PSC-induced Galectin-1 Promotes the Malignant Behavior of Pancreatic Ductal Adenocarcinoma

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Abstract: Background Galectin-1 is a β-galactoside-binding protein overexpressed in the pancreatic stellate cells (PSCs) of pancreatic ductal adenocarcinoma (PDAC), while its expression is typically low in pancreatic cancer cells (PCCs). The point at which galectin-1 expression in PCCs increases, and its association with PDAC progression, have been unclear. Methods Galectin-1 expression in PDAC and metastatic lymph nodes was investigated using an immunohistochemical assay. PANC-1 PCC cells were co-cultured with PSCs expressing different levels of galectin-1. Subsequently, galectin-1 was overexpressed in PANC-1 cells using recombinant lentiviruses, and their proliferation, invasion, anchorage-independent growth, and in vivo tumorigenicity were evaluated. Results There was intermediate galectin-1 expression in PCCs, and it was positively associated with galectin-1 expression in PSCs in the PDAC tissues. Galectin-1 was strongly expressed in the metastatic lymph nodes. In the co-culture, high galectin-1 expression in the PSCs increased the galectin-1 expression in the PANC-1 cells. The galectin-1 overexpression in the PANC-1 cells enhanced their clone formation ability, proliferation, and invasion, increased the expression of proliferating cell nuclear antigen (PCNA) and BCL-2, and decreased Bax expression, promoting the establishment and growth of tumors. Conclusion High galectin-1 expression in PSCs induces galectin-1 expression in PCCs and subsequently promotes the malignant biological behavior of PDAC.

Key words: Pancreatic ductal adenocarcinoma; Pancreatic stellate cells; Galectin-1; Invasion; Metastasis

Introduction

Pancreatic ductal adenocarcinoma (PDAC) has a high mortality rate [1]. The poor outcome of PDAC is related to its aggressive growth and rapid progression of distant metastases, low rate of surgery, and resistance to chemoradiation, making treatment extremely challenging [2]. At diagnosis, 50%–60% of patients already have advanced disease with distant metastases, and of the 10% or so of patients who are able to undergo curative resection, many will relapse with distant metastasis and/or locoregional disease [3,4]. In terms of histopathology, PDAC often occurs with a dense desmoplastic reaction [5]. This histological type composes approximately 80% of the tumor, and is not just a passive tumor cell scaffold, but is actively involved in carcinogenesis [6]. The cellular component driving this desmoplastic response is pancreatic stellate cells (PSCs), pancreatic stromal cells with quiescent and activated states [7,8]. Growing evidence has demonstrated that the interaction between PDAC cells and activated PSCs plays an important role in PDAC development. The PSCs create a hypoxic and desmoplasia microenvironment that can promote PDAC initiation, invasion, evasion of immune surveillance, chemoradiation resistance and metastasis by generating high levels of chemotactic factors, cytokines, growth factors, and excessive extracellular matrix (ECM) [9-14]. Therefore, targeting the PSC and PDAC cell interaction could represent a new treatment option for advanced PDAC, especially treatments that target PSCs in the microenvironment of PDAC [2,6,15]. However, despite increasing attention being focused on the tumor microenvironment, there is not a comprehensive understanding of the specific functions and effects of PSCs in PDAC.

Galectin-1 (LGALS1), a member of the galectin family of β-galactoside-binding proteins, is a homodimer comprising 14-kDa subunits with two β-galactoside-binding sites [14]. The biological functions of galectin-1 include cell-matrix and cell-cell communication and cell proliferation [16]. Increased evidence has indicated that the
expression of galectin-1 is increased in tumors, indicating that it is involved in tumor cell invasion and the formation of metastasis [17], increasing tumor angiogenesis [18], and defending tumors from host immune responses [14,19]. Galectin-1 also plays a role in the desmoplastic reaction that appears around PDAC cells [20]. Galectin-1 was overexpressed in single culture activated PSCs and promoted PSC proliferation and chemokine production [20,21]. We have shown that, in PDAC, galectin-1 is mostly expressed in activated PSCs and can significantly boost tumor cell growth, progression, and proliferation, and contributes to a poorer outcome [22,23]. In addition, galectin-1 is weakly expressed in pancreatic cancer cells (PCCs), but is strongly expressed in metastatic peripancreatic lymph nodes [23]. Although the galectin-1 expression in PDAC tissues has been profiled, the specific function of endogenous galectin-1 expressed by PSCs in the PDAC microenvironment and its expression in PCCs have not been characterized.

