Galectin-1 is an inductor of pancreatic stellate cell activation

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Abstract

Pancreatic stellate cells (PSCs) play a key role in the development of pancreatic fibrosis, a pathological feature of chronic pancreatitis and pancreatic cancer. Here, we show that activation of rat PSCs in vitro is associated with increased expression of galectin-1 (gal-1) and that gal-1 modulates PSC function. Expression of the lectin was stimulated by fetal calf serum and platelet-derived growth factor. PSCs exposed to exogenous gal-1 proliferated at a higher rate and synthesised more collagen than controls. Gal-1-dependent collagen synthesis was blocked by lactose but not by cellobiose, suggesting that gal-1 acts on PSCs through targeting β-galactoside-containing glycoconjugates. Analysis of gal-1 signalling in PSCs revealed an activation of the extracellular signal-regulated kinases 1 and 2 and enhanced DNA binding of AP-1 transcription factors. Together, our data implicate gal-1 in PSC activation and suggest further studies to analyse the role of endogenous lectins in the development of pancreatic fibrosis in vivo.

Keywords: Galectin-1; Pancreatic stellate cells; Proliferation; Collagen synthesis; ERK signalling; AP-1

1. Introduction

Activation of pancreatic stellate cells (PSCs) plays a pivotal role in pancreatic fibrosis [1–3], a pathological feature of chronic pancreatitis (CP) and pancreatic cancer [4,5]. Frequently, progression of fibrosis is associated with the development of an exocrine and endocrine insufficiency of the gland. In recent years, the molecular basics of PSC activation have been studied, and two groups of activators have been identified: (I) ethanol and its metabolites (most of all acetaldehyde) [6,7] and (II) cytokines such as the strong PSC-mitogen platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β), the main stimulator of extracellular matrix (ECM) protein synthesis [2,8,9]. Potential sources of cytokines stimulating PSC activation in the inflamed pancreas are, for example, activated macrophages (secretion of TGF-β1) [10], platelets (containing PDGF and TGF-β1) [8] and acinar cells (expressing tumor necrosis factor-α (TNF-α) [11], interleukin-1 and interleukin-6 [12], which have also been implicated in PSC activation [13]). In response to pro-fibrogenic mediators, quiescent PSCs undergo a myofibroblast-like transformation, a process associated with proliferation and increased ECM production [1,14]. In the fibrotic pancreas, activated PSCs are the main source of ECM proteins [3]. In vitro, isolated PSCs undergo an activation process that mimics the situation in vivo. After some days in culture, the cells proliferate and display typical features of activated myofibroblasts (e.g., expression of α-smooth muscle actin [α-SMA], increased secretion of ECM proteins) [1,14].

Galectins are a family of β-galactoside-binding animal lectins [15,16] with a wide range of biological functions, including regulation of adhesion, cellular growth and apoptosis [17,18] as well as modulation of the immune response [19,20]. Through their carbohydrate recognition domains, galectins specifically interact with glycoconjugates of counter-receptors on the cell surface [18,21]. One
of the best-characterised galectins in mammals, galectin-1 (gal-1), forms a homodimer of 14-kDa non-covalently linked subunits [22]. Glycoproteins that have been suggested to be involved in mediation of gal-1 effects include CD2, CD3, CD4, CD7, CD43 and CD45 on T-cells [19,23–25], as well as the ECM proteins laminin [26] and fibronectin [27]. It is likely that the molecular targets of gal-1 vary in a cell-type-characteristic manner. Two recent studies have implicated gal-1 in the activation of hepatic stellate cells (HSCs), the principle effector cells in liver fibrosis. Analysis of the rat HSC proteome revealed increased gal-1 expression and secretion in culture-activated HSCs [28]. Gal-1 was also up-regulated when HSCs were activated in vivo by carbon tetrachloride. A similar expression profile was found for gal-3 [29]. Gal-1 and gal-3 also modulate HSC function [29]. Both lectins stimulated HSC proliferation in a dose-dependent manner, with the stimulation by the former higher than that by the latter. Furthermore, gal-1 enhanced the migratory activity of the cells. We have previously shown that the mitogen-activated protein (MAP) kinases ERK 1 and 2 (extracellular signal-regulated kinases 1 and 2) mediate mitogenic signals in rat PSCs [30]. Interestingly, gal-1 and gal-3 were found to exert growth-promoting effects on HSCs, at least in part, through activating the ERK 1/2 signal transduction pathway [29].

