Galectin-1 induces chemokine production and proliferation in pancreatic stellate cells

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Masamune, Atsushi, Masahiro Satoh, Jun Hirabayashi, Kenichi Kasai, Kennichi Satoh, and Tooru Shimosegawa. Galectin-1 induces chemokine production and proliferation in pancreatic stellate cells. Am J Physiol Gastrointest Liver Physiol 290: G729–G736, 2006. First published December 22, 2005; doi:10.1152/ajpgi.00511.2005.—Galectin-1 is a β-galactoside-binding lectin. Previous studies have shown that galectin-1 was expressed in fibroblasts of chronic pancreatitis and of desmoplastic reaction associated with pancreatic cancer. These fibroblasts are now recognized as activated pancreatic stellate cells (PSCs). Here, we examined the role of galectin-1 in cell functions of PSCs. PSCs were isolated from rat pancreatic tissue and used in their culture-activated phenotype unless otherwise stated. Expression of galectin-1 was assessed by Western blot analysis, RT-PCR, and immunofluorescent staining. The effects of recombinant galectin-1 on chemokine production and proliferation were evaluated. Activation of transcription factors was assessed by EMSA. Activation of MAPKs was examined by Western blot analysis using anti-phosphospecific antibodies. Galectin-1 was strongly expressed in culture-activated but not freshly isolated PSCs. Recombinant galectin-1 increased proliferation and production of monocyte chemoattractant protein-1 and cytokine-induced neutrophil chemoattractant-1. Galectin-1 activated ERK, JNK, activator protein-1, and NF-κB, but not p38 MAPK or Akt. Galectin-1 induced proliferation through ERK and chemokine production mainly through the activation of NF-κB and in part by JNK and ERK pathways. These effects of galectin-1 were abolished in the presence of thiodigalactoside, an inhibitor of β-galactoside binding. In conclusion, our results suggest a role of galectin-1 in chemokine production and proliferation through its β-galactoside binding activity in activated PSCs.

IN 1998, star-shaped cells in the pancreas, namely, pancreatic stellate cells (PSCs), were identified and characterized (2, 5). In the normal pancreas, stellate cells are quiescent and can be identified by the presence of vitamin A-containing lipid droplets in the cytoplasm. In response to pancreatic injury or inflammation, they are transformed (“activated”) from their quiescent phenotype into myofibroblast-like cells, which actively proliferate, express the cytoskeletal protein α-smooth muscle actin (α-SMA), and produce extracellular matrix components including type I collagen. Many of the morphological and metabolic changes associated with the activation of PSCs in animal models of fibrosis also occur when these cells are grown in serum-containing medium in culture on plastic. There is accumulating evidence that activated PSCs play a pivotal role in the development of pancreatic fibrosis (2, 5, 13, 24). In addition, PSCs may participate in the pathogenesis of acute pancreatitis (13, 28). The activation of signaling pathways such as p38 MAPK (29), Rho-Rho kinase (25), and JNK (27) are likely to play a role in PSC activation. However, intracellular signaling pathways in PSCs remain largely unknown.

Galectin-1 is a member of the galectin family of β-galactoside-binding animal lectins (8). Galectin-1 is a homodimer of 14-kDa subunits that possesses two β-galactoside-binding sites and is expressed at various levels in many tissues under normal and pathological conditions (34). Galectin-1 is localized in the cell nucleus, in the cytoplasm, on the cell surface, and in the extracellular matrix into which galectin-1 is secreted (18, 34). Galectin-1 acts both within the cells via sugar-independent interactions with other proteins and outside of the cells via sugar-dependent interactions with β-galactoside-containing glycoconjugates expressed by the same cell or by neighboring cells after their secretion, which is independent of a classical signal sequence (8, 18, 34). The sugar binding activity of galectin-1 is inhibited by thiodigalactoside (TDG) or lactose. Galectin-1 plays a role in a variety of cell functions including proliferation, migration, adhesion, immune responses, apoptosis, inflammation, and carcinogenesis (17, 18, 21, 34, 35, 39, 45, 51).