We investigated the influence of different galectin-1 levels in primary PSCs on the galectin-1 protein expression profile of PCCs and examined the correlations of the molecular findings to determine whether the different galectin-1 levels in PCCs: i) promote cancer cell proliferation, anti-apoptosis, and invasion; ii) have the capacity to promote tumor establishment and growth.

Materials and Methods

Patients and pancreatic tissues
A total of 66 malignant pancreatic tumor samples and 18 chronic pancreatitis tissue specimens were included in the present study. The pancreatic cancer patients comprised 45 men and 21 women with a median age of 55 years (range, 37–83 years), and the chronic patients comprised 13 men and 5 women with a median age of 54.5 years (range, 27–71 years). The tissues adjacent to the specimens were evaluated histologically according to the criteria of the World Health Organization, and the tumor pathological stage was classified according to the International Union Against Cancer Classification (UICC). More detailed information on the clinicopathological characteristics of patients, PDAC tissues, and the histological evaluation of the PDAC tissue specimens have been described previously [14,23,24].

Ethics statements
All patients provided informed consent for their cooperation in the research study, which was authorized by the Ethics Committees of each institution. A copy of the consent form for each patient is readily available upon request.

All animal experiments were accomplished in line with the guidelines of the Experimental Animal Center Institutional Committee of Yangzhou University for Care and Use of Laboratory Animals. The procedures performed on animals, feeding conditions and the method of execution have been described previously [23].

Immunohistochemical staining and evaluation

Hematoxylin and eosin (H&E) staining and immunohistochemical staining were carried out as previously reported [14,23]. The primary antibodies were mouse monoclonal anti-galectin-1 (sc-166618; 1:200; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) and anti-cytokeratin 19 antibodies (1:200; MA106329; ABR, USA). Five fields (~×200) were randomly counted for each sample.

Cells and culture conditions
The methods of primary human PSCs identification, isolation, maintenance were described previously [14,23,24]. Passage number 2–5 PSCs were utilized for all analyses. PANC-1 cells were maintained in DMEM supplemented with 10% FBS. Co-culture experiments were carried out as follows: monolayers of PSCs (1 × 10^5 cells) and PANC-1 cells (5 × 10^5 cells) were respectively cultured in the upper and lower chambers of transwells (six-well, 0.4-Km, Corning, NY, USA) at 37°C. Proteins were extracted from PANC-1 cell lysates after the co-culture.

Quantitative reverse transcription-polymerase chain reaction
Total RNA was extracted from cultured cells using the Trizol reagent (Invitrogen, Beijing, China) according to the manufacturer’s instructions. QRT-PCR was performed using a SYBR Premix Ex Taq Reverse Transcription-PCR kit (TaKaRa, Shiga, Japan). The following primers were used: galectin-1 forward 5’-GAGGTGGCTCCTGACGCTAA-3’ and reverse 5’-CCTTGGCTTTGACACAGT-3’, and b-actin forward 5’-AGAAAACTGTGCACCACACC-3’ and reverse 5’-TACACAGCTGGATACGA-3’. β-actin was used as an internal control for comparison of the data. Quantitative PCR was performed using the ABI PRISM 7500 Fast Real-Time PCR System (Applied Biosystems, Carlsbad, CA, USA), and the comparative Ct method was used to assess relative differences in mRNA levels between two samples. All samples were run in triplicate.