The functions of galectins in physiologic and pathologic processes of the pancreas are poorly characterised. Increased gal-1 and gal-3 levels have been detected in tissue samples of CP [31]. While gal-1 was found to be up-regulated in fibroblasts, intense gal-3 signals were observed in cells of ductular complexes. Based on these data, a role of gal-1 and gal-3 in ECM changes in CP has been suggested. Here, we have characterised the expression profile of gal-1 in the course of PSC activation in vitro and analysed how gal-1 expression is regulated. Furthermore, we have studied the biological effects of gal-1 in PSCs and the molecular principles of its action.

2. Materials and methods

2.1. Reagents

Nitrocellulose, the enhanced chemoluminescence (ECL) Plus kit, peroxidase-labelled antibodies and radiochemicals were purchased from Amersham Biosciences (Freiburg, Germany), the phospho-ERK 1/2 (Thr 202/Tyr 204 of human ERK 1) and the ERK 1/2 protein antibody from New England BioLabs (Frankfurt, Germany), and antibodies used for supershift analysis from Santa Cruz Biotechnologies (Santa Cruz, CA, USA). Genistein, PD98059 as well as calphostin C were delivered by Merck Biosciences (Schwalbach, Germany) and Hybridoma Express medium by PAA Laboratories (Cöln, Germany). Hank’s buffered salt solution (HBSS), Iscove’s modified Dulbecco’s medium (IMDM) and all supplements for cell culture were obtained from Life Technologies (Eggenstein, Germany), Nycomed from Nycomed (Oslo, Norway), collagenase P, deoxyribonuclease, polynucleotide kinase as well as poly(dI-dC) from Roche Diagnostics (Mannheim, Germany), the recombinant cytokines PDGF-BB (rat), TNF-α (human; rat-reactive) as well as TGF-β1 (human; rat-reactive) from R & D Systems (Minneapolis, MN, USA), and rat tail collagen from Tebu (Frankfurt, Germany). Ascorbat, β-aminopropionitrile and the α-SMA antibody as well as standard laboratory chemicals were from Sigma-Aldrich (St. Louis, MO, USA), Oligonucleotides (probes and PCR primer) were purchased from BioTeZ (Berlin, Germany).

Gal-1 was obtained from human placenta exactly as previously described [32]. Gal-1 polyclonal antibodies (pAbs) were produced in rabbits immunised with gal-1. From serum the IgG-fraction was separated by affinity chromatography on protein A agarose. For separation of gal-1 specific pAbs, the IgG fraction was passed through gal-1 agarose prepared by coupling the lectin to NHS-activated Sepharose™ 4 Fast Flow according to the manufacturer’s instructions (Amersham Pharmacia). The pAb cross-reacts with gal-1 from pig and cow as detected by antibody binding to the proteins on blots as well as after spotting the lectins to nitrocellulose membranes (not shown).

2.2. Cell culture

Stellate cells were isolated from the pancreas of male LEW.1W inbred rats as previously described [30]. Briefly, the organ was digested with a mixture of collagenase P (0.05%), protease IX (0.02%) and deoxyribonuclease (0.1%) in HBSS. PSCs were separated from acinar and other cells by Nycodenz (12%) density gradient centrifugation. After collection from the top of the gradient, PSCs were washed, resuspended in IMDM supplemented with 10% fetal calf serum (FCS), 1% non-essential amino acids (dilution of a 100 X stock solution), 100 U/ml penicillin and 100 μg/ml streptomycin. Isolated PSCs were cultured at 37 °C in a 5% CO₂ humidified atmosphere. All experiments were performed with cells growing in primary culture, or, depending on the experimental settings, with cells of the first passage. If replating of the cells was required, PSCs were harvested by trypsinisation on day 7 after isolation and recultured at equal seeding densities. To avoid gal-1 binding to serum proteins, serum-free culture media (IMDM or Hybridoma Express medium) were used in all lectin-stimulation experiments as indicated.