There have been a few reports on the expression of galectin-1 in the pancreas, but with some conflicting results (10, 47, 50). mRNA and protein analyses of galectin-1 revealed its low abundance in the normal pancreas (10, 50). Wang et al. (50) reported that galectin-1 was moderately to intensely expressed in fibroblasts of chronic pancreatitis samples. Berberat et al. (10) reported that fibroblasts and extracellular matrix cells around the cancer mass showed strong galectin-1 expression, but cancer cells were negative. Shen et al. (47) reported that galectin-1 was strongly expressed in the stroma surrounding the cancer mass but negative in samples of chronic pancreatitis. Fibroblasts in the area of fibrosis in chronic pancreatitis and of desmoplastic reaction associated with pancreatic cancer are now recognized as activated PSCs (4, 6). However, the galectin-1 expression and possible regulation of cell functions by galectin-1 in PSCs are largely unknown. We here report that PSCs expressed galectin-1 upon activation and that recombinant galectin-1 induced chemokine production and proliferation in activated PSCs.

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MATERIALS AND METHODS

Materials

Collagenase P and recombinant human interleukin-1β were from Roche Applied Science (Mannheim, Germany). [γ-32P]ATP was from Amersham Biosciences (Buckinghamshire, UK). Rat recombinant PDGF-BB and human recombinant galectin-1 were from R&D Systems (Minneapolis, MN). Double-stranded consensus oligonucleotides probes for NF-κB and activator protein-1 (AP-1) were from Promega (Madison, WI). Goat anti-galectin-1 antibody and antibodies used for suphershift assays were from Santa Cruz Biotechnology (Santa Cruz, CA). Rabbit antibodies against phosphorylated and total MEK, ERK, JNK, p38, MAPK, Akt, inhibitor of NF-κB (IkB)-α, and biotinylated protein ladder detection pack were from Cell Signaling Technology (Beverly, MA). Bay 11-7082, U-0126, and SP-600125 were from Calbiochem (La Jolla, CA). Rabbit antibody against GAPDH was from Trevigen (Gaithersburg, MD). All other reagents were from Sigma-Aldrich (St. Louis, MO) unless specifically described.

Production of Recombinant Galectin-1

Recombinant human galectin-1 was generated as previously described (15). Briefly, the DNA fragment encoding human galectin-1 was amplified by PCR using cloned cDNA as a template. The amplified fragment was ligated to the pET21a vector. Generated prokaryotic expression vector was used to transform Escherichia coli BL21(DE3). Recombinant protein was induced in the cells by 1 mM isopropyl-β-D-thiogalactopyranoside. They were purified by affinity chromatography on asialofetuin-Sepharose 4B. Experiments were performed using “house-made” recombinant galectin-1 or commercial galectin-1.

Cell Culture

All animal procedures were performed in accordance with National Institutes of Health Animal Care and Use guidelines. Rat PSCs were prepared from pancreas tissues of male Wistar rats (Japan SLC; Kobe, Japan) weighing 200–250 g as previously described (26). Cells were lysed in SDS buffer, and nuclear extracts were prepared from pancreas tissues of male Wistar rats (Japan SLC; Kobe, Japan), and counted at 4°C overnight. A 100-fold excess of unlabeled oligonucleotide was added before the addition of experimental reagents. For experiments using the inhibitors of MAPKs, nuclear extracts from galectin-1-treated PSCs were incubated with p50, p65, c-Rel, or the p52 complexes or without antibody before incubation for 2 h at 4°C with antibodies against p50, p65, c-Rel, or the p52 subunit of NF-κB complexes or without antibody before incubation with the radiolabeled probe.

Western blot analysis. Western blot analysis was performed as previously described (26). Cells were lysed in SDS buffer, and cellular proteins (~25 μg) were fractionated with a biotinylated protein ladder on a 10–20% gradient SDS-polyacrylamide gel. They were transferred to a nitrocellulose membrane (Bio-Rad; Hercules, CA), and the membrane was incubated overnight at 4°C with goat anti-galectin-1 antibody. After incubation with peroxidase-conjugated rabbit anti-goat IgG antibody. After incubation with peroxidase-conjugated rabbit anti-goat IgG antibody, proteins were visualized using an ECL kit (Amersham Biosciences). Peroxidase-linked anti-biotin antibody was added to the secondary antibody to simultaneously detect biotinylated marker proteins. Levels of α-SMA and GAPDH were determined in a similar manner.

Cell Proliferation Assay

5-Bromo-2′-deoxyuridine ELISA. DNA synthesis was measured by the incorporation of 5-bromo-2′-deoxyuridine (BrdU) using a commercial kit (Cell Proliferation ELISA, BrdU; Roche Applied Science) according to the manufacturer’s instruction. BrdU incorporation was quantitated by differences in absorbance at wavelengths of 370–492 nm.