Western blotting analysis
Western blotting was carried out as reported previously [14,23,25]. In brief, cells were lysed in SDS buffer and total cellular proteins (100 mg) were separated on 10% or 10%–20% gradient SDS polyacrylamide gels, transferred to PVDF membranes, and incubated with mouse anti-Galectin-1 (sc-166618; 1:200; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), anti-Bax (1:200; sc-509, Santa Cruz Biotechnology, Santa Cruz, CA), anti-Bax (1:200; sc-7480, Santa Cruz Biotechnology, Santa Cruz, CA), and anti-PCNA (1:200; sc-53407, Santa Cruz Biotechnology, Santa Cruz, CA) antibodies overnight at 4°C. After incubation with peroxidase-conjugated rabbit anti-mouse IgG antibody (Cell Signaling Technologies, Beverly, MA, USA), proteins
were visualized using an ECL system (GE Healthcare, Chalfont St. Giles, UK). Alpha-tubulin was used as a loading control.

**Preparation and transduction of recombinant lentiviruses**

The plasmids utilized to prepare recombinant lentiviruses to induce galectin-1 overexpression in PSCs or PANC-1 cells have been described previously [14]. In brief, the human galectin-1 gene fragment was cut out from a human cDNA library and cloned into pHAGE-CMV-MCS-I/Zs-Green between the BamHI and Xhol restriction enzyme sites. The methods used to infect the PSCs or PANC-1 cells, perform cell selection, and identify the viruses were reported previously [14].

**In vitro proliferation assay**

The proliferation of PANC-1 cells was tested using the methyl thiazolyl tetrazolium (MTT) (Sigma, USA) assay as described in a previous publication [22]. Galectin-1 overexpressing PANC-1 cells (2.5 × 10⁴) or normal control cells were placed in 96-well plates. We used 10% FCS to culture PANC-1 cells for 12 hours, until the cells adhered to the plate. The culture medium was then changed, and the proliferation levels were detected after 24, 48, 72, and 96 h. The results were determined by absorbance (570 nm) in a microtiter plate reader.

**In vitro invasion assay**

The Matrigel invasion assay was applied to evaluate the ability of PANC-1 cells with and without overexpression of galectin-1 to infiltrate the ECM. The method used has been described in a previous publication [23].

**Wound healing assay**

PANC-1 cells were placed in 24-well plates and allowed to proliferate to confluence. After being serum-starved for 12 hours, the monolayer cells were wounded by scraping off a band of cells with a pipette tip (200 µL). The cells were cultured for an additional 24 hours after wounding, then cells were fixed. Photographs were taken of three different segments of the ‘wound’ area after wounding, and the cell numbers inside the wound boundaries were counted.

**Assay for Ethynyl Deoxyuridine (EdU) incorporation**

To assess the *in vivo* cell proliferation, mice were intraperitoneally injected with 100 µg of EdU in PBS. After 4 hours, the tumor tissues (PANC-1) were collected from mice, sectioned, subjected to immunohistochemistry and observed under an optical microscope as described previously [26].

We randomly selected 5 groups of confluent cells from each sample image to count the number of EdU-positive cells. The relative positive ratio was determined from the average of the five group values.

**Assay for anchorage-independent growth**

An anchorage-independent growth assay was performed as described previously [27]. Experiments were carried out in 6-well dishes coated with 1% agarose (in DMEM with 10% FBS). One thousand cells were equally suspended in 0.5% agarose/DMEM and allowed to adhere to the bottom. The dishes were kept at 4°C for 2 hours to allow the agarose to solidify, then were incubated at 37°C. After 24 hours, fresh medium (2 ml) was added on the agar. The medium was replaced every 2 days. After 14 days, we counted the colony numbers and photographed the colonies by phase-contrast photomicrography. Only colonies ≥ 0.1 mm in diameter were counted.

**TUNEL assay**

The TUNEL assay was performed as described previously [28]. We counted the apoptotic cells (green staining) under a microscope (x200), and the apoptosis rate was calculated based on the proportion of stained cells among all cells in each sample. Four fields (100 cells/field) were randomly counted for each group.

