattice) formation within membranes by crosslinking ligands in a glycoside cluster effect that greatly increases its affinity. However, as a matter of fact, Gal-1 is able to recognize only about 1/40 of the total N-glycans present in human serum glycoproteins (Kita et al., 2007), and around 1/8 of the sites supposed to be galectin specific. It is believed that part of Gal-1 specificity is mediated by additional binding sites

Gal-1 partners	Biological context	Functional Outcome
CA-125	cervical cancer cells	Gal-1 export to cell surface
CD2/CD3	T cells	T cell activation and apoptosis
CD4	T cells	Unclear
CD43, CD45	T cells	Gal-1 induced T cell death (depending on specific receptor glycosylation). Redistribution of the receptors in the cell surface.
CD7	T cells	Induction of apoptosis
CEA	colon carcinoma cells	Unclear
Chondroitin sulphate	SMC	Incorporation of ECM components important for SMC
Fibronectin	placenta ovary carcinoma cells	Control of cell adhesion
1B2 glycolipid	olfactory neurons	Adhesion between adjacent axons and with the ECM resulting in olfactory axon fasciculation
Glycoprotein 90K	melanoma cells	Formation of multicell aggregates
GM1 ganglioside	neuroblastoma cells	Sialidase dependent cell growth inhibition
HBGp82	brain	Unknown
INTEGRINS		
$\alpha_1\beta_1$, $\alpha_7\beta_1$	SMC skeletal myocytes	Intracellular signaling leading to adhesion, FAK activation, migration
$\alpha_5\beta_1$	colon, breast, ovarian, hepatocellular carcinoma cells,	Antiproliferative effects, induction of anoikis
$\alpha_M\beta_2$	macrophages	Possibly crosslinking receptors or affecting receptor-ligand binding affinity
Laminin	placenta, smooth muscle cells, leydig cells	Assembly of ECM, adhesion, migration, apoptosis
LAMP-1, LAMP-2	ovarian, colon carcinoma cells	Tumor cell adhesion and metastasis
Mucin	gastrointestinal tract	Protection from the epithelial surface
NRP-1	endothelial cells	Signaling pathway activation, migration and adhesion
Pre-B cell receptor	B cells	Cell differentiation, adhesion
Osteopontin, vitronectin	SMC	Adhesion, ECM assembly
Thrombospondin	SMC	Adhesion

Table 1. Proteins that are known to interact with Gal-1 through their CRD. Description of the best characterized Gal-1 interactors, specifying the biological context in which the interaction has been identified, and the consequent functional outcome. SMC: smooth muscle cells. Detailed references can be found at (Camby et al., 2006).

recognizing more than the canonical galactose. Thus, the particular structural context of galectin binding sites depicts a complex scenario and impairs stating generalizations. For instance, Gal-1 is able to induce T cell death by binding a glycan ligand without lactosamine units, that is very abundant but less preferred (Hernandez et al., 2006). Normally though, Gal-1 recognition capacity is deeply influenced by specific conditions regarding carbohydrate content and linkage. Minor alterations in N-glycan chains have been reported to influence Gal-1 binding in such a way that changes the overall biological outcome (Andre et al., 2007). Cell type specific expression patterns of several proteins and their glycans can modulate different Gal-1 mediated effects (Gu et al., 1994; Moiseeva et al., 1999). Particular glycosylation structures are known to mask glycans to Gal-1, which impede Gal-1 induced T-lymphocyte (Liu & Rabinovich, 2010) and cancer cell (Valenzuela et al., 2007) death. For instance, in contrast to Th1 and Th17 cells, Th2 cells are protected from Gal-1 induced apoptosis by presenting α2-6 sialylation of cell surface glycoproteins (Toscano et al., 2007).

In the ECM, Gal-1 displays high affinity for laminin, fibronectin, thrombospondin, vitronectin, osteopontin and glycosamine glycans such as chondroitin sulfate (Table 1). Depending on the cell type and cell activation status, these interactions finally lead to a pro-adhesive or an anti-adhesive effect.