2.3. Cell proliferation assay

Cell proliferation was quantitated through the measurement of 5-bromo-2’-deoxyuridine (BrdU) incorporation into newly synthesised DNA, using the BrdU labelling and detection enzyme-linked immunosorbent assay kit (Roche
2.4. Measurement of collagen synthesis

Collagen synthesis was assessed by measuring [3H]-proline incorporation into acetic acid-soluble proteins. Therefore, PSCs were plated in 12-well plates and allowed to adhere. FCS-containing culture medium was then replaced by Hybridoma Express medium. Gal-1, lactose and cellobiose (alone or in combination as indicated) were preincubated with medium for 4 h at 37 °C before they were added to the cells for 72 h. During the last 24 h, the medium also contained 2.5 μCi/ml [3H]-proline (48 Ci/mmol), 50 μg/ml ascorbate and 50 μg/ml β-aminopropionitrile. All further steps were performed essentially as described before [33]. Briefly, proline incorporation was stopped by the addition of 50 μl/ml 10 N acetic acid (overnight incubation at 4 °C). Culture supernatants were mixed with 100 μl/ml FCS, 5 μg/ml rat tail collagen as well as 250 μl/ml 25% NaCl dissolved in 0.5 N acetic acid and incubated at 4 °C for 30 min. Protein precipitates received by centrifugation (30 min at 10,000 g) were washed with 5% NaCl and dissolved in 0.5 N acetic acid. [3H]-proline incorporation was measured by liquid scintillation counting. Raw data were normalised on the basis of absolute cell counts determined by trypan blue staining of PSCs cultured in parallel under identical conditions, except that no [3H]-proline was present during the last 24 h of incubation.

2.5. Immunoblotting

Protein extracts of PSCs growing in 6-well plates (pretreated as indicated) were prepared and adjusted to identical protein concentrations as previously described [30]. After separation by SDS-PAGE, proteins (15 μg per lane) were blotted onto nitrocellulose filters. The membranes were blocked with 1% bovine serum albumin (BSA), exposed to the indicated protein-specific antibodies overnight at 4 °C, and finally incubated for 2 h at room temperature with a horseradish peroxidase-labelled anti-rabbit or anti-mouse Ig antibody. Afterwards, the blots were developed using the ECL Plus kit. For reprobing with additional antibodies, membranes were stripped by incubation in stripping buffer (62.5 mM Tris–HCl, pH 6.7, 2% SDS, 100 mM 2-mercaptoethanol) at 50 °C for 30 min. Chemoluminescence signals on X-ray films were quantitated by scanning densitometry using an imaging densitometer.

2.6. Search for transcription factor binding motifs

In order to identify putative transcription factor binding motifs in the gal-1 promoter, positions −624 to +50 of the rat galectin-1 genomic sequence (GenBank accession number U40624) were analysed using the program MATCH™ (public version 1.0; Biologische Datenbanken GmbH, Wolfenbüttel, Germany).

2.7. Electrophoretic mobility shift assays (EMSA)

PSCs growing in 6-well plates were cultured for 12 h in serum-free IMDM before they were stimulated as indicated. Cell lysis and preparation of nuclear extracts were performed essentially as previously described [30,34]. For EMSA experiments, nuclear proteins of 105 cells were incubated with 16 fmol of the following double-stranded oligonucleotides: 5′-CGC TTG ATG ACT CAG CCG ATC-3′ (AP-1 probe, consensus binding motif underlined), 5′-CTT CCG GGG AAA ACC CGG GGG GCC-3′ (corresponding to positions −565 to −542 of the rat galectin-1 gene, putative NF-κB binding motif underlined), 5′-CCA-GAAGCCCACAGAAGGAGCAATA-3′ (corresponding to positions −512 to −488 of the rat galectin-1 gene, putative serum response element [SRE] underlined). Oligonucleotides were end-labelled with [γ32P]-ATP by polynucleotide kinase. The binding reactions (30 min at room temperature) were performed in a total volume of 20 μl in the following buffer: 10 mM Tris–HCl (pH7.5), 50 mM NaCl, 0.1 mM EDTA, 1 mM diethiothreitol, 5% glycerol, 0.1% NP40, 1 mg/ml BSA, and 100 μg/ml poly(dI–dC). Afterwards, supershift analysis was initiated by adding 1 μg antibody as indicated. After an incubation period of 20 min, protein–DNA complexes were analysed by electrophoretic separation on a 6% non-denaturing polyacrylamide gel. Dried gels were exposed to X-ray film. All results shown are representative of at least three independent experiments.