Cell number counts. PSCs (1 × 10^5 cells/well) were plated in six-well culture plates and serum starved for 24 h. Cells were treated with galectin-1 (at 10 μg/ml) or PDGF-BB (at 25 ng/ml) for 24 or 48 h. They were fixed in methanol, stained with DifQuick (Sysmex; Kobe, Japan), and counted at ×100 magnification. Six microscopic fields were randomly chosen for each specimen.

Nuclear Extract Preparation and EMSA

Nuclear extracts were prepared, and EMSA was performed as previously described (22). Double-stranded oligonucleotide probes for AP-1 (5′-CGCTTGATGATCGCCGGGA-3′) and NF-κB (5′-AGTGGGAGCCACTTGGCAGG-3′) were end labeled with [γ-32P]ATP. Nuclear extracts (~10 μg) were incubated with the labeled oligonucleotide probe for 20 min and electrophoresed through a 4% polyacrylamide gel. The gel was dried and autoradiographed at ~80°C overnight. A 100-fold excess of unlabeled oligonucleotide was incubated with nuclear extracts for 10 min before the addition of the radiolabeled probe in the competition experiments. For supershift assays, nuclear extracts from galectin-1-treated PSCs were incubated for 2 h at 4°C with antibodies against p50, p65, c-Rel, or the p52 subunit of NF-κB complexes or without antibody before incubation with the radiolabeled probe.

Activation of MAPKs was examined by Western blot analysis using anti-phosphospecific MAPK antibodies. These antibodies recognize only phosphorylated forms of MAPKs, thus allowing the assessment of activation of the kinases. Levels of total MAPKs, Akt (phosphorylated at Ser473 and total), and IκB-α were also determined by Western blot analysis.
Chemokine Measurement

ELISA. After a 24-h incubation, cell culture supernatants were harvested and stored at −80°C until measurements. Levels of monocyte chemotactic protein-1 (MCP-1) and cytokine-induced neutrophil chemoattractant-1 (CINC-1) in culture supernatants were measured using commercial kits (Pierce Chemical; Rockford, IL; and Panapharm Laboratories; Udo, Japan) according to the manufacturers’ instructions.

Northern blot analysis. After a 4-h incubation, total RNA was prepared from PSCs using the RNAeasy total RNA preparation kit (Qiagen). Ten micrograms of total RNA were separated on a 1% agarose-2.2 M formaldehyde gel and transferred to a nylon membrane filter (Amersham Biosciences). Blots were hybridized at 42°C for 16 h to 32P-labeled DNA probes of MCP-1, CINC-1, or β-actin generated by PCR. Specific primer sets were as follows: MCP-1 sense 5′-AGCCAGATGCAATGATGC-3′ and 5′-GGAAAAGAGATGGAATTG-3′, CINC-1 sense 5′-ATGGTCTCAGCACCACCCGCT-3′, and antisense 5′-ACTTTGGGACACCCATTGAC-3′, and β-actin sense 5′-GATGTTGGGTGTTGGTGTCAGAAGAGA-3′ and antisense 5′-GCTCATTGCCGATAGTGATGACCT-3′. MCP-1, CINC-1, and β-actin were amplified with 30 cycles at 94°C (for 1 min), at 55°C (for 1 min), and at 72°C (for 1 min). The identity of the RT-PCR was confirmed by direct sequencing. After the hybridization, the filter was washed and subjected to autoradiography at −80°C overnight.

Effect of Galectin-1 on the Transformation of Freshly Isolated PSCs in Culture

Freshly isolated PSCs were treated with serum-free medium, 10% FBS, galectin-1 (at 10 μg/ml), or an oxidation-resistant galectin-1 mutant (C2S; at 10 μg/ml). After a 5-day incubation, morphological changes were assessed after cells were stained with glial fibrillary acidic protein (GFAP) as previously described (30) using a streptavidin-biotin-peroxidase complex detection kit (Histofine Kit, Nichirei; Tokyo, Japan). Briefly, cells were directly plated on slides and fixed with methanol at −20°C, and endogenous peroxidase activity was then blocked by an incubation with 0.3% hydrogen peroxide. After an immersion in normal goat serum, slides were incubated with rabbit anti-GFAP antibody, followed by peroxidase-conjugated streptavidin. Finally, color was developed by incubating the slides for several minutes with diaminobenzidine (Dojindo; Kumamoto, Japan).