In the cell membrane, Gal-1 has many interactors resulting in very different effects (Table 1). Glycosylated cell surface receptors are closely linked to the adhesive properties mediated by Gal-1. For instance, Gal-1 interaction with $\alpha_7\beta_1$ integrin interferes with integrin/laminin binding and controls cell adhesion. Gal-1 interaction with NRP-1 has been involved in migration and adhesion of endothelial cells (Hsieh et al., 2008). Gal-1 can also function as a regulator of the immune response through its interaction with CD7, CD45 and CD43. Moreover, Gal-1 has also been involved in cell growth inhibition through its interaction with $\alpha_5\beta_1$ integrin, GM1 ganglioside or the glycoprotein 90K/MAC-2BP. Gal-1 can also recognize HBGp82 in the brain, CA125 in ovarian cancer cells, LAMP-1, LAMP-2 and CEA in colon carcinoma cells and 1B2 glycolipid in olfactory axons.

4. tPA: Connecting galectins and cancer protein glycosylation?

4.1 tPA: General features

Our group has recently characterized how an interaction between Gal-1 and a glycosylated protein –tPA- is involved in pancreatic cancer progression (Roda et al., 2009). tPA is mainly synthesized by endothelial cells, but it has also been detected in the central nervous system, being secreted by neurons and glial cells and it can also be produced by keratinocytes, melanocytes and various tumor cells. tPA best documented role is the conversion of plasminogen into plasmin, which degrades fibrin clots in blood vessels after thrombosis through a well-orchestrated process involving several regulators. Besides, tPA is also involved –by its catalytic activity- in the activation of growth factors and matrix metalloproteinases in the ECM (Fig.6). In addition to these proteolytic activities, we and others have demonstrated that tPA can exert catalytic-independent functions in different cell types, including neurons (Medina M.G. et al., 2005), kidney fibroblasts (Hu et al., 2006) and tumors (Ortiz-Zapater et al., 2007).

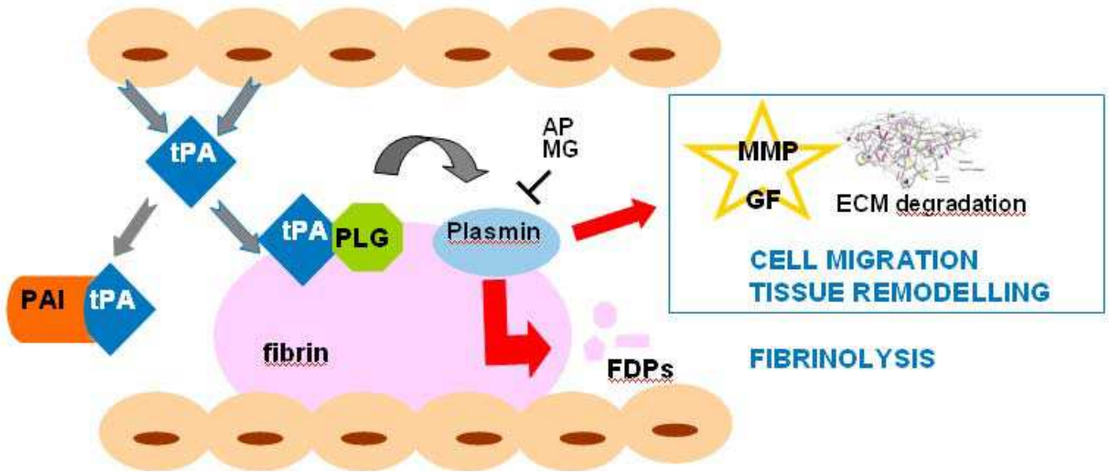

Fig. 6. tPA physiological functions. The best documented role for tPA is the activation of the zymogen plasminogen into plasmin, which degrades fibrin clots in blood vessels after thrombosis. Moreover, tPA is also involved in activation of matrix metalloproteinases and growth factors and in the ECM degradation, participating in cell migration and tissue remodeling events.

tPA is a glycoprotein of 527 aminoacids and around 70 KDa, depending on specific glycosylation. tPA is synthesized as a single-chain protein but it is quickly hydrolyzed by plasmin, forming a two-chain structure maintained together by a disulfide bond. Structurally, apart from a typical signal peptide and a prosequence, tPA is formed by 5 different autonomous domains, which are encoded by separate exons or sets of exons: 1) The fibronectin type I domain in the amino terminus, which mediates fibrin affinity; 2) An EGF-like domain which is probably involved in cell surface receptor binding; 3) Two kringle regions with a triple looped structure, with a high degree of homology with plasminogen kringle domains and 4) A serine protease domain with the active site residues His322, Asp371 and Ser478.