2.8. Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated from cells pretreated as indicated using the RNeasy kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. One microgram (1 μg) of total RNA was reverse transcribed into cDNA using oligo(dT)12-18 primer and superscript II-reverse transcriptase according to the manufacturer’s instructions (Invitrogen, Karlsruhe, Germany). mRNA expression was evaluated by PCR using the Taq PCR Master Mix Kit (Qiagen). To determine expression of the housekeeping gene hypoxanthine phosphoribosyl transferase (HPRT), the respective cDNA samples were co-amplified with a defined concentration of a synthetic DNA control fragment (CF) as internal standard containing HPRT specific primer sequences [35,36]. The following primers were used: 5′-TCC CAG CGT CGT GAT TAG TG-3′ (forward) and 3′-GGC TTT...
TCC ACT TTC GCT GA-5’ (reverse). The molecular weights of the standard and cDNA amplicons were different, allowing electrophoretic separation in a 1.8% agarose gel containing 0.1 μg/ml ethidium bromide. Ethidium bromide fluorescence intensities were measured with an electronic camera and analysed with the GEL-PRO™ Analyzer (version 4.0) program (Media Cybernetics, Silver Spring, MD, USA).

Gal-1 mRNA expression was studied using the following primers: 5’-ATG GCC TGT GGT CTG GTG CT-3’ (forward) and 3’-AAT TCA CAC ACC GGA AAC TC-3’ (reverse) [29]. For quantitative evaluation, fluorescence intensities were analysed as described above. Subsequently, raw data were normalised for differences in input cDNA concentrations. Therefore, (I) the ratio of HPRT/CF band intensities was calculated for each sample, and (II) the resulting values were expressed as percentage of the control indicated in the figure legend and (III) used to correct gal-1 fluorescence band intensities.

2.9. Statistical analysis

Results are expressed as means±standard error of the mean (SEM) for the indicated number of separate cultures per experimental protocol. Statistical significance was checked using Wilcoxon’s rank sum test. p<0.05 was considered to be statistically significant.

3. Results

3.1. Expression of gal-1 in PSCs

Freshly isolated PSCs were cultured for different periods of time (3–10 days), and RNA as well as protein extracts were subjected to RT-PCR and Western blot analysis, respectively. The results indicate expression of the gal-1 mRNA (Fig. 1A) and protein (Fig. 1B) in all PSC cultures. Interestingly, a culture time-dependent increase of gal-1 mRNA and protein expression was observed (compare lane 1 in 1A and B with 2–4). Enhanced expression of gal-1 in late PSC cultures correlated with the increase of α-SMA expression in the course of culture-induced myofibroblastic transdifferentiation. In contrast, expression of the proteins ERK 1 and 2 remained unchanged, excluding a non-specific effect (Fig. 1B). The differences between early and late PSC cultures regarding gal-1 mRNA and protein expression were statistically significant (Fig. 1C).

3.2. Regulation of gal-1 expression

To gain insight into the mechanisms underlying induction of gal-1 expression in PSCs, we studied the effects of FCS and three cytokines that have been implicated in PSC activation, PDGF, TGF-β1 and TNF-α. Stimulation of serum-starved PSCs with either FCS at 10–20% (Fig. 2A) or PDGF (10 ng/ml; Fig. 2B) induced a significant increase of gal-1 mRNA expression, whereas TGF-β1 and TNF-α had no significant effect (Fig. 2B). The PDGF-effect on gal-1 expression was almost abolished by the tyrosine kinase inhibitor genistein at the non-toxic concentration of 10 μM and non-significantly reduced by the protein kinase C inhibitor calphostin C as well as PD98059, a specific inhibitor of MEK (mitogen/extracellular signal regulated kinase) (Fig. 2B).

In an attempt to identify transcription factors that may be involved in the induction of gal-1 expression in PSCs, we screened the region from −624 to +50 of the rat gal-1 gene...
for putative binding motifs and designed two probes for EMSA analysis. As shown in Fig. 3A, re-stimulation of serum-starved PSCs with FCS induced the binding of at least three distinct protein complexes to an oligonucleotide probe (covering positions −512 to −488) with a putative SRE. The protein/DNA complexes, however, were not supershifted by an antibody against the serum response factor (SRF; lane 6), although the antibody was capable of detecting SRF in nuclear extracts of activated PSCs (data not shown).