**In vivo models**

The animal experiments were performed according to our previously described research [23,29]. In brief, xenograft tumors (n = 4/group) were generated by embedding PANC-1 cells subcutaneously (SC) on both flanks of nude mice (the left sides were injected with the galectin-1 overexpressing PANC-1 cells, and the right sides were injected with normal control PANC-1 cells) utilizing a 25-gauge needle. We measured the tumor size using calipers, and the volume was estimated by the formula: length × width × depth × 0.5236, as described previously [23]. The animals were sacrificed on day 30 post-implantation using an overdose of Nembutal.

**Statistical analysis**

The results are shown as the means ± standard deviation. The differences between groups were evaluated using t-tests and one-way ANOVAs. The relationship between the expression of galectin-1 in PSCs and in PCCs was evaluated by Pearson’s correlation test. *P*-values ≤ 0.05 were considered to be statistically significant. All statistical analyses were carried out using the SPSS 19.0 software.

**Results**

**Galectin-1 in PCCs is linked to galectin-1 expression in PSCs and the malignant behavior of PDAC**

We previously showed that galectin-1 staining was mostly detected in the PDAC and chronic pancreatitis stroma (the activated PSCs) [30] (Figure 1a), and that the total galectin-1 staining was associated with lymph node metastasis, perineural invasion, increased tumor size,
tumor differentiation, and the International Union Against Cancer (UICC) stage, with high expression predicting a poor outcome for PDAC [23,24]. However, in the present work, there was obvious galectin-1 staining in some PSC-adjacent PCCs (Figure 1), and the number of PCCs with galectin-1 staining correlated significantly with PDAC differentiation, where there were markedly more PCCs with galectin-1 staining in poorly differentiated tissue than in well- or moderately-differentiated tissue \( (P = 1.01 \times 10^{-13}, P = 6.54 \times 10^{-8}, \text{respectively}) \). There were also more PCCs with galectin-1 staining in moderately-differentiated tissues than in well-differentiated tissues \( (P = 0.0014) \) (Figure 1b-d). A Pearson correlation analysis showed positive correlations between the number of PCCs with galectin-1 staining and the average density of galectin-1 staining in PDAC \( (r^2 = 0.78, P = 9.78 \times 10^{-23}) \) (Figure 1f).

In addition, when there was high expression of galectin-1 in the PDAC stroma, it was also strongly expressed in the metastatic PCCs in the metastatic lymph nodes (Figure 1e-g). These results imply that high galectin-1 expression in PSCs may induce galectin-1 expression in some PCCs that are in proximity to PSCs, which may then initiate or support the malignant biological behavior of PDAC and eventually lead to metastasis.

**High galectin-1 expression in PSCs upregulates galectin-1 expression in PCCs**

To clarify the impact of PSC-derived galectin-1 on the galectin-1 expression profile in PCCs, we first detected the galectin-1 levels in normal human PSCs (hNPSCs) and PCC lines (SW1990, BxPC-3, PANC-1, CFPAC-1). qRT-PCR and Western blotting showed that the hNPSC galectin-1 levels were obviously higher than those in the PCC lines (Figure 2a). However, co-culture with PSCs exhibiting high galectin-1 expression significantly increased the galectin-1 levels in the PCC lines compared to the levels in single-cultured PCCs \( (P < 0.05) \) (Figure 2b). To verify that endogenous galectin-1 in PSCs induces galectin-1 expression in PCCs, β-lactose (50 mM) was used as a galectin-1 antagonist in the co-culture system. Treatment with β-lactose reduced the galectin-1 expression levels in the PSCs to a level similar to that in the single PCC line culture \( (P > 0.05) \) (Figure 2a, b). This finding indicates that, as an inhibitor of galectin-1, β-lactose hindered the ability of PSCs-secreted galectin-1 to act on PCCs, confirming that the increased expression of galectin-1 in PCC lines was at
least partly induced by the galectin-1 expression of PSCs.