Due to the size of tPA and the presence of glycosylated chains in the molecule, the complete structure of the protease remains still undetermined. However, the detailed structure has been revealed for some of the domains by NMR or X-Ray diffraction as for the fibronectin type I domain (Downing et al., 1992), EGF domain (Smith et al., 1995), kringle 2 (Byeon et al., 1991; Byeon & Llinas, 1991; de Vos et al., 1992) and the catalytic domain (Lamba et al., 1996; Renatus et al., 1997a; Renatus et al., 1997b).

Glycosylation differences describe two different tPA isoforms (type I and type II), displaying species, cell and site-specific patterns of these post-translational modifications. In type I tPA, 4 glycosylation sites are occupied in separate domains, which play a role in different biological and pathological tPA functions: an O-linked fucose in Thr61 (EGF domain) and three N-linked carbohydrate chains; an oligomannosidic structure highly conserved between species at Asn117 (in kringle 1), and two complex and hybrid type structures at Asn184 (in kringle 2) and Asn448 (in the catalytic domain). Type II tPA lacks the glycosylation at Asn184 and this absence allows the conversion of single-chain to two-chain tPA, through plasmin mediated cleavage of the polypeptide backbone between Arg275 and Ile276. The presence of glycan chains at site Asn184 affects the structure of the

glycan population at Asn448, being two-chain tPA a more active tPA regarding clot lytic activity and fibrin-binding capacity.

4.2 Role of tPA and tPA receptors in pancreatic cancer

tPA overexpression correlates with poor prognosis in several cancers. In pancreatic cancer studies, tPA is found to be highly expressed in well differentiated human pancreatic cancer cultures and overexpressed in 95% of pancreatic ductal adenocarcinomas (PDAs), being absent in normal pancreas (Paciucci et al., 1996, 1998; Ryu et al., 2002) (Fig.7).

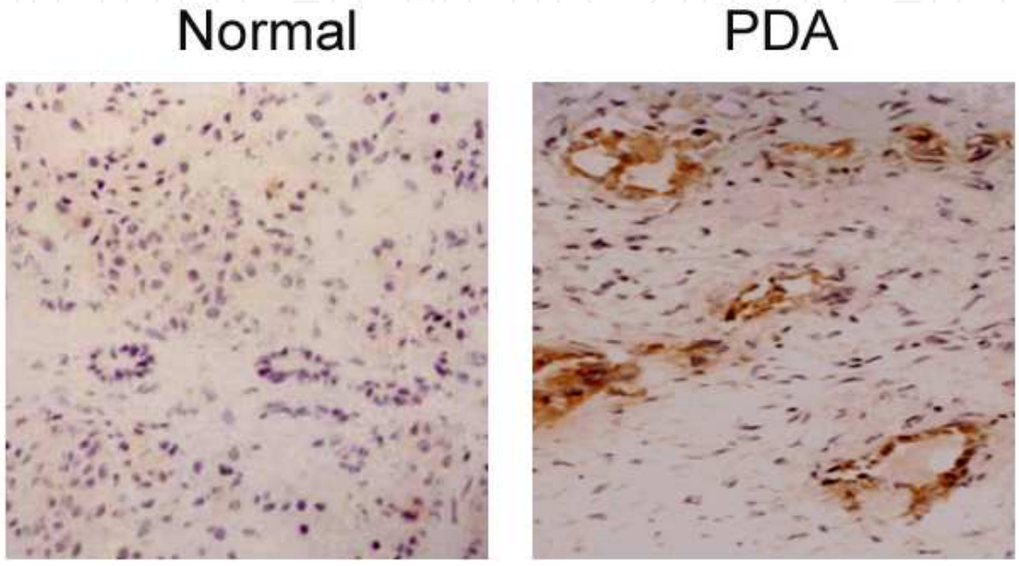

Fig. 7. tPA is overexpressed in human pancreatic cancer. tPA expression assessed by immunohistochemistry in normal pancreas (normal), showing no tPA expression, whereas, in human pancreatic ductal adenocarcinoma (PDA), high expression levels of tPA are detected in ducts.