Furthermore, TNF-α stimulation of serum-starved PSCs induced transient protein binding to a probe (corresponding to positions −565 to −542) with a NF-κB binding motif (Fig. 3B). A supershift analysis revealed that the probe-binding protein complex contains the NF-κB subunit p65.

3.3. Gal-1 stimulates PSC proliferation and collagen synthesis

Having shown that PSCs express gal-1, we also sought to determine whether PSCs are gal-1-responsive. In all subsequent experiments, gal-1 isolated from human placenta [32] was used for cell stimulation.

To study PSC growth, cell proliferation after exposure to gal-1 concentrations that did not affect cell viability (analysed by trypan blue staining, data not shown) was assessed using the BrdU DNA-incorporation assay. Control cells were either left untreated or stimulated with the strong PSC mitogen PDGF (Fig. 4). Gal-1 significantly enhanced PSC proliferation in the concentration range 10–20 μg/ml.
A comparison with the effect of PDGF, however, revealed that the cytokine was the more potent mitogen.

A key effector function of activated PSCs is the synthesis of large amounts of ECM proteins such as collagen. To address the question how gal-1 affects collagen synthesis in PSCs, the effect of the lectin on \(^{3}H\)-proline incorporation into newly synthesised acetic acid-soluble proteins was determined.

Fig. 5 shows that gal-1 (at 5–10 \(\mu\)g/ml) significantly enhanced \(^{3}H\)-proline incorporation. The gal-1-effect was blocked by lactose, but not by cellobiose.

Together, the data indicate that gal-1 exerts stimulatory effects on differentiated PSCs.

3.4. Effects of gal-1 on ERK 1 and AP-1 signalling

Previous data from our laboratory have implicated ERKs in PSC activation [30]. To study possible effects of gal-1 on the ERK 1/2 signalling pathway, phospho-ERK levels in PSCs were determined by Western blot analysis. As shown in Fig. 6, gal-1 stimulation for 20–180 min induced phosphorylation of both ERK 1 and 2 (compare lanes 2–4 with 1 and 6). The strongest increase of the phospho-ERK 1 and 2 levels was observed in response to PDGF treatment (60 min; lane 5).

To further analyse the signal transduction pathways involved in gal-1 signalling in PSCs, EMSA experiments were performed. The results (Fig. 7) indicate that gal-1-stimulation for 1–4 h induced protein binding to an AP-1 consensus sequence (panel A, lanes 1–5). Again, the effect of PDGF was qualitatively identical but more pronounced (lane 6). The gal-1-induced protein/DNA complex super-
shifted with a c-Fos antibody (panel B), indicating the presence of this protein and providing evidence that the probe-binding transcription factor was indeed AP-1. In contrast, gal-1 had no effect on the DNA-binding of NF-κB and Smad transcription factors (data not shown).

4. Discussion

A growing body of evidence suggests that activated PSCs are essentially involved in pancreatic fibrosis, but the molecular mechanisms underlying PSC activation in the diseased organ are incompletely known. In this study, we have addressed the question how the animal lectin gal-1, a protein previously shown to activate hepatic stellate cells [29], affects key effector functions of PSCs in vitro. Our results indicate that gal-1 significantly stimulates both PSC proliferation and collagen synthesis. The gal-1-dependent increase of collagen production was blocked by lactose, but not by cellobiose, suggesting that β-galactoside-containing glycoconjugates mediate the gal-1 effect. The specific counter-receptors for gal-1 in PSCs, however, remain to be identified. In pilote co-immunoprecipitation experiments, we were unable to show gal-1 binding to two attractive candidate cell surface molecules, α5β1 integrin and the PDGF receptor, respectively.

Importantly, PSCs are not only gal-1-responsive but also a source of the lectin. In the course of PSC activation induced by sustained culture, expression of gal-1 and α-SMA increased in parallel. Gal-1 mRNA expression was stimulated by FCS and PDGF, implicating serum growth factors in the induction of gal-1 expression. In future studies, it will be interesting to test if gal-1 acts on PSCs through an autocrine loop, thereby promoting activation. The polyclonal gal-1 antibody used in this study did not inhibit PSC growth (data not shown). In the absence of additional data (e.g., from antisense gal-1 studies), this observation alone does not allow definitive conclusions.