**Endogenous galectin-1 promotes the malignant biological behavior of PANC-1 cells**

To assess the influence of endogenous galectin-1 on the biological behavior of PCCs, galectin-1 was overexpressed in PANC-1 cells using recombinant lentiviruses. qRT-PCR and Western blotting showed that the galectin-1 expression in PANC-1 cells was > 4-fold that of the untransfected control PANC-1 cells (Figure 2c-g). The colony formation

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**Figure 2** Galectin-1 expression was detected in PSC and PCC lines. Galectin-1 was mainly expressed in PSCs, while low levels of galectin-1 expression were detected in the PCC lines (a). Co-culturing galectin-1-overexpressing PSCs (GO-PSC) with PCC lines increased the galectin-1 expression in the PCCs (b). *$P < 0.01$ vs. single culture, $\#P < 0.05$ vs. single culture. (c, d) Green fluorescent protein-expressing PANC-1 cells transduced with pHAGE lentivirus were selected using flow cytometry and were observed under epifluorescence and light microscopy, which confirmed the presence of the galectin-1-expressing lentivirus in all cells. (e, f) Representative Western blot results showing the increased human galectin-1 expression in PANC-1 cells transduced with the galectin-1 lentivirus (Over). (g) Quantitative analysis of the differentiated phenotype of PANC-1 cells. Human galectin-1 mRNA expression was increased in the Over group. *$P < 0.01$ vs. control PCCs.
assay showed that 14 days after seeding under anchorage-independent conditions, the PANC-1 cells overexpressing galectin-1 formed significantly more colonies than the control PANC-1 cells ($P = 0.0176$) (Figure 3a, 3g). The wound healing assay similarly showed more migration of the galectin-1-overexpressing PANC-1 cells compared to the control cells 24 h after wounding ($P < 0.01$) (Figure 3b, 3h). PANC-1 cells overexpressing galectin-1 had significantly increased invasive ability compared to the control cells ($P = 0.0469$) (Figure 3c, 3i).

The MTT assay showed that PANC-1 cells overexpressing galectin-1 had significantly increased proliferation compared to the control cells at 48 h ($P < 0.05$), 72 h ($P < 0.01$), and 96 h ($P < 0.01$) (Figure 3f). More importantly, galectin-1-overexpressing PANC-1 cells had increased BCL-2 and proliferating cell nuclear antigen (PCNA) expression ($P < 0.01$) (Figure 3e, 3k) and decreased Bax expression ($P < 0.05$) (Figure 3e, 3k), further supporting the pro-proliferation effect of galectin-1 on PANC-1 cells and also indicating that galectin-1 might have anti-apoptotic effects. In addition, the TUNEL assay showed that galectin-1-overexpressing PANC-1 cells had an obviously decreased apoptosis rate ($P = 0.0465$), which further supports the anti-apoptotic activity of galectin-1 (Figure 3d, 3j). These results indicate that endogenous galectin-1 promotes PANC-1 cell proliferation, growth, migration, invasion, and anti-apoptosis effects, which contribute to the malignant biological behavior of the cells.

![Figure 3](image-url)Effects of galectin-1 overexpression on PCC cells. (a, g) The colony formation assay showing that galectin-1-overexpressing PANC-1 cells (Over) had a significantly increased ability to form cells clones. (b, h) The wound-healing assay showing that galectin-1 overexpression significantly increased PANC-1 cell migration. (c, i) Transwell assays showed that galectin-1 overexpression significantly increased PANC-1 cell invasion. (f) MTT assays showed that galectin-1 overexpression significantly increased PANC-1 cell proliferation. (d, j) The TUNEL assay showed that galectin-1 overexpression significantly decreased PANC-1 cell apoptosis. *$P < 0.05$ vs. Control (normal) PCCs. (e, k) Galectin-1 overexpression in PANC-1 cells increased BCL-2 and PCNA expression, and decreased Bax expression, indicating that it promotes PANC-1 cell proliferation and decreases apoptosis. *$P < 0.01$ vs. Control (normal) PCCs, *$P < 0.05$ vs. Control (normal) PCCs. OD, optical density.
Influence of endogenous galectin-1 in PCCs on the establishment and growth of tumors