In vitro and *in vivo* studies have determined that tPA contributes to pancreatic cancer progression by increasing cell invasion, Erk1/2 phosphorylation, cell proliferation and angiogenesis (Aguilar S et al., 2004; Diaz et al., 2004; Paciucci et al., 1998). These effects are mediated through tPA interaction with different cell membrane receptors. In this regard, EGFR is overexpressed in pancreatic cancer and it has been demonstrated to participate in tPA effects in cell proliferation (Hurtado et al., 2007; Ortiz-Zapater et al., 2007). AnxA2 -the best characterized tPA receptor and its major receptor in endothelial cells- has also been clearly involved in tPA-mediated pancreatic cancer cell invasion, proliferation and angiogenesis (Diaz et al., 2004; Ortiz-Zapater et al., 2007). Nevertheless, AnxA2 does not seem to be the only functional tPA pancreatic cancer receptor as its interaction with the protease only explains part of the tPA found in the cell membrane (Diaz et al., 2004; Ortiz-Zapater et al., 2007). These data and the fact that AnxA2 seems to be inappropriate as a target for pancreatic therapy due to its important physiological functions in blood coagulation homeostasis moved us to find new tPA receptors that could be involved in tPA protumoral functions in pancreatic cancer. As described in the next section, we have recently demonstrated that Gal-1 is a new functional tPA receptor (Roda et al., 2006, 2009).

4.3 tPA/Gal-1 interaction: Glycosylation involvement and role in pancreatic cancer

Interaction between tPA and Gal-1 was first identified in total tumoral pancreatic cell lysates by affinity capture with tPA-sepharose followed by 2D- electrophoresis (Roda et al., 2006). However these data did not prove whether tPA/Gal-1 interaction was direct or mediated through other proteins. In a more recent work, using recombinant proteins and surface plasmon resonance, we proved that tPA/Gal-1 interaction was direct and specific (Roda et al., 2009). Furthermore, Gal-1 was able to increase tPA mediated plasmin generation, suggesting interesting functional outcomes from their interaction.

Taken into account that 1) galectins are lectins with high affinity for β-galactosides, 2) Gal-1 binds galactose, and lactose with even higher affinity, through its CRD, and 3) tPA is a glycoprotein, we hypothesized that tPA and Gal-1 interaction was N-glycan mediated. In order to know whether that was the case, surface plasmon resonance was used to determine if carbohydrates were able to interfere with this interaction. Galactose (in a dose dependent manner) and lactose (with even higher effectiveness), inhibited tPA/Gal-1 interaction (Roda et al., 2009). Proving galactose specificity, neither glucose nor cellobiose was able to do so. These data demonstrated that the Gal-1 CRD was involved in tPA interaction and as expected, pointed at galactose in a β-anomeric position as its high affinity epitope.

Importantly, our results showed that this Gal-1/tPA interaction was not only relevant *in vitro,* but also *in vivo* where the lectin was actively involved in tPA induced Erk1/2 activation, proliferation, migration and invasion. tPA/Gal-1 effects were not restricted to pancreatic cells but were also found in tPA-mediated protumoral effects in fibroblasts from the tumor stroma, demonstrating the important role for tPA/Gal-1 interaction in the epithelial/fibroblast crosstalk and in pancreatic cancer tumor progression (Roda et al., 2009).