Statistical Analysis

Results were expressed as means ± SD. Experiments were performed at least three times, and similar results were obtained. Representative luminograms and autoradiograms are shown. Differences between the groups were evaluated by ANOVA, followed by Fisher’s test for post hoc analysis. A P value of <0.05 was considered statistically significant.

RESULTS

Galectin-1 Was Strongly Expressed in Culture-Activated But Not Freshly Isolated PSCs

Freshly isolated PSCs were induced to transform to a myofibroblast-like phenotype by culture on plastic in serum-containing medium. Progressive activation of PSCs was demonstrated by their expression of α-SMA (Fig. 1A). Western blot analysis showed that galectin-1 was undetectable in PSCs on day 1 but was strongly detected as a single band of ~14 kDa in culture-activated PSCs on day 14 (Fig. 1B). In agreement with the results of the Western blot analysis, mRNA of galectin-1 was barely detectable in PSCs on day 1 but was strongly expressed in PSCs on day 14 (Fig. 1C). Immunofluorescent staining of PSCs on day 14 showed both cytoplasmic and nuclear expression of galectin-1 (Fig. 1D).

Galectin-1 Induced Chemokine Production

Galectin-1 induced MCP-1 and CINC-1 production in a dose-dependent manner (Fig. 2A). The effects were significant at as low as 1 μg/ml galectin-1. We also examined the expression of MCP-1 and CINC-1 mRNAs by Northern blot analysis. Galectin-1 induced mRNA expression of MCP-1 and CINC-1, which was inhibited in the presence of TDG (Fig. 2B).

Galectin-1 Induced Proliferation of PSCs

Galectin-1 increased cell proliferation in a dose-dependent manner as assessed by DNA synthesis and cell number counts (Fig. 3). The proliferative effect of galectin-1 at 10 μg/ml was less potent than PDGF-BB at 25 ng/ml, which is one of the most potent mitogen for PSCs in vitro (3).

Galectin-1 Activated AP-1 and NF-κB

Galectin-1 increased AP-1 and NF-κB binding activities (Fig. 4, A and B). The specificity of DNA binding activities was demonstrated by the addition of a 100-fold molar excess of unlabeled AP-1 or NF-κB oligonucleotide in the competition assays as shown in lane 3 of Fig. 4, A and B. The activation of these transcription factors was abolished in the presence of...
TDG, as shown in lane 4 of Fig. 4, A and B. We also examined the effect of galectin-1 on the degradation of IκB-α by Western blot analysis. Galectin-1 induced degradation of IκB-α, further supporting that galectin-1 activated NF-κB (Fig. 4C). The supershift assay showed that pretreatment with antibodies against p50 and p65, but not c-Rel or p52, subunits induced the supershift, suggesting that the inducible NF-κB complex consisted of p50 and p65 heterodimer (Fig. 4D).

**Galectin-1 Activated MEK-ERK and JNK Pathways**

Galectin-1 activated MEK and ERK in a time-dependent manner peaking around 1 h (Fig. 5A). Activation of ERK by galectin-1 was observed in a dose-dependent manner and inhibited in the presence of TDG and U-0126, an inhibitor of the ERK pathway (Fig. 5, B and C). Galectin-1 activated 46-kDa JNK but not the 54-kDa isoform, whereas IL-1β activated both 46- and 54-kDa JNK (Fig. 5D). Galectin-1 did not activate p38 MAPK or Akt.

**Differential Roles of Intracellular Signaling Pathways in Proliferation and Chemokine Production of PSCs**

To clarify the roles of activated MAPKs and NF-κB in galectin-1-induced proliferation and chemokine production, we employed specific inhibitors of MAPKs and NF-κB. U-0126 and TDG abolished PSC proliferation in response to galectin-1 (Fig. 6A). Bay 11-7082, an inhibitor of NF-κB (36), and SP-600125, an inhibitor of the JNK pathway, did not affect galectin-1-induced proliferation. Galectin-1-induced chemokine production was abolished by Bay 11-7082 and TDG and partially inhibited by SP-600125 and U-0126 (Fig. 6B). Thus galectin-1 induced proliferation mainly through the activation of the MEK-ERK pathway and chemokine production mainly through the activation of NF-κB and in part by JNK and ERK pathways.