In order to evaluate the influence of endogenous galectin-1 in PCCs on tumor progression in vivo, we embedded PANC-1 cells subcutaneously into the flanks (left, galectin-1-overexpressing cells; right, control cells) of male nude mice. Compared with the control side, the tumor volume of the galectin-overexpressing side increased significantly more beginning on day 10 after implantation (Figure 4a, b). At 25 days after implantation, the mean volume and weight of tumors on the galectin-overexpressing sides were 877.25 ± 89.75 mm$^3$ and 0.85 ± 0.10 g, respectively, while those of the control side were 549.75 ± 63.80 mm$^3$ and 0.38 ± 0.25 g, respectively (Figure 4b, c). The results clearly showed that the tumors grew more quickly and were when the cells overexpressed galectin-1 ($P < 0.01$). In addition, the EdU incorporation assay revealed that the galectin-overexpressing tumors had higher proliferation than the control tumors (Figure 4d, e). The results show that the overexpression of endogenous galectin-1 in PCCs increase tumor formation and growth.

Discussion

It was previously established that PSCs in the PDAC tumor microenvironment can boost tumor growth, invasion, metastasis, tumor hypoxia, immune evasion, and drug resistance [31,32]. We evaluated the influence of galectin-1 in PSCs, and found that they induced galectin-1 expression in PCCs, which in turn promoted the malignant biological behavior of PDAC. Histologically, galectin-1 is mostly expressed in the PDAC stroma (the source of PSCs) [23], but in the current study, some PSC-adjacent PCCs also had obvious galectin-1 staining, and there was a strong positive association between this staining and the malignant behavior of PDAC. Furthermore, we showed that high galectin-1 level in PSCs increased the expression of galectin-1 in the PANC-1 PCC cells, and galectin-1 overexpression in the PANC-1 cells enhanced PCC growth, proliferation, and invasion, increased PCNA and BCL-2 expression, decreased Bax expression and increased tumor establishment and growth.

PCNA is a known biomarker of proliferation, and its overexpression is linked to the progression of PDAC [33].

![Figure 4 In vivo animal model.](image-url)

(a, g) The colony formation assay showing that galectin-1-overexpressing PANC-1 cells (Over) had a significantly increased ability to form cells clones. (b, h) The wound-healing assay showing that galectin-1 overexpression significantly increased PANC-1 cell migration. (c, i) Transwell assays showed that galectin-1 overexpression significantly increased PANC-1 cell invasion. (f) MTT assays showed that galectin-1 overexpression significantly increased PANC-1 cell proliferation. (d, j) The TUNEL assay showed that galectin-1 overexpression significantly decreased PANC-1 cell apoptosis. *$P < 0.05$ vs. Control (normal) PCCs. (e, k) Galectin-1 overexpression in PANC-1 cells increased BCL-2 and PCNA expression, and decreased Bax expression, indicating that it promotes PANC-1 cell proliferation and decreases apoptosis. *$P < 0.01$ vs. Control (normal) PCCs, **$P < 0.05$ vs. Control (normal) PCCs.

OD, optical density
BCL-2 is a typical anti-apoptotic protein and its overexpression contributes to the progression and metastasis of various cancers [34,35]. Bax has 40% homology with Bcl-2, but its overexpression was proven to promote cell apoptosis in PDAC, and it is currently considered to be a pro-apoptotic protein. In addition, the TUNEL assay confirmed that galectin-1 has anti-apoptotic effects in PCC cells [36]. In summary, the results indicate that the expression of galectin-1 by PSCs and the interaction between PSCs and PCC cells may be important in the initiation and progression of PDAC.