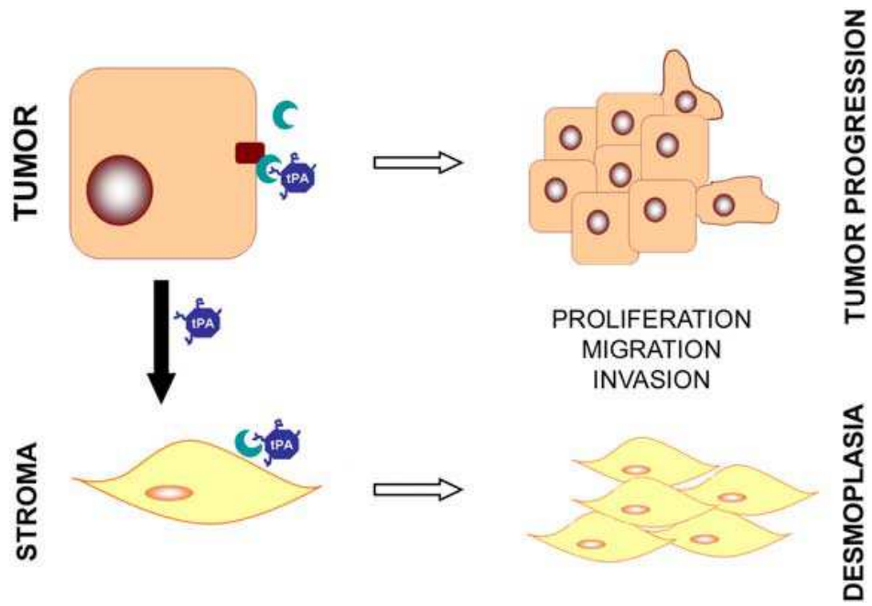

Fig. 8. Gal-1 is acting as a functional tPA receptor in pancreatic cell lines and fibroblasts. Gal-1 in pancreatic cancer cells can activate Erk1/2, induce proliferation, migration and invasion by binding to tPA in an autocrine fashion. Gal-1 can also act in a paracrine fashion over fibroblasts, triggering the same pathological effects that could be involved in the desmoplastic reaction.

Thus, we propose a model in which tPA secreted from pancreatic epithelial cells could act both in a paracrine and in an autocrine manner. In the latter, it would bind to Gal-1 in the cell surface of pancreatic tumoral cells triggering Erk1/2 activation and subsequent proliferation, as well as invasion. These events would be favoring tumor progression. On the other hand, tPA could bind in a paracrine fashion to Gal-1 from fibroblasts, what would induce the same events but in this mesenchymal cell line, leading to the desmoplastic reaction (Fig.8).

5. Glycans and Gal-1 in pancreatic cancer diagnosis and therapy

5.1 Glycans in diagnosis and therapy

Serum glycoproteins constitute the most frequent family of current tumor markers (Ludwig & Weinstein, 2005). Among others, two main characteristics highlight glycans in diagnosis: their altered structure upon tumorigenesis and the fact that they are frequently found in secreted proteins, what might facilitate their accessibility in the clinics. The most frequently used glycoproteins in this context are highly glycosylated mucins like CA19-9, CA125, CA27-29, CA15-3 but other proteins like PSA, AFP, CEA, RNase1 and hCG-β have also been considered (Peracaula et al., 2008).

Glycans have been closely involved in several events driving tumor progression, so therapeutic strategies targeting them have been studied with special attention (Dube & Bertozzi, 2005; Fuster & Esko, 2005). For example, the carbohydrate moiety of growth factor receptors is key in the regulation of cell signaling towards proliferation. Besides, several molecules like mucins, proteoglycans and gangliosides, modulate growth factor receptor activity through their glycan structures. Thus, different approaches directed to these glycans have already been designed and are being tested in the clinics such as peptide-based vaccines and monoclonal antibodies against mucins or gangliosides. The possibility of altering glycan synthesis and their maduration has also been proposed. One of the most studied effects of glycans over tumor development is their role in invasion due to their structurally altered presence in proteins well known for their effects upon adhesion and migration like E-cadherin, integrins, syndecans, proteoglycans and hyaluronan. Therefore, several strategies with the aim to block tumor specific patterns of glycosylation have been planned such as the inhibition of GnTV (responsible of increased β1,6-branched N-glycans) or polysialyltransferases. The reduction of tumor angiogenesis has also been addressed through glycan-based therapy by the use of modified heparin fragments or compounds inhibiting heparanase. Anti-selectin antibodies or mimetics of selectin ligands have been proposed to be useful against metastasis.

5.2 Gal-1 in diagnosis and therapy

Galectins are overexpressed in many different tumors and their expresssion has been related to poor prognosis suggesting their possible use as markers for diagnosis (Lahm et al., 2001; Rabinovich, 2005; Salatino et al., 2008). Indeed, Gal-1 detection in serum has been proven to be useful to monitor tumor progression and clinical severity in patients with head and neck squamous cell carcinoma (Saussez et al., 2008) and ovarian carcinoma (Allen et al., 1993).