**Galectin-1 Did Not Induce the Transformation to Myofibroblast-Like Cells**

After 5 days, PSCs treated with 10% FBS showed transformation into cells with a myofibroblast-like phenotype, whereas PSCs treated with serum-free medium were small and circular, with slender dendritic processes (Fig. 7, A and B). PSCs treated with recombinant galectin-1 did not show the myofibroblast-like appearance but showed cell spreading compared with cells treated with serum-free medium only (Fig. 7C). Because galectin-1 loses its carbohydrate-binding activity by oxidation, we also examined the effect of the oxidation-resistant mutant galectin-1 on the degradation of IκB-α by Western blot analysis. Galectin-1 induced degradation of IκB-α, further supporting that galectin-1 activated NF-κB (Fig. 4C). The supershift assay showed that pretreatment with antibodies against p50 and p65, but not c-Rel or p52, subunits induced the supershift, suggesting that the inducible NF-κB complex consisted of p50 and p65 heterodimer (Fig. 4D).

Fig. 2. Gal-1 induced chemokine production. A: serum-starved, culture-activated PSCs were treated with Gal-1 at the indicated concentrations in serum-free medium. After 24 h of incubation, culture supernatants were harvested, and the levels of monocyte chemoattractant protein (MCP)-1 and cytokine-induced neutrophil chemoattractant (CINC)-1 were determined by ELISA. **P < 0.01 vs. Gal-1 at 0 μg/ml. B: PSCs were left untreated (Cont) or treated with Gal-1 (at 10 μg/ml) in the absence or presence of thiodigalactoside (TDG; at 20 mM) in serum-free medium. After 4 h of incubation, total RNA was prepared, and the levels of MCP-1, CINC-1, and β-actin were determined by Northern blot analysis.

Fig. 3. Gal-1 induced proliferation of PSCs. Serum-starved, culture-activated PSCs were stimulated with Gal-1 at the concentrations or PDGF-BB (at 25 ng/ml) in serum-free medium. A: after 24 h of incubation, DNA synthesis was assessed by BrdU incorporation and ELISA. Data are shown as means ± SD (as a percentage of the control); n = 6. **P < 0.01 vs. Gal-1 at 0 μg/ml. B: PSCs were then stimulated with Gal-1 (at 10 μg/ml) or PDGF-BB (at 25 ng/ml) for 2 or 4 days. Cells were stained with Diff-Quick and counted at ×100 magnification. Six microscopic fields were randomly chosen for each specimen. **P < 0.01 vs. Cont (serum-free medium only). OD, optical densiometric units.

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and appears to be rather anti-inflammatory (17, 39). Galectin-1 was shown to suppress the inflammatory and autoimmune responses mainly through the induction of apoptosis in T cells in collagen-induced arthritis (41) and 2,4,6-trinitrobenzene sulfonic acid-induced colitis (43). Galectin-1 inhibited leukocyte-endothelial cell interactions in an experimental model of peritonitis (19). Galectin-1 inhibited T-cell activation and promoted apoptosis of activated T cells (35). Galectin-1 inhibited T cell adhesion to the extracellular matrix and abrogated the production of proinflammatory cytokines such as tumor necrosis factor (TNF)-α and interferon-γ by activated T cells (38). Our results are in striking contrast to these studies showing anti-inflammatory roles of galectin-1, and it would be interesting to see whether this phenomenon is specific to PSCs or also observed in other myofibroblast-like cells in the liver, lung, kidney, or intestine.

The effects of galectin-1 on cell proliferation are multifaceted. It can function in both a carbohydrate-dependent and carbohydrate-independent fashion, and its effects can be either positive or negative, depending on the responder cell types or its subcellular localization (17, 45). Galectin-1 has been shown to be mitogenic for vascular smooth muscle cells (31), pulmonary arterial endothelial cells (42), and hepatic stellate cells (21). These mitogenic activities were related to the lectin property of galectin-1. Exogenously added galectin-1 inhibited the growth of embryonic fibroblasts (51). Inhibition of galectin-1 gene expression by antisense cDNA transfection in a rat glioma cell line arrested tumor growth, suggesting that endogenous galectin-1 had growth-promoting activity (52). In addition, galectin-1 might exert biphasic modulation of cell growth

C2S, in which the second cysteine residue is changed to serine (16). Like wild-type galectin-1, C2S did not induce the transformation to myofibroblast-like cells (Fig. 7D).