Cancer cells typically overexpress galectin-1, and it functions in tumor cell adhesion, invasion, angiogenesis, the epithelial–mesenchymal transition (EMT) and metastasis [37-39]. It is being increasingly recognized as a vital protein that mediates the development of an immunosuppressive microenvironment, allowing for tumor growth via promoting angiogenesis and local immunosuppression [40]. Galectin-1 can function via the TGF-β1/Smad, TNF-α/NF-κB, and Notch signaling pathways, promoting a variety of malignant behaviors [41]. Galectin-1 has been considered a potential target for cancer treatment by using relevant inhibitors [42]. Moreover, our previous research [2,23,24] demonstrated that β-lactose, an antagonist of galectin-1, could reduce the malignant behavior of PDAC. In PDAC, galectin-1 induced a series of tumor-related processes, such as invasion, angiogenesis, MMP2/MMP9 expression and the EMT, which could all contribute to the growth of pancreatic cancer cells [43]. Galectin-1 was initially thought to be expressed exclusively in activated PSCs, where its expression promoted cancer cell growth and correlated with a poorer survival [22,23]. Further observations revealed galectin-1 staining in some PSC-adjacent PCCs, and this positively correlated with the degree of PDAC differentiation. In addition, cancer cells in the metastatic lymph nodes in PDAC showed strong galectin-1 staining. Moreover, galectin-1 staining within the lymphocyte areas of the tumor was significantly associated with a poorer outcome [44]. These results indicate that increased galectin-1 expression in PCCs may induce the malignant behavior of cancer cells and promote PDAC progression. Increased evidence indicates that PSCs in the tumor microenvironment cross-talk with tumor cells to promote PDAC development [45], and the proteins or cytokines expressed by the PSCs and PCCs, and induced by their interactions, play a vital role in malignancy, but the mechanism(s) have been unclear.

To clarify whether PSCs with high galectin-1 expression can induce galectin-1 expression in PCCs and whether it is related to the malignant biological behavior of PDAC, we co-cultured PSCs and PCCs. We discovered that PSCs with high galectin-1 expression could promote galectin-1 expression in PCCs, while treatment of the cultures with β-lactose decreased the expression, confirming that the galectin-1 expressed by PSCs was at least partly responsible for the increase in galectin-1 in PCCs. Furthermore, PANC-1 cells transfected with recombinant adenovirus in vitro to overexpress galectin-1 had increased growth, proliferation, invasion, and anti-apoptotic activity compared to control cells, which was confirmed in the PANC-1 xenograft model.

However, the full impact of the interaction between PSCs and PCCs with regard to galectin-1 induction and the subsequent promotion of malignant behavior by PDAC requires further investigation. At present, the following are considered to be implicated in these effects: first, activated PSCs can induce the epithelial–mesenchymal transition (EMT) in PCCs [45-47], and galectin-1 can also trigger the EMT in cancer cells [38,39,48-50], therefore, mesenchymal-phenotype PCCs may have increased galectin-1 expression. Second, PSCs can also induce stem cell-like phenotypes in PCCs, resulting in resistance to conventional therapies, distant metastasis, and recurrence [51-54]. Third, galectin-1 is overexpressed in cancer stem cells [55], and these cells are usually found on the tumor periphery, where the invasive front is located. This finding indicates that the stem cell-like cancer cells may have increased galectin-1 expression and may induce or increase the progression of PDAC.

There have been a few previous studies on the biological influence of galectin-1-overexpressing PSCs on PCCs [23,30,56,57]. Based on those studies and the present findings, we hypothesize that after they come into contact with the surrounding stroma, the PSCs begin to express galectin-1 on the membrane. Moreover, high galectin-1 expression in the stroma can lead to the desmoplastic reaction via paracrine signaling. We believe that the galectin-1 secreted into the tumor microenvironment by PSCs acts on fibroblasts via both paracrine and autocrine pathways to promote cell proliferation, invasion and migration.

In conclusion, our study indicates that the overexpression of galectin-1 in PSCs enhances the expression of galectin-1 in PCCs. This process promotes PCC proliferation, anti-apoptosis, invasion, and tumor establishment and growth in an autocrine manner. Our previous study reported that PSC-derived galectin-1 can increase PDAC progression and lead to a poorer survival [23]. Thus, targeting galectin-1 on both PSCs and PCCs might inhibit PDAC progression. However, the specific mechanism and method to specifically target these cells requires further study.

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Conflicts of Interests
The Authors declare that there is no conflict of interest.

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