In pancreatic cancer therapy, Gal-1 fulfills interesting requirements to be considered for targeting such as not being expressed in normal pancreas, increasing drug selectivity. Moreover, the use of Gal-1 inhibitors is particularly appealing because Gal-1 knockout mice are viable and fertile and do not show overt abnormalities (Poirier & Robertson, 1993), probably due to redundant functions from other members of the galectin family. Nevertheless, the dichotomous effects of Gal-1 must be well considered for efficient targeting, as depending on many intrinsic and extrinsic factors, the lectin can exert contrary effects (mitogenic or antiproliferative and pro or anti-adhesive). That is so the case that even Gal-1 and Gal-1 mimetic compounds have been also proposed for anticancer therapy (Fischer et al., 2005). Thus, special attention must be paid concerning Gal-1 conformation, quaternary structure, oxidation state, concentration, subcellular localization, ability to establish protein/protein or protein/glycan interactions, target cell type and presence of specific glycan receptors with certain glycosylation signatures, among others. Another interesting aspect to take into account for the use of Gal-1 in cancer therapy is its role as a master regulator of the immune response. Indeed, downregulating Gal-1 expression inhibits migration and restores susceptibility to apoptosis and so to cytotoxic drugs, making its inhibition a promising target in cancer therapy (Salatino et al., 2008; Rabinovich, 2005).

Finally, it has been reported that the huge stromal reaction accompanied with an important lack of angiogenesis impairs drug delivery and cause pancreatic cancer resistance. The stroma has been shown to be decisive in tumor progression, which can be inhibited maintaining a normal context. Different stromal cells have been under the scope for therapy as they are more accessible to pharmacological agents and genetically stable, which makes them less prone to acquire resistance. Indeed, therapies targeting other molecules involved in the desmoplastic reaction and vasculature have proven to improve efficiency delivery of gemcitabine in a pancreatic cancer mice model (Olive et al., 2009). Interestingly, silencing Gal-1 results in increased chemotherapy toxicity in glioblastoma cell lines (Le Mercier et al., 2008; Puchades et al., 2007). Gal-1 importance in tumor microenvironment immunosuppression is also considered in treatment. As a matter of fact, Gal-1 inhibition as adjuvant with vaccine immunotherapy significantly reduces breast tumor progression in mice (Stannard et al., 2010).

6. Conclusion

Overall, this context provides us with a whole universe of possibilities that might help in the design of new diagnosis markers and therapies directed to hamper tumor development. Still, the huge versatility of most of the molecules containing a glycan fraction forces research to deeply evaluate the molecular mechanisms affected upon targeting in order to avoid undesirable secondary effects that might prevent their use in treatment. Regarding Gal-1, the same precautions must be taken, considering the vast amount of partners and biological outcomes to which it is link. This complexity impairs analyzing the role of molecules independently and requires that each and every interaction is studied in detail. In this sense, a much finer approach in cancer therapy would result from targeting specific protein/protein interactions instead of individual proteins.

Our work has made an important contribution by specifically deciphering the relevance of Gal-1 interaction with a glycosylated protein – tPA- in the context of pancreatic tumor progression. Our data add valuable knowledge to enable a better understanding of

pancreatic cancer molecular biology. The relevant functional outcomes from Gal-1/tPA interplay open the door to new therapeutic strategies targeting the complex without interfering with tPA and Gal-1 independent physiological functions. Therefore, we stand for tPA/Gal-1 interaction as a promising target for pancreatic cancer, which could delay or even revert tumoral progression in this devastating disease.

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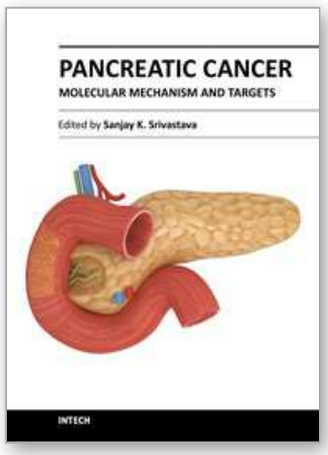

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This book provides the reader with an overall understanding of the biology of pancreatic cancer, hereditary, complex signaling pathways and alternative therapies. The book explains nutrigenomics and epigenetics mechanisms such as DNA methylation, which may explain the etiology or progression of pancreatic cancer. Book also summarizes the molecular control of oncogenic pathways such as K-Ras and KLF4. Since pancreatic cancer metastasizes to vital organs resulting in poor prognosis, special emphasis is given to the mechanism of tumor cell invasion and metastasis. Role of nitric oxide and Syk kinase in tumor metastasis is discussed in detail. Prevention strategies for pancreatic cancer are also described. The molecular mechanisms of the anti-cancer effects of curcumin, benzyl isothiocyante and vitamin D are discussed in detail. Furthermore, this book covers the basic mechanisms of resistance of pancreatic cancer to chemotherapy drugs such as gemcitabine and 5-flourouracil.

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