DISCUSSION

We showed here that galectin-1 expression was strongly induced upon activation in PSCs and that recombinant galectin-1 induced chemokine production and proliferation. Galectin-1 induced proliferation mainly through the activation of the ERK pathway, whereas chemokine production was mediated mainly by the activation of NF-κB and in part by JNK and ERK pathways. Thus intracellular signaling pathways play differential roles in galectin-1-induced regulation of PSC functions. Galectin-1 may act both extracellularly after secretion and intracellularly (8, 18, 34). Extracellular functions of galectin-1 have been revealed by studies employing recombinant galectin-1, whereas many intracellular properties have been elucidated by extrinsic control of gene expression in cells via transfection and inhibition (e.g., antisense). This study focused on the exogenous role of galectin-1 in PSC functions. It is uncertain whether high levels (~μg/ml) of soluble galectin-1 can be achieved in the pancreas in vivo. Of note, recent evidence indicates that the amount of galectin-1 secreted by different cell types is sufficient to kill T cells when galectin-1 is presented in the context of the extracellular matrix (14). Regarding its endogenous role, we have recently found that inhibition of endogenous galectin-1 gene by the introduction of antisense galectin-1 cDNA reduced the expression of α-SMA and collagen genes (A. Masamune, M. Satoh, and T. Shimosegawa, unpublished observations).

The ability of galectin-1 to activate NF-κB and induce chemokine production was unexpected, especially because it implies proinflammatory roles of galectin-1 in PSCs. To our knowledge, this is the first study showing the ability of galectin-1 to activate NF-κB and induce chemokine production. The role of galectin-1 in inflammation has been extensively studied

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Fig. 4. Gal-1 activated activator protein (AP)-1 and NF-κB. A and B: culture-activated PSCs were left untreated (lane 1) or treated with Gal-1 (at 10 μg/ml) in the absence (lane 2) or presence (lane 4) of TDG (at 20 mM) in serum-free medium for 1 h. Nuclear extracts were prepared and subjected to EMSA using AP-1 (A) or NF-κB (B) consensus oligonucleotide probes. Arrows denote specific complexes competitive with cold double-stranded oligonucleotide probes (lane 3). Free probe; *nonspecific band. C: culture-activated PSCs were treated with Gal-1 (at 10 μg/ml) for the indicated times. Total cell lysates were prepared, and the level of IκB-α was determined by Western blot analysis. D: for supershift assays, nuclear extracts from Gal-1-treated PSCs were incubated for 2 h at 4 °C with antibodies against p50, p65, c-Rel, or p52 subunits of NF-κB complexes or without antibody (no Ab) before incubation with the radiolabeled probe. Arrows denote further gel retardations (supershifts).

Fig. 5. Gal-1-activated ERK and JNK. A and B: culture-activated PSCs were treated with Gal-1 (at 10 μg/ml) for the indicated times (A) or at the indicated concentrations (in μg/ml) for 1 h (B) in serum-free medium. C: PSCs were left untreated (Cont) or treated with Gal-1 (at 10 μg/ml) in the absence or presence of TDG (at 20 mM) or an inhibitor of ERK pathway [U-0126 (U), at 5 μM] for 1 h. D: PSCs were treated with Gal-1 (at 10 μg/ml) for the indicated times or with positive controls (PC; IL-1β at 2 ng/ml for JNK and p38 MAPK, and PDGF-BB at 25 ng/ml for Akt) for 10 min. Total cell lysates (~100 μg) were prepared, and the levels of phosphorylated and total MEK, ERK, JNK, p38 MAPK, and Akt were determined by Western blot analysis.
depending on its concentrations (1). Although high doses of galectin-1 inhibited cell proliferation independent of its sugar-binding activity, low doses of galectin-1 were mitogenic through its H9252-galactosi-1 binding activity (1). In general, cells regulate their susceptibility to galectins by regulating the expression of glycoprotein counterreceptors (46). Galectin-1 does not have specific receptors, but possible counterreceptors for galectin-1 include fibronectin (33), laminin (53), β1-integrin (32), and T cell glycoproteins CD43 and CD45 (35). Although we have not yet identified the counterreceptors for galectin-1 in activated PSCs, possible receptors include fibronectin and laminin (5). Galectin-1 may not be a potent activator because some cell surface glycoproteins recognized by galectin-1 may not deliver any signals to the cell, and it is also possible different glycoproteins transmit opposing signals that result in net cancellation. This is one explanation as to why relatively high concentrations of galectin-1 are often required to demonstrate effects and why galectin-1 has different effects on different cells, because different glycoconjugates may be engaged in different cell types (17). As pointed out by Scott and Weinberg (45), the bifunctional nature of galectin-1, in conjunction with other experimental variables, makes it difficult to assess overall outcomes and the significance of growth-regulatory actions in many previous investigations.