The signalling pathways mediating the induction of gal-1 expression in the course of stellate cell activation in liver and pancreas are currently unknown. In this study, we used a tyrosine kinase inhibitor (genistein), a PKC inhibitor (calphostin C) and the MEK inhibitor PD98059 to analyse regulation of gal-1 expression in vitro. Although all three inhibitors interfered with PDGF-induced gal-1 expression, only genistein displayed a significant effect, suggesting that tyrosine phosphorylation is required for the induction.

The rat gal-1 promoter region contains a variety of putative binding motifs but, so far, to the best of our knowledge, no trans-acting factors have been identified. It has been shown, however, that basal mouse gal-1 promoter activity is determined by sequences encompassing the transcription start site (−50/+50) [37]. In pilote experiments, we analysed protein binding to two oligonucleotides, derived from the gal-1 promoter region, with putative binding sites for NF-κB and SRF, respectively. The results indicate that NF-κB binds to the corresponding motif in a TNF-α-dependent manner. Previous studies have implicated both TNF-α and NF-κB in PSC activation [34,38]. However, we also found that TNF-α did not induce expression of the gal-1 mRNA. Activation of NF-κB is therefore either not sufficient or not required for gal-1 expression. Interestingly, we furthermore observed a serum-dependent protein binding to the oligonucleotide with a putative SRE. The binding protein(s) remains to be identified because, according to our data, SRF is not involved.

PSCs have been shown to participate in regeneration early after acute necrotising pancreatitis in humans [39]. It is therefore likely that perpetuation of PSC activation under persistent pathological conditions, rather than PSC activation itself, is the pathological process that leads to fibrosis. In this scenario, it is conceivable that gal-1 secretion by activated PSCs plays an important role in balancing the inflammatory reaction in the pancreas: various recent studies have indicated that gal-1 exerts complex regulatory effects on cells of the immune system (reviewed in Ref. [40]). Thus, gal-1 was found to induce apoptotic death of activated (but not resting) T-cells [19]. Furthermore, gal-1 blocks lipopolysaccharide-induced nitric oxide production in macrophages [41] and inhibits leucocyte rolling and extravasation in experimental inflammation [42]. Considering that immunological mechanisms have been implicated in the pathogenesis of CP [43,44], we suggest that gal-1 might be involved in restricting the wound-healing response in the inflamed pancreas. Under persistent pathological conditions (such as secretion of growth factors by tumor cells and long-term exposure to ethanol metabolites), the activating effects of gal-1 on PSCs may, on the other hand, promote fibrosis. Clearly, this hypothesis needs to be tested in animal models of CP. In this regard, it is interesting to note that gal-1 has recently been reported to be a potent suppressor of experimental colitis in mice [45].

In the past few years, the intracellular signal transduction pathways in PSCs have become a focus of attention. Recently, we and others have shown that the MAP kinases ERK 1 and 2 are involved in the mediation of activation signals in PSCs. Thus, PDGF exerts its mitogenic effects on PSCs, at least in part, through the ERK 1/2 signal transduction pathway [30], and ethanol induces a fast [46] and long-lasting [47] activation of both enzymes. Furthermore, induction of myofibroblastic transdifferentiation of PSCs is associated with an early activation of the ERK 1/2 signalling cascade [30]. Here, we found that gal-1 is a potent inducer of ERK 1/2 activation in PSCs. The result is in agreement with a previous report on ERK activation by gal-1 in HSCs [29], suggesting a more general role of ERK 1 and 2 in the mediation of activating gal-1 effects in stellate cells. Our data also indicate that gal-1 induces an activation of AP-1. Previous studies of our group have implicated AP-1, a dimeric transcription factor composed of Fos and Jun proteins [48], in the transcriptional control of
PSC activation [30,33,34]. Thus, we found that DNA-binding of AP-1 is stimulated by PDGF, while all-trans retinoic acid inhibits PSC proliferation and acts as a transrepressor of AP-1. A gal-1-dependent activation of AP-1 has also been observed in human Jurkat T-lymphocytes [32]. The gal-1 effect on AP-1 was specific because two other transcription factors that have previously been implicated in PSC activation, Smad proteins and NF-κB [30,34], were not activated by the lectin. In view of these data, AP-1 is an important candidate to couple gal-1 signalling with the control of transcription in PSCs.

Until now, specific therapies to prevent, retard or even reverse pancreatic fibrosis do not exist. Their development essentially depends on the further elucidation of the molecular basics of fibrogenesis in the pancreas. In this regard, further studies on the role of lectins in the regulation of PSC activation are required.

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