Galectin-1 expression was strongly induced upon activation of PSCs. The underlying mechanisms of this upregulation remain unknown. It has been shown that galectin-1 expression was upregulated upon activation in endothelial cells (9), macrophages (40), and T cells (12). Fuertes et al. (12) reported that increased galectin-1 expression during T cell activation involved MEK-ERK and p38 MAPK pathways. It has been shown that activation of p38 MAPK plays a role in the activation of PSCs (29). On the other hand, galectin-1 did not induce transformation to myofibroblast-like cells. This finding does not exclude the role of galectin-1 in the development of pancreatic fibrosis, because it has been shown that cytokines and growth factors such as transforming growth factor-β1 and TNF-α induced the transformation of quiescent PSCs (44). These mediators are released from inflammatory cells, platelets, and pancreatic acinar cells as well as pancreatic cancer cells (6), leading to the activation and galectin-1 expression in PSCs. Along this line, ovarian carcinoma cells have been shown to induce galectin-1 expression in fibroblasts (49). Interestingly, cells treated with galectin-1 showed more cell spreading. Similar effects on cell spreading were reported in macrophages (40). It is unknown whether this is merely a result of altered adhesion and modifications in the organization of actin cytoskeleton or resembles a transition to the myofibroblast-like phenotype.

Very recently, Fitzner et al. (11) reported that galectin-1 induced proliferation and collagen production in PSCs. Although they showed that galectin-1 activated ERK and AP-1, the roles of intracellular signaling pathways in galectin-1-mediated cell functions were not pursued in detail. We show here for the first time that galectin-1 induced MCP-1 and

![Fig. 6. Differential roles of signaling pathways in Gal-1-induced proliferation and chemokine production in PSCs.](image-url)
CINC-1 production in cultured cells. In addition to ERK and AP-1, galectin-1 activated JNK and NF-kB but not p38 MAPK or Akt in PSCs. Galectin-1 induced proliferation mainly through the activation of the ERK pathway, whereas chemokine production was mediated mainly by the activation of NF-kB and in part by JNK and ERK pathways. Of note, in disagreement with our study, galectin-1 failed to activate NF-kB in their study. The reason for this discrepancy remains unknown, but the different preparations of galectin-1 might be one explanation. While we employed recombinant galectin-1 expressed in Escherichia coli, they prepared galectin-1 from the human placenta.

Galectin-1 has attracted attention as a potential cancer target because of its contribution to a variety of events including tumor transformation, cell cycle regulation, apoptosis, cell adhesion, migration, and inflammation (37). Overexpression of galectin-1 in the stromal component of tumors has been proposed to act as an “immunological shield” surrounding tumor cells (48, 49). Galectin-1 might modulate interactions between the extracellular matrix and glycoconjugates located on the cell surface and hence affect invasion (48, 49). In a murine model of melanoma, blockade of galectin-1 resulted in an enhanced tumor-specific T cell-mediated response and consequently tumor rejection, suggesting a role of galectin-1 in tumor immune escape through its prosapopotic activity on T cells (35). Our results suggest a novel role of galectin-1 expressed in the stroma, i.e., the recruitment of inflammatory cells in part by the production of MCP-1 and CINC-1. Recruited inflammatory cells then produce cytokines and growth factors, leading to the sustenance of pancreatic inflammation and the development of pancreatic fibrosis. Although the immune system can reduce tumor incidence through immune-surveillance mechanisms, it can also promote tumor progression through inflammation-dependent mechanisms (20). This is along with the concept that inflammation plays a central role in tumor progression, particularly in the context of host-tumor interaction (7). It is also possible that galectin-1, produced by activated PSCs, might directly affect the behavior of pancreatic cancer cells (48). Elucidation of the roles of galectin-1 in the desmoplastic reaction will facilitate better understanding and rational approaches for the treatment of pancreatic cancer